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(29) This isotope effect, an "α effect at the β carbon", is presumed to be a measure of ring opening in the transition state. A value of 1.00 would correspond to a reactant-like transition state. For a ring-opened product-like transition state the calculated value of 0.86 for two D is based on the fractionation factors for propane (1.103) and cyclopropane, i.e., $(1.029)\cdot(1.103)^{-1} = 0.93$ and $0.93^2 = 0.86$. *Between these limits the isotope effect may or may not vary linearly with the degree of ring opening in the transition state.* In this regard it was brought to our attention by a referee that the fractionation factor given by Hartshorn and Shiner[35] for cyclobutane is in fact identical with that given for cyclopropane. This somewhat surprising fact may have several interpretations. One is that the "α effect at the β carbon" is *not* linearly related to the degree of ring opening, but instead varies little from unity as the O–C–C angle increases from 60 to 90° and then begins to rise steeply as the O–C–C angle passes beyond 90° toward the tetrahedral limit. This would imply an *extremely* late or product-like transition state for the reaction of **1** at C-7 in acid. A second interpretation would be that the equality of the fractionation factors for cyclopropane and cyclobutane is fortuitous. The plausibility of this interpretation is supported by the fact that, for none of the terms which enter the calculation of the fractionation factor, are the values for cyclopropane and cyclobutane equal; only their products are equal (Table VIII of ref 35). Nevertheless, the issue of the manner in which the "α effect at the β carbon" varies with geometry is one which requires careful consideration in attempts to deduce transition state structures from kinetic secondary deuterium isotope effects.
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Robert P. Hanzlik,* Richard B. Westkaemper[38]
Department of Medicinal Chemistry
University of Kansas, Lawrence, Kansas 66045

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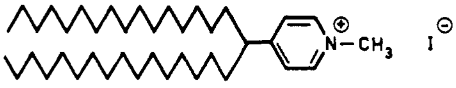
Vesicle Formation by Two Novel Synthetic Amphiphiles Carrying Micropolarity Reporter Head Groups

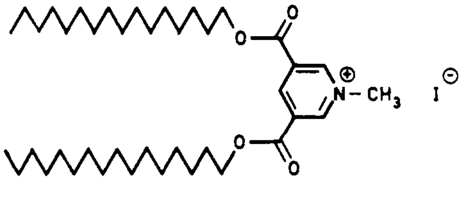
Sir:

Phospholipid vesicles or liposomes are of great interest as models for biological membranes.[1,2] Usually the vesicles were built up from naturally occurring or synthetically prepared phospholipid molecules. Recently it was shown that vesicles can also be obtained from simple synthetic organic amphiphiles,[3] which are in most cases composed of long-chain dialkyldimethylammonium halides.[4–7] There are, however, also reports on stable bilayer structures formed from amphiphiles with anionic,[8,9] zwitterionic,[10] or nonionic[10] head groups.

Herein we report a study of the aggregation behavior of two novel synthetic doubly chained surfactants, 17-(4-*N*-methylpyridinium)tritriacontane iodide (**1**)[11] and 3,5-dicarbo-*n*-hexadecyloxy-1-methylpyridinium iodide (**2**).[11] These amphiphilic molecules were chosen for investigation since the head groups exhibit charge-transfer (CT) transitions which are very sensitive to changes in the immediate microenvironment.[12] Thus, sonication of **1** and **2** in Tris·NaCl buffer solutions[16] gave transparant mixtures of uni- and multilamellar vesicles as revealed by electron microscopy.[17] Typical electron micrographs are shown in Figure 1. The diameter of these stable vesicles ranges from 500 to 5000 Å (**1**) and from 500 to 2500 Å (**2**), respectively. The micrographs resemble closely the patterns found for vesicles formed from phospholipids[18] and from other synthetic organic amphiphiles.[3a,4,5,8] When the sonication was performed in the presence of 1% (by weight) uranyl acetate, the micrographs showed lamellae of parallel and curved hydrocarbon layers which evoke a "fingerprint" impression (Figure 1).[19] The thickness of the bilayer (35 ± 5 Å) is estimated from the micrograph and corresponds with the expected value of ~37 Å for twice a fully extended *n*-hexadecyl hydrocarbon chain. The spacing between the bilayers is ~20 Å. It was also possible to prepare mixed vesicles (diameter 200–4000 Å) from both **1** and **2** with egg yolk phosphatidylcholine (PC).



1



2

The presence of *closed* vesicles was established by using the technique of Weinstein et al.[20] which takes advantage of the efficient self-quenching of the fluorescence of carboxyfluorescein (CF) upon entrapment of the dye in vesicles in relatively high concentration (100 mM). Escape of CF from the vesicle compartments results in vast dilution of the dye and the rapid buildup of fluorescence. Thus, after sonic dispersal of **1** or **2** in 1.5 mL of 0.1 M aqueous CF solution (pH 7.4) and centrifugation, nonentrapped CF was removed from the supernatant by column chromatography on Sephadex G-100 using a Tris·NaCl buffer solution[16] as the eluant. The vesicles were recovered in the void volume of the column. After dilution with a Hepes·NaCl buffer solution[21] (pH 7.4), the appearance of fluorescence, which monitores leakage of CF through the vesicle membranes, was recorded as a function of time at room temperature.[22,23] It is found that CF molecules are rapidly released from vesicles prepared from **1** by sonication at 0 °C (80% release after 20 min). No vesicles could be obtained upon sonication of **1** at 50 °C. By contrast, vesicles were readily formed upon sonic dispersal of **2** at 50 °C and these vesicles released CF only very slowly (9% release after 20 min). This temperature-dependent effect is reminescent of that observed previously for phospholipid vesicles[24] and probably originates from the formation of unannealed vesicles when sonication is

