ner. On chromatography, there was isolated 75 mg (34.2%) of **21** and 164 mg (41.8%) of **19**.

For **19**: IR (neat, cm^{-1}) 3048, 2950, 2870, 1691, 1465, 1391, 1370, 1164, 856, 715; ^1H NMR (300 MHz, C_6D_6) δ 5.92 (t, J = 8.2 Hz, 1 H), 5.75 (t, J = 8.3 Hz, 1 H), 3.14 (t, J = 7.0 Hz, 1 H), 2.77-2.68 (m, 1 H), 2.45-2.27 (m, 2 H), 1.58 (dd, J = 11.2, 3.4 Hz, 1 H), 1.72-1.37 (m, 5 H), 1.30-1.23 (m, 1 H), 1.08-0.98 (m, 1 H), 0.84 (s, 3 H), 0.67 (s, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$, ppm) 208.28, 137.47, 124.89, 59.10, 51.33, 48.57, 42.53, 39.88, 32.89, 28.59, 27.63, 25.08, 24.11, 22.22; MS m/z (M^+) calcd 204.1514, obsd 204.1520.

Anal. Calcd for $C_{14}H_{20}O$: C, 82.30; H, 9.87. Found: C, 82.38; H, 9.87.

Exposure of **14e** (90 mg, 0.47 mmol) to the identical reaction conditions furnished after chromatographic separation the pair of ketones **27** (7 mg, 7.3%) and **24** (22 mg, 24.3%).

For **27**: IR (neat, cm^{-1}) 2940, 2867, 1690, 1446, 1159, 875, 841; ^1H NMR (300 MHz, C_6D_6) δ 5.64 (d, J = 7.6 Hz, 1 H), 2.89 (d, J = 5.9 Hz, 1 H), 2.64-2.48 (m, 2 H), 2.26-2.22 (m, 1 H), 1.96-1.89 (m, 1 H), 1.70 (d, J = 1.6 Hz, 3 H), 1.75-1.17 (series of m, 9 H); ^{13}C NMR (75 MHz, $CDCl_3$, ppm) 207.12, 134.89, 129.24, 54.36, 51.65, 51.43, 35.40, 30.61, 27.18, 25.86, 24.77, 21.98, 21.71.

The reaction involving **14f** (232 mg, 1.06 mmol) resulted in the formation of **29** (11 mg, 4.8%) and **28** (49 mg, 21.3%).

For **28**: IR (neat, cm^{-1}) 2944, 2870, 1693, 1464, 1446, 1366, 1140, 869; ^1H NMR (300 MHz, C_6D_6) δ 5.57 (d, J = 7.9 Hz, 1 H), 3.11 (dd, J = 7.8, 5.3 Hz, 1 H), 2.75-2.67 (m, 1 H), 2.37 (dd, J = 13.6, 5.4 Hz, 1 H), 2.29-2.18 (m, 1 H), 1.99 (m, 1 H), 1.52 (d, J = 1.6 Hz, 3 H), 1.66-1.40 (m, 5 H), 1.16-1.12 (m, 2 H), 1.04 (s, 3 H), 0.86 (s, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$, ppm) 207.47, 145.03, 119.10, 51.27, 48.97, 47.32, 45.49, 41.88, 40.99, 36.71, 29.88, 28.80, 26.03, 24.40, 21.70; MS m/z (M^+) calcd 218.1670, obsd 218.1697.

Anal. Calcd for $C_{15}H_{22}O$: C, 82.52; H, 10.15. Found: C, 82.37; 10.06.

For **29**: IR (neat, cm^{-1}) 2942, 2866, 1688, 1463, 1443, 1365, 843; ^1H NMR (300 MHz, C_6D_6) δ 5.68 (dd, J = 7.5, 1.5 Hz, 1 H), 2.92 (d, J = 6.0 Hz, 1 H), 2.76-2.70 (m, 1 H), 2.31-2.18 (m, 3 H), 1.770 (d, J = 1.6 Hz, 3 H), 1.72-1.65 (m, 1 H), 1.60-1.41 (m, 4 H), 1.39-1.30 (m, 1 H), 1.19-1.12 (m, 1 H), 1.03 (s, 3 H), 0.85 (s, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$, ppm) 207.39, 134.52, 129.34, 54.50, 51.20, 48.64, 45.88, 43.45, 37.36, 35.65, 29.63, 28.34, 26.34, 21.72 (2C); MS m/z (M^+) calcd 218.1670, obsd 218.1681.

Acknowledgment. Support of this work by the National Institutes of Health (Grant GM-28468) and the Ministere des Relations Exterieures (Paris) is gratefully acknowledged. We also thank Dr. M.-A. Poupart for carrying out the MM2 calculations.

Surface-Specific Cleavage of a Cationic Carbonate-Functionalized Vesicular Surfactant

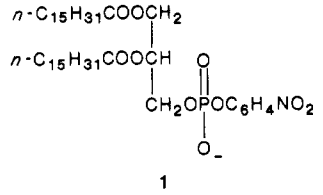
Robert A. Moss,* Santanu Bhattacharya, Paolo Scrimin,[1] and Shanti Swarup

Contribution from the Wright and Rieman Laboratories, Department of Chemistry, Rutgers, The State University of New Jersey, New Brunswick, New Jersey 08903. *Received April 8, 1987*

Abstract: The cationic, *p*-nitrophenyl carbonate functionalized 1,2-dipalmitoylglyceryl surfactant **2** was synthesized. Vesicles created at pH 3.9 from this surfactant gave rapid partial *p*-nitrophenol release at pH 7.9, attributed to site-specific hydrolysis of exovesicular *p*-nitrophenyl carbonate moieties. Endovesicular *p*-nitrophenyl carbonates are cleaved ~700 times more slowly under these conditions, probably because they are protected by the maintenance of a pH gradient across the outer vesicle bilayer.

The ubiquity of morphological and functional asymmetry between the two halves of bilayer biological membranes[2] has stimulated attempts to create *synthetic* vesicular (liposomal) membranes that are chemically differentiated at their exovesicular and endovesicular surfaces.[3] Although glyceryl diester lipids are major constituents of biological membranes,[4] very little has been done in the way of generating exo/endo-differentiated, synthetic glyceryl diester vesicles.

Recently, we reported that anionic vesicles created from the *p*-nitrophenyl phosphate derivative of 1,2-dipalmitoylglycerol (**1**) could be specifically cleaved at their exovesicular surfaces by hydroxide ions at pH 11.8.[5] The endovesicular *p*-nitrophenyl



1

phosphate moieties became accessible only after "damaging" the vesicles with the cationic, single-chain surfactant cetyltrimethylammonium chloride (CTACl). Exo/endo surface specificity was attributed to the inability of hydroxide ions to cross the vesicular bilayers, a suggestion supported by the finding that vesicle-entrapped riboflavin (at pH 5.5) was not deprotonated by bulk aqueous hydroxide ion at pH 11.8, although this reaction, and the attendant loss of riboflavin fluorescence, occurred immediately upon the addition of CTACl.[5]

Despite the novel, locus-specific chemistry observed with vesicular **1**, there remained the suspicion that it constituted somewhat of a special case even among liposomes, which, as a class, are known to maintain a variety of chemical gradients.[6] Thus, the *p*-nitrophenyl phosphate scissile groups present in vesicular **1** ensure that the initial cleavage of *p*-nitrophenoxide (PNPO) from the monoanionic disubstituted phosphate will leave behind a less reactive, dianionic monoalkylphosphate ester and that these residual phosphatidic acid dianionic surfactants will form particularly stable vesicles.

In order to generalize the hydrolytic procedure for the creation of surface-differentiated glyceryl diester vesicles, we have now prepared a cationic, nonphospholipid, diester, reactive carbonate surfactant, **2**. Vesicles of **2**, created at pH 4, support surface-specific exovesicular cleavage of $PNPO^-$ by bulk aqueous hydroxide ions at pH 8. The resulting vesicles should carry "choline" hydroxyl at their exovesicular surfaces but retain *p*-nitrophenyl carbonate functionalities at their endovesicular surfaces. The new results are particularly interesting in contrast to the inability of vesicles created from the analogously functionalized dihexadecylmethylammonium surfactant, **3**, to support surface-specific

(1) Visiting Professor from Università di Padova, Fulbright Scholar, 1985-1986.

(2) Jain, M. K.; Wagner, R. C. *Introduction to Biological Membranes*; Wiley: New York, 1980; pp 105f.

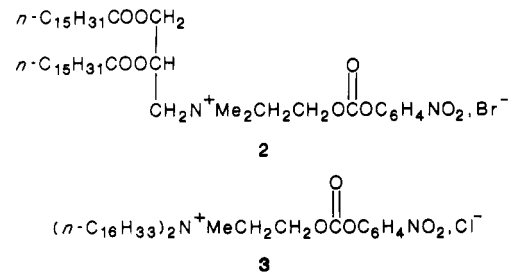
(3) Reviews: (a) Fuhrhop, J.-H.; Fritsch, D. *Acc. Chem. Res.* **1986**, *19*, 130. (b) Fuhrhop, J.-H.; Mathieu, J. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 100. (c) Fendler, J. H. *Membrane Mimetic Chemistry*; Wiley: New York, 1982.

(4) Eibl, H. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 257.

(5) Moss, R. A.; Swarup, S. *J. Am. Chem. Soc.* **1986**, *108*, 5341. A full account of this work will appear in: *Surfactants in Solution*, Vol. 7; Mittal, K. L., Ed.; Plenum: New York, in press.

(6) See ref 2, pp 117-142, and ref 3c, pp 141-158.

0002-7863/87/1509-5740$01.50/0



2

3

hydrolysis under similar reaction conditions.[7]

Results

Synthesis. Surfactant **2** was prepared by the sequence outlined in Scheme I. *dl*-3-Bromo-1,2-propanediol was diesterified with palmitoyl chloride in chloroform/pyridine at 25 °C, affording solid bromo diester **4** in 70% yield after recrystallization from ether/methanol. This material was pure by TLC and had the appropriate properties.[8] Bromide **4** was converted to the *N,N*-dimethyl tertiary amine **5** by reaction with excess dimethylamine in acetone (sealed tube). The amine was purified by chromatography over silica gel ($CHCl_3$ eluent) to afford a waxy solid (83%) that, without further purification, was quaternized with excess 2-bromoethanol in acetone (containing a trace of diisopropylethylamine to remove adventitious HBr), affording crystalline ammonium salt **6**. Chromatography afforded pure **6** in 82% yield.

Finally, choline analogue **6** was converted to the desired *p*-nitrophenyl carbonate, **2**, by reaction with excess *p*-nitrophenyl chloroformate in chloroform/pyridine. Recrystallization afforded **2** with bromide counterions. The structure was secured by NMR spectroscopy and elemental analysis; cf. Experimental Section.

Vesicles. These were prepared from solid surfactant **2** under carefully controlled sonication conditions: pH 3.9, 20 °C, sonication at 40 W for 25 min. The *p*-nitrophenyl carbonate group is readily cleaved, so reproducible vesicle formation without extensive concomitant esterolysis requires care. This problem is discussed further later. Vesicle preparations were filtered through 0.8-μM Millex (Millipore) filters before use in order to remove any titanium particles from the sonicator probe.

Dynamic light scattering studies (Ar laser, 488 nm, 90° scattering angle) gave a hydrodynamic diameter of 1400–1500 Å for vesicular **2** at pH 3.9. Covesicallization under these conditions with 5-fold excess CTACl or sodium dodecyl sulfate (SDS) afforded aggregates with d = 1250 or 900 Å, respectively. Adjustment of vesicular **2** to a bulk aqueous pH of 8.1 gave aggregates with $d \sim 1000$ Å, but extensive PNPOH esterolysis occurs under these conditions (vide infra).

In general, electron microscopic examination of air-dried vesicular **2**, stained with 2% uranyl acetate at pH 4 and dried on a carbon–formvar coated copper grid, substantiated the light scattering results. We observed mostly spherical aggregates with $d \sim$ 1100–1400 Å. The slightly lower diameter (vs. ~1400 Å in solution) may reflect dehydration of the vesicle. It was difficult to decide on the average number of lamellae in these vesicles from the electron micrographs, but the size is suggestive of multilamellar character.[9]

Vesicular solutions of **2** at pH 3.9 appeared by light scattering to be in size stable for ~50 h, but, even at this pH, slow cleavage of *p*-nitrophenol occurred.[10] Therefore, all kinetic and characterization studies employed freshly prepared vesicular **2**.

The temperature associated with the gel to liquid crystalline phase transition of vesicular **2** (T_c) was determined from the discontinuity in the fluorescence polarization (P) of covesicular

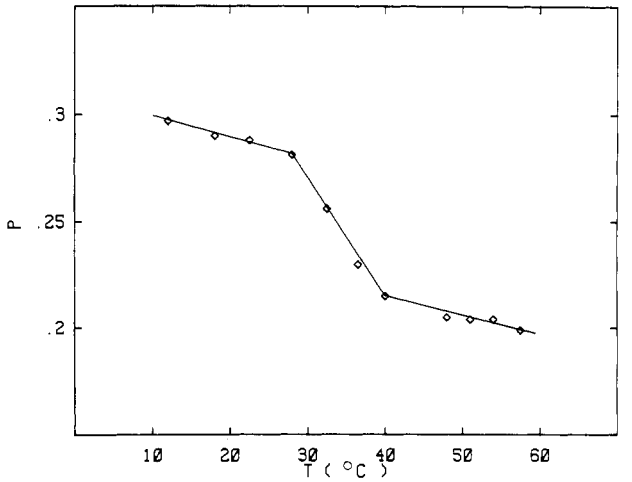
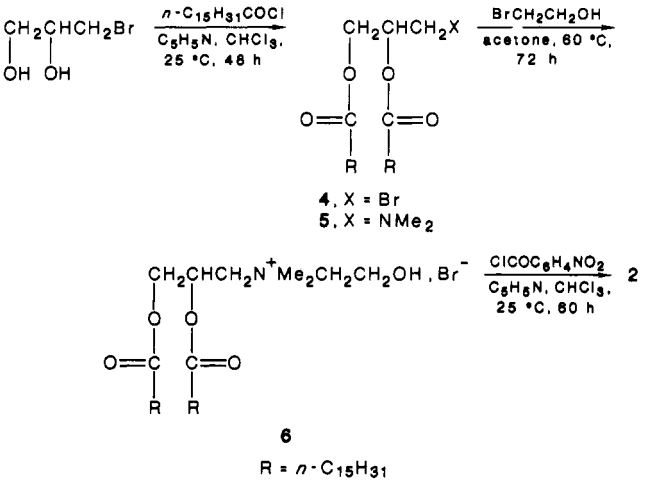


Figure 1. Fluorescence polarization (P) of 1,6-diphenyl-1,3,5-hexatriene in vesicular **2** as a function of temperature (T). The midpoint (34 °C) of the "transition region" is taken as the gel to liquid crystal phase transition temperature (T_c) of vesicular **2**.

Scheme I



1,6-diphenyl-1,3,5-hexatriene as a function of temperature, according to the method of Andrich and Vanderkooi.[11] Figure 1 shows that P changes sharply between ~28 and 40 °C. The midpoint of the transition, 34 °C, is taken as T_c. As a test of the accuracy of the fluorescence method, we obtained similar T_c values (25–27 °C) for sonicated dihexadecyldimethylammonium bromide vesicles by either the fluorescence method or differential scanning calorimetry.

Attempts to prepare vesicular **2** by injection methods largely failed; unavoidable esterolysis or other decomposition occurred upon dissolving **2** in warm ethanol, acetonitrile, dioxane, or dimethyl sulfoxide preparatory to the injection step, and the surfactant was insoluble in the cold solvents. We also examined sonication procedures at 0 °C,[12] but these vesicle preparations were similar in the extent of accompanying esterolysis to preparations from sonication at 20 °C; see below.

Kinetic Studies. Esterolysis reactions of vesicular **2** were generally initiated by mixing a pH 3.9 solution of vesicular **2** with a Tris buffer solution adjusted to pH 8.14, 9.26, or 10.02 to afford a reaction solution with an appropriately basic final pH. The vesicle and buffer solutions were both maintained at μ = 0.01 (KCl) to ensure constant ionic strength before and after mixing. The kinetics of the cleavage of *p*-nitrophenoxide (PNPO) ion from

(7) Moss, R. A.; Schreck, R. P. *Tetrahedron Lett.* **1985**, *26*, 6305.
(8) Moschidis, M. C. *Chem. Phys. Lipids* **1985**, *36*, 297.
(9) Hope, M. J.; Bally, M. B.; Mayer, L. D.; Janoff, A. S.; Cullis, P. R. *Chem. Phys. Lipids* **1986**, *40*, 89.
(10) This could be followed spectrophotometrically at 317 nm. Alternatively, released *p*-nitrophenol appeared as an "instantaneous" absorption increase at 400 nm when the pH was adjusted to 8 to inititate kinetic runs; see below.
(11) Andrich, M. P.; Vanderkooi, J. M. *Biochemistry* **1976**, *15*, 1257. See also: Swarup, S.; Moss, R. A. In *Surfactants in Solution*, Vol. 7; Mittal, K. L., Ed.; Plenum: New York, in press.
(12) Berenholz, Y.; Gibbes, D.; Litman, B. J.; Goll, J.; Thompson, T. E.; Carlson, F. D. *Biochemistry* **1977**, *16*, 2806.

Table I. Cleavage of *p*-Nitrophenoxide from Vesicular **2** at 25 °C[a]

run	final pH	additive	10^4(concn), M	$10^2 k_\psi^{f}$,[b] s^{-1}	$10^2 k_\psi^{s}$,[c] s^{-1}
1	7.94	none		3.1	0.0044
2	8.75	none		14.4	0.032
3	9.59	none		[d]	0.099
4	7.94	none		3.1	6.1[e]
5	11.6	SDS	33.3	0.0065	[f]
6	9.6	SDS	2.5	0.031	[f]
7	7.94	SDS	0.5	0.010	0.54[e]
8	8.75	SDS	0.5	0.034	[g]
9	9.6	SDS	0.5	0.28	[g]
10	7.9	CTACl	2.5	5.8	6.9[e]
11	7.9	CTACl	5.0	5.8	8.9[e]
12	7.9	CTACl	7.5	5.8	10[e]
13	7.9	$NH_4^+Cl^-$	1330	5.3	none[h]
14	7.9	$Me_4N^+Cl^-$	1500	1.7	3.3[e,i]

[a] Conditions: [**2**] = 5×10^{-5} M in all runs except run 2, where it is 3.2×10^{-5} M; surfactant additives were cosonicated with **2** at pH 3.92. [b] Initial "fast" pseudo-first-order cleavage of PNPO. Total absorbance ~50% of theory, including "prior cleavage"; see text. [c] Subsequent "slow" cleavage. [d] Too fast to monitor by conventional kinetics. [e] 0.1 mL of 1 M aqueous NaOH solution was added at the end of the "fast" reaction. The second kinetic step proceeded at pH 12.3. The first reaction proceeded at the indicated "final pH". [f] No slow reaction; the first reaction accounted for quantitative release of PNPO in a monoexponential process. [g] No distinct biphasic behavior; k_ψ^{f} accounted for 65–70% of the absorption change. [h] The fast reaction accounted for 95% of PNPO release. [i] The kinetic phasing was 55%:45% for the two successive steps.

2 were followed spectrophotometrically at 400 nm and pH ≥8. Under these conditions, PNPO that had been cleaved during vesicle preparation afforded an "instantaneous" absorption "jump", whereas vesicular cleavage occurring subsequent to mixing displayed a time-dependent absorption increase. In all experiments, reaction solutions ultimately afforded quantitative release of PNPO. Prior cleavage during vesicle preparation was ≤25%.

Time-dependent cleavages of PNPO from vesicular **2** at pH ≥7.9 were kinetically complex, generally consisting of consecutive "fast" and "slow" pseudo-first-order processes. This behavior is exemplified by runs 1–4 of Table I at pH 7.94, 8.75, or 9.59. The fast and slow kinetics phases each account for ~50% of the total absorbance change. However, we have included prior cleavage (from vesicle preparation) with the total absorbance change attributed to the fast step. Also, our manual mixing technique probably causes us to miss ~10% of the fast reaction absorbance change. Therefore, a more accurate accounting of the fast/slow absorbance changes would probably be ~40:60, suggestive of multilamellar vesicles; cf. above.[13].

Both the fast and slow processes increase with pH. [Indeed, log k_ψ^{s} vs. pH is *linear* (runs 1–4) at pH 7.9, 8.8, 9.6, and 12.3.] At pH 7.9, k_ψ^{f} is ~700 times greater than k_ψ^{s} (run 1). A graphical rendering of the data for run 2 of Table I appears in Figure 2 and clearly illustrates the biphasic kinetic behavior. Here, at pH 8.75, $k_\psi^{f} = 0.14$ s^{-1} and it is ~400 times greater than k_ψ^{s} (3.2×10^{-4} s^{-1}). Note that although there is a clear time differential between these consecutive reactions (i.e., $\tau_{1/2}^{f} = 4.8$ s, $\tau_{1/2}^{s} = 36$ min), the slow process is always proceeding, albeit much more slowly than the initial process. In fact, we conclude that the slow cleavage of PNPO is an endovesicular reaction that is rate limited by the permeation of OH^- into the vesicles; see below and the Discussion.

Runs 5–9 of Table I report the effects of cosonicating the single-chain anionic surfactant SDS with **2**. The resulting

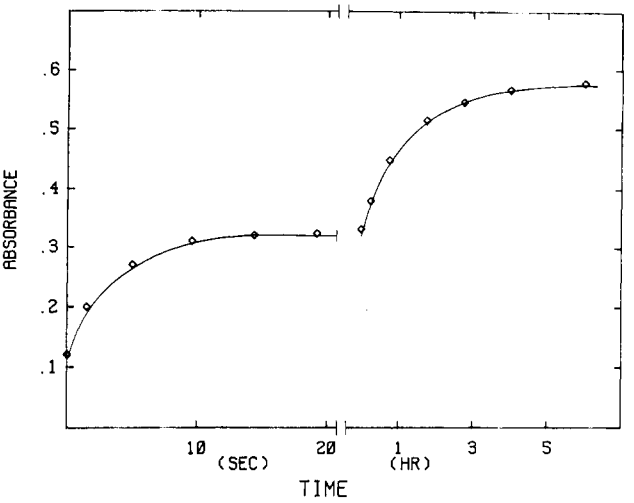


Figure 2. Absorbance at 400 nm vs. time for the cleavage of *p*-nitrophenoxide ions from vesicular **2** at a bulk aqueous (external) pH of 8.75 (Table I, run 2). Note the discontinuous time axis. The initial (exovesicular) reaction is complete within 20 s and has $k_4 = 0.14$ s^{-1}; the subsequent reaction requires several hours for completion with an apparent $k_\psi = 3.2 \times 10^{-4}$ s^{-1}.

coaggregates are now net negatively charged (runs 5 and 6) or they are charge neutralized (runs 7–9). They require either higher pH for efficient PNPO cleavage than does cationic vesicular **2**, or they afford lower values of "k_ψ^{f}" at comparable pH. These are consequences of the loss of the favorable **2**/OH^- electrostatic attraction with the **2**–SDS coaggregates. Most importantly, the coaggregates have *lost* the clearly differentiated biphasic kinetic character evident in the reactions of holovesicular **2**. In contrast, runs 10–12, involving coaggregates of **2** and the single-chain cationic surfactant CTACl, *retain* their biphasic cleavage kinetics.

Crucially, when a large excess of ammonium chloride was included in the pH 7.9 Tris buffer (run 13) vesicular **2** lost its biphasic kinetics; only k_ψ^{f} was observed, and it accounted for 95% of the $PNPO^-$ cleavage. Ammonium chloride is known to discharge OH^- gradients across bilayer membranes,[14] so this experiment suggests that the biphasic kinetics observed in runs 1–4 depend upon slow OH^- permeation to the endovesicular *p*-nitrophenyl carbonate target groups. The permeability of $NH_4^+Cl^-$ probably depends on its ability to dissociate to the neutral ammonia molecule. Accordingly, it is interesting to note that $Me_4N^+Cl^-$, which cannot so dissociate, does not alter the biphasic nature of the cleavage of vesicular **2**; cf. Table I, run 14.[15]

Discussion

The principal conclusion suggested by the data of Table I is that vesicular **2** supports surface-specific exovesicular carbonate hydrolysis. About half of the scissile PNPO moieties can be cleaved at a mildly basic pH and at rates that are hundreds of times faster than cleavage rates of the residual (endovesicular) PNPO groups (runs 1 and 2). The latter can be readily cleaved at higher pH (runs 3 and 4). The rate constants for (both) exovesicular (and endovesicular) cleavage increase with increasing pH (runs 1–4), and, as expected, exovesicular hydroxide ion cleavage of cationic carbonate **2** (k_ψ at pH 8.8 = 0.14 s^{-1}) is much faster than the analogous cleavage of anionic vesicular phosphate **1** (k_ψ at pH 11.8 = 4.7×10^{-4} s^{-1}).[5] It is remarkable that the hydrolytically sensitive, reactive ester moieties of **2** can be protected from rapid hydrolysis by confinement to inner loci of these surfactant vesicles. However, this "protection " is not as complete

(13) Employing reasonable values for the molecular volume of **2** (~1200 Å^3) and the thickness of vesicular lamellae (30–40 Å), we can estimate the distribution of molecules of **2** in (e.g.) a trilamellar vesicle with an overall diameter of 1400 Å (light scattering). We calculate that ~36% of the surfactant molecules reside in the exovesicular leaflet of the outermost bilayer, whereas the remaining 64% are distributed between the endovesicular leaflet of this bilayer (26%) and the interior second (30%) and third (8%) lamellae. This rough calculation is in reasonable accord with the implications of the kinetic results. For a model analysis of the geometry of egg phosphatidylcholine vesicles, see: Huang, C.; Mason, J. T. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 308.

(14) Bramhall, J. *Biochim. Biophys. Acta* **1984**, *778*, 393 and references therein.

(15) We note that the use of entrapped riboflavin as a probe of OH^- permeation to endovesicular sites (as with **1**; see above) is impractical with vesicular **2** because of the high pK_a of riboflavin (10.2). The resulting high pH needed to deprotonate the riboflavin and quench the fluorescence of its acidic form extensively cleaves $PNPO^-$ from **2**. Strong absorption at ~400 nm by the $PNPO^-$ prevents efficient excitation of the riboflavin.

as it is for the active phosphate groups of anionic vesicular **1**, where (at an external pH of 11.6) endovesicular cleavage is effectively nil, even after 24 h. With cationic vesicular **2**, however, endovesicular cleavage is appreciable at an external pH of 8, with $k_\psi = 4.4 \times 10^{-5}$ s^{-1} and $\tau_{1/2} = 4.4$ h.

It is of further interest that these protective properties are not afforded by covesicles of **3** and (n-$C_{16}H_{33})_2N^+Me_2$,Br^- (**16**$_2$), where OH^- cleavage at pH ~8 ($k\psi = 0.03$ s^{-1}) is similar in rate to the exovesicular cleavage of **2** but is quantitative and monophasic.[7] Indeed, we have recently been able to prepare holovesicles of **3** by the sonication protocol described in the Experimental Section. The p-nitrophenyl carbonate groups of these vesicles were also cleaved by OH^- at pH 8 with quantitative, uniphasic kinetics ($k_\psi = 0.057$ s^{-1}). We will return below to this key difference between **2** and **3**.

Obviously, the exo/endo hydrolytic kinetic differentiation previously observed[5] with vesicular **1** is not limited to anionic, phosphatidic acid derived surfactant vesicles. This phenomenon has now been observed with cationic nonphospholipid vesicles. Note that upon completion of the exovesicular hydrolysis of **2** (~20 s; cf. Figure 2), the outer surface of the vesicles should be functionalized with choline moieties (cf. **6** in Scheme I) resulting from rapid decarboxylation after the PNPO cleavage. However, the endovesicular surface(s) will still bear p-nitrophenyl carbonate groups. We plan various experiments to make use of this surface-specific chemical differentiation.

As in the example of vesicular **1**,[5] we suggest that the origin of the surface-specific kinetic differentiation experienced with vesicular **2** resides in its ability to maintain a pH gradient across its bilayers. Thus, the exovesicular p-nitrophenyl carbonate groups can be subjected to an externally imposed, basic, hydrolytic, bulk aqueous medium, while the analogous endovesicular groups remain "protected" at or near the acidic pH (~4) associated with vesicle creation.[16] This scheme effectively mandates that slow permeation of OH^- be rate limiting in the cleavage of the otherwise highly reactive endovesicular p-nitrophenyl carbonate groups.

Support for this scenario comes from two lines of experimentation described above. First, endovesicular PNPO groups are cleaved at rates similar to those of the exovesicular groups (i.e., biphasic kinetics disappear) when vesicular **2** is rendered "porous" by coaggregation with the anionic, single-chain surfactant SDS (Table I, runs 5–9).[17] This observation parallels the loss of exo/endo kinetic differentiation experienced by anionic vesicular **1** upon covesicallization with excess, cationic, single-chain CTACl.[5] Presumably, these reagents create regions of enhanced OH^- permeability in the bilayer packing arrangement, thus providing reagent access to the endovesicular target groups. The vesicles of **1** or **2** are thus "damaged", but light scattering studies indicate that sizeable aggregates of **1** and **2** persist after cosonication with CTACl or SDS.

Second, the biphasic kinetics of vesicular **2** disappear in the presence of excess $NH_4^+Cl^-$, which is known to discharge transvesicular pH gradients.[14] This is not a salt effect; comparable concentrations of $Me_4N^+Cl^-$ are not able to "dephase" the kinetics (runs 13 and 14 in Table I).

We return now to the marked differences in the abilities of vesicular **2** and covesicular (**3** + **16**$_2$) to support kinetically differentiated exo and endovesicular cleavages. Clearly, the backbone architectures of these surfactants are very different, and we may immediately ask whether this feature alone, reflected in differing packings of the membrane bilayer derived from **2** and (**3** + **16**$_2$), suffices to explain their divergent chemical properties. More subtly, we determind the T_c of vesicular (**3** + **16**$_2$) as 24 °C, whereas that of vesicular **2** is ~34 °C (see above). Might the reactivity differences between these vesicular systems at 25 °C simply reflect the fact that (**3** + **16**$_2$) is at its gel to liquid crystalline transition temperature, and consequently more fluid and permeable to OH^- than vesicular **2**, which is below its T_c at 25 °C.?

Although the T_c factor may enter into the relative abilities of vesicular **2** and (**3** + **16**$_2$) to maintain OH^- gradients and to afford exo/endovesicular differentiation, it is unlikely to be the entire story. Vesicular (**3** + **16**$_2$) at 15 °C, below its T_c, is still unable to support kinetically biphasic carbonate cleavage at pH 8,[7] and, more importantly, vesicular **2** at 40 °C, above its T_c, still cleaves with distinct biphasic kinetics, albeit that the rate constants are greater than at 25 °C. Thus, $k_\psi^f = 0.090$ s^{-1} and $k_\psi^s = 7.4 \times 10^{-4}$ s^{-1} at 40 °C, whereas at 25 °C, the comparable values are 0.031 and 4.4×10^{-5} s^{-1}) (Table I, run 1). Additionally, the fast/slow phasing shifts toward the fast process (70%) at 40 °C.

We conclude that the differences between vesicular **2** and **3** (or **3** + **16**$_2$) are structure dependent to a significant degree and that the "natural backbone" architecture of **2** is supportive of the observed exo/endovesicular kinetic differentiation. These conclusions are subject to refinement by further experimental tests that are now under way. Most importantly, we have demonstrated that exo/endo surface chemical differentiation can be carried out with cationic, nonphospholipid, synthetic surfactant vesicles.

Experimental Section

Materials. 1,2-Dihydroxy-3-bromopropane, palmitoyl chloride, bromoethanol, and p-nitrophenyl chloroformate were obtained from Aldrich Chemical Co. The chloroformate was recrystallized (CCl_4) and sublimed before use. 1,6-Diphenyl-1,3,5-hexatriene was purchased from Fluka, and dimethylamine gas was obtained from Matheson. All solvents were Fisher spectral grade. Melting points and boiling points are uncorrected.

***rac*-1,2-Dipalmitoylglycerol Bromohydrin (4).** To a solution of 3-bromo-1,2-propanediol (1.55 g, 10 mmol) in 15 mL of freshly distilled $CHCl_3$ (from P_2O_5) and 1.58 g (20 mmol) of anhydrous pyridine at 0 °C was added dropwise with stirring a solution of 5.4 g (20 mmol) of palmitoyl chloride in 20 mL of dry $CHCl_3$ over ~1 h. After the addition was complete, the reaction solution was stirred magnetically for 48 h at 25 °C. Then the reaction solution was diluted with 120 mL of Et_2O and poured into 25 mL of ice cold 0.5 N H_2SO_4. The ethereal layer was separted, and then washed several times with 0.5 N H_2SO_4, with water, twice with 10-mL portions of 0.1 N $NaHCO_3$ solution, and again with water. The organic solution was dried over anhydrous Na_2SO_4, filtered, and stripped of solvent on a rotary evaporator. The white residual solid was dissolved in 25 mL of Et_2O, diluted to turbidity with 5 mL of dry CH_3OH, and stored at −5 °C for crystallization. The product was recrystallized in a similar manner to give 4.4 g (6.97 mmol; 69.7%) of **4**: mp 40.5–41 °C (lit.[8] mp 40.5–41 °C); R_f ~0.68 (silica gel TLC, 5:1 n-hexane/EtOAc, one spot); NMR (400 MHz, 99:1 $CDCl_3$, CD_3OD) δ 0.90 (t, $J = 7$ Hz, 6 H, 2 CH_3), 1.30 ("s", 48 H, 2 $(CH_2)_{12}$), 1.65 (q, $J = 7$ Hz, 4 H, 2 CH_2 β to C=O), 2.34 (q, $J = 6$ Hz, 4 H, 2 CH_2 α to C=O), 3.32 (2 AB q, 2 H, CH_2Br), 4.28 (2 AB q, 2 H, OCH_2CH), 5.03 (m, 1 H, OCH_2CH).

***dl*-1,2-Bis(palmitoyloxy)-3-(dimethylamino)propane (5).** A solution of 2.0 g (3.17 mmol) of **4** in 25 mL of dry acetone was placed in a screw-top pressure tube at 3–4 °C, and 3.9 g (86.7 mmol) of dimethylamine was added. The tube was sealed and its contents were heated with magnetic stirring at 60 °C for 40 h. Solvent was stripped and the oily residue was taken up in 20 mL of dry Et_2O, extracted with 2 × 15 mL of saturated aqueous $NaHCO_3$, washed with dilute brine, and dried over Na_2SO_4. After filtration, solvent was stripped to give crude **5**, which was purified by chromatography over 100 g of 230–400-mesh Sigma silica gel, eluted with $CHCl_3$. The desired **5** had R_f ~0.75 (silica gel TLC, 8:1 $CHCl_3$/MeOH) and was a waxy, semisolid at room temperature. We obtained 1.56 g (2.62 mmol; 82.6%) of **5**: NMR (400 MHz, 98:2 $CDCl_3$, CD_3OD) δ 0.90 (t, $J = 7$ Hz, 6 H, 2 CH_3), 1.30 ("s", 48 H, 2 $(CH_2)_{12}$), 1.62 (m, 4 H, 2 CH_2 β to C=O), 2.30 and 2.32 (s + m, 10 H, NMe_2 + 2 CH_2 α to C=O), 2.45 (m, 2 H, CH_2N), 4.1, 4.4 (m's, 2 H, OCH_2CH), 5.21 (m, 1 H, OCH_2CH).

(1,2-Bis(palmitoyloxy)-3-propyl)dimethyl(β-hydroxyethyl)ammonium Bromide (6). A solution of 395 mg of **5** (0.66 mmol), 400 mg (3.2 mmol) of bromoethanol, 1.0 μL of diisopropylamine, and 15 mL of dry acetone was prepared in a screw-top pressure tube. The tube was sealed and its contents were magnetically stirred and heated at 60 °C for 72 h. Cooling afforded white crystals that were filtered and washed with cold, dry acetone. Quaternary salt **6** was purified by chromatography over 50 g of 230–400-mesh silica gel with elution by 9:1 $CHCl_3$/MeOH to afford 390 mg (0.54 mmol; 82%) of pure **6**: R_f ~0.1 (TLC, silica gel, 9:1 $CHCl_3$/MeOH); mp ~95 °C (gel), 190 °C (liquid crystal); NMR (400 MHz, 80:20 $CDCl_3$, CD_3OD) δ 0.82 (t, $J = 7$ Hz, 6 H, 2 CH_3), 1.2 ("s", 48 H, 2 $(CH_2)_{12}$), 1.53 (m, 4 H, 2 CH_2 β to C=O), 2.27 (m, 4 H, 2 CH_2

(16) On the maintenance of gradients by vesicles, see: Kunitake, T.; Okahata, Y.; Yasunami, S. *Chem. Lett.* **1981**, 1397. See, also, note 6.

(17) Vesicles persist in these coaggregates as demonstrated by light scattering; see above.

α to C=O), 3.21, 3.23 (2 s, 6 H, 2 MeN^+), 3.60 (m, 3 H, CH_2N + 1 OCH_2CH), 3.97 (m, 4 H, $N^+CH_2CH_2O$), 4.47 (m, 1 H, 1 OCH_2CH), 5.64 (m, H, OCH_2CH). The OH signal was not observed.

Anal. Calcd for $C_{39}H_{78}O_5NBr$: C, 64.9; H, 10.9; N, 1.94. Found: C, 64.9; H, 11.2; N, 1.76.

(1,2-Bis(palmitoyloxy)-3-propyl)dimethyl(β-(p-nitrophenyl carbonato)ethyl)ammonium Bromide (2). To a solution of 350 mg (0.49 mmol) of **6** in 15 mL of freshly distilled $CHCl_3$ was added 400 mg (1.98 mmol) of p-nitrophenyl chloroformate and 39 mg (0.49 mmol) of dry pyridine (distilled from KOH and BaO). The solution was stirred magnetically for 60 h at 25 °C in a flask protected from moisture. Solvent was stripped, and the solid residue was recrystallized from acetone. Traces of pyridinium hydrochloride were removed by percolation with 5 mL of cold water (pH 5.5). The solid was dried under vacuum to give 250 mg (0.28 mmol; 57%) of carbonate **2**: R_f = 0.3 (TLC on silica gel, 9:1 $CHCl_3$/MeOH); mp ~105 °C (gel), 150 °C (dec); NMR (400 MHz, 70:30 $CDCl_3$, CD_3OD) δ 0.82 (crude t, J ~ 7 Hz, 6 H, 2 CH_3), 1.2 ("s", 48 H, 2 $(CH_2)_{12}$), 1.53 (m, 4 H, 2 CH_2 β to C=O), 2.27 (m, 4 H, 2 CH_2 α to C=O), 3.25, 3.27 (2 s, 6 H, 2 MeN^+), 3.76 (m, 2 H, $CHCH_2N^+$), 4.02 (m, 4 H, $N^+CH_2CH_2O$), 4.40 (d, J = 7 Hz, 1 H, 1 OCH_2CH), 4.70 (s, 1 H, 1 OCH_2CH) 5.56 (m, 1 H, OCH_2CH), 7.40 and 8.22 (A_2B_2 "doublets", 4 H, aromatic).

Anal. Calcd for $C_{46}H_{81}O_9N_2Br$: C, 62.3; H, 9.22; N, 3.16; Br, 9.02. Found: C, 62.3; H, 8.93; N, 2.95; Br, 9.25.

Generation of Vesicles. **2** (3.15 mg) was placed in a 50-mL beaker with 25 mL of distilled water adjusted to pH 3.92 with HCl. The water was added so that all of the solid was under water, with none floating on the surface. The beaker was put in a water bath at 20 °C, and after 5 min, its contents were sonicated with the 108 mm × 19 mm (diameter) immersion probe of the Braunsonic Model 1510 sonicator. The sonicator probe was immersed to three-fourths of its length, and sonication at 40 W was carried out for 25 min, during which the temperature of the solution rose to 38 °C. The vesicle solution was cooled to ~20 °C and filtered through a 0.8-μm Millex (Millipore) filter. See above for physical characterization of vesicular **2**.

Light Scattering. Light scattering data were collected at 25 °C and a 90° scattering angle with a Nicomp Model TC-100 computing autocorrelator, an argon laser light source (488 nm), and a Hazeltine microcomputer that used the cumulant program. The channel width was adjusted to produce a decay of 1.5-2.0 s. Vesicles were generated as described above, using [**2**] = 5×10^{-5} M. Variance in the observed hydrodynamic diameter ranged from 0.4-0.7. A variance <1.0 indicates a unidisperse system.

Electron Microscopy. One drop of a 5×10^{-5} M aqueous solution of vesicular **2**, prepared at pH 3.9, was placed on a 200-mesh carbon-formvar coated copper grid. One drop of 2% aqueous uranyl acetate (pH 4) was added. After 2 min, excess liquid was carefully blotted, and the sample was air-dried for 5-10 min more. The specimen was examined with a JEOL 100CX electron microscope at 80 kV. Micrographs were recorded at magnifications of 50 000 and 66 000.

T_c Measurement. To 3.15 mg (0.0036 mmol) of **2** in a 50-mL beaker was added 5 μL of a 1×10^{-5} M THF solution of 1,6-diphenyl-1,3,5-hexatriene (Fluka). Solvent was removed in a stream of nitrogen, and 25 mL of a pH 3.9 (HCl) solution of 0.01 M KCl was added. Sonication and filtration were carried out as described above. Fluorescence polarization measurements were carried out on 2-3-mL samples as described in ref 11, using a Perkin-Elmer Model MPF-3L fluorescence spectrometer and Polacoat 4B polarizers for both excitation and emission beams. Temperature (±1 °C) was regulated by a thermostated, circulating-water bath. The data appear in Figure 1 and are interpreted in the Discussion.

Kinetic Methods. Reactions were initiated by mixing vesicle solutions with Tris buffer solutions at various pHs. Kinetics were followed at 400 nm ($PNPO^-$ release) on a Gilford Model 250 spectrophotometer and a Gilford Model 6051 recorder. Pseudo-first-order rate constants were obtained from the traces by computer analysis in the standard manner. Kinetic runs are summarized in Table I.

Acknowledgment. We are grateful to Prof. Lee Simon (Waksman Institute) for electron microscopy and to the U.S. Army Research Office for financial support. The XL-400 NMR spectrometer used in this study was purchased in part with a grant from the National Science Foundation.

Structural Studies of 4-Phenyl-2,4,6-triazatetracyclo[6.3.2.$0^{2,6}$.$0^{7,9}$]trideca-10,12-diene-3,5-dione, a Dihydrodiazabullvalene. Preference for a Bisected Aminocyclopropane Derivative Having an Unusual Planar Triazolidinedione Ring

Grant R. Krow,*† Yoon B. Lee,† Steven W. Szczepanski,† David E. Zacharias,‡ and David B. Bailey§

Contribution from the Department of Chemistry, Temple University, Philadelphia, Pennsylvania 19122, Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111, and Technology and Development Center, ARCO Chemical Co., Newtown Square, Pennsylvania 19073. *Received March 14, 1986. Revised Manuscript Received May 5, 1987*

Abstract: X-ray structural analyses of dihydrodiazabullvalene derivative **2** have disclosed a nearly planar triazolidinedione ring. This geometry corresponds to a bisected aminocyclopropane with a planar amino group; prior calculations indicated this to be the least stable geometry for an aminocyclopropane. The analyses also reveal a long distal cyclopropyl bond of 1.688 (4) Å at 293 K or 1.586 (2) Å at 153 K with shorter adjacent cyclopropyl bonds of average value 1.477 (3) Å at 293 K or 1.499 (2) Å at 153 K. The long distal bond length at 293 K is associated with a high degree of atomic thermal motion. σ-Acceptor effects associated with the electronegative N3 atom of the triazolidinedione best account for distortions of the cyclopropyl bond lengths. Interatomic distances revealed by the X-ray diffraction analyses of crystalline **2** are consistent with a static structure, which at 293 K shows a step in the progression to the fluxional state; **2** is fluxional in solution at 30 °C.

The [4 + 2]-cycloaddition product **1** of 4-phenyl-1,2,4-triazoline-3,5-dione and cyclooctatetraene has been reported to form a photoproduct upon acetone-sensitized irradiation.[1] A rearranged dihydrodiazabullvalene **2** was suggested; however, confirming spectral data for **2** were not reported.[1,2] Further investigation

† Temple University.
‡ Fox Chase Cancer Center.
§ ARCO Chemical Co.

(1) Evin, A. B.; Miller, R. D.; Evanega, G. R. *Tetrahedron Lett.* **1968**, 5863-5866.

0002-7863/87/1509-5744$01.50/0