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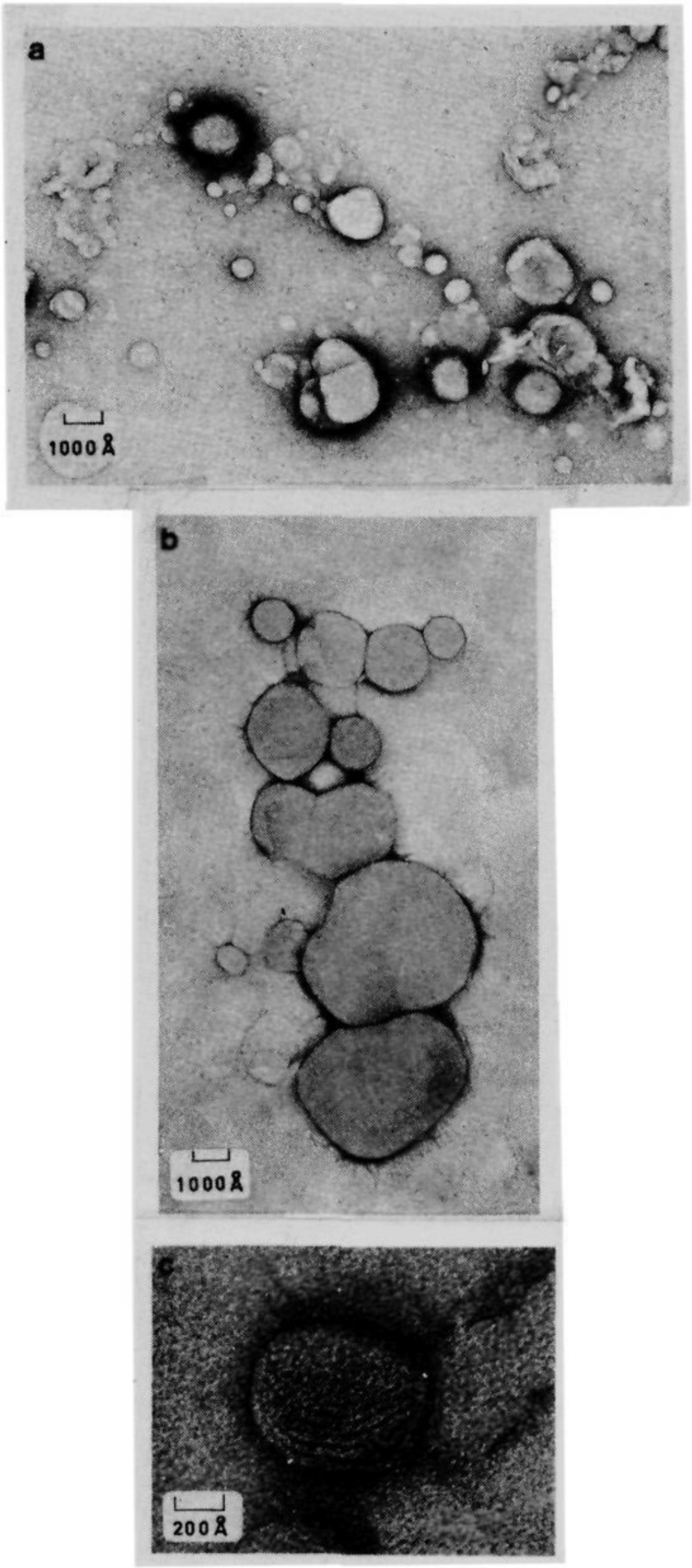

Figure 1. Electron micrographs of (a) sonicated **2** at 25.000-fold magnification; (b) sonicated **1** at 25.000-fold magnification; (c) **2** sonicated in the presence of 1% (by weight) uranyl acetate at 50.000-fold magnification.

carried out below its phase-transition temperature. Evidence, but no proof,[25] for the occurrence of a thermotropic phase transition between 25 and 50 °C for vesicles formed from **2** comes from a preliminary ^1H NMR investigation.[26] At 70 °C the observed line width of the 56 methylene protons at δ 1.35 ppm is 12 Hz. Upon decreasing temperature this signal broadens until the lines totally disappear at 25 °C. Restricted mobility of the hydrocarbon chains at reduced temperatures is commonly invoked to rationalize these effects.[4,27] Similar effects were observed below 10 °C in the ^1H NMR spectrum of vesicles generated from **1**.

Considering now the question of the micropolarity near the head groups in the electrical double layer of the vesicle membrane, we exploit the pronounced solvatochromism[28] of the first long-wavelength intramolecular CT absorption of **1** and **2**. In all cases the Beer–Lambert law was obeyed. The observed transition energies (E_T) were correlated with Kosower's Z values using 1,4-dimethylpyridinium iodide (**3**) and 3,5-dicarbomethoxy-1-methylpyridinium iodide (**4**) as model compounds.[29] The results have been utilized for the correlation of the transition energies obtained for vesicles prepared from **1** and **2** as well as for mixed vesicles of these amphiphiles with PC (Table I). Vesicles formed from pure **1** show λ_m 358 nm (E_T = 80.1 kcal mol^{-1}) and comparison with **3** yields Z = 64.2. Since E_T for monomeric **1** in water is estimated to be 115.6 kcal mol^{-1}, the transfer of the head group of **1** from water to the electrical double layer of the vesicles leads to a reduction of E_T by ~35.5 kcal mol^{-1} or a change in micropolarity from that in water to one comparable with that in dichloromethane! The effect is somewhat less dramatic for vesicles formed from pure **2** [λ_m 364 nm (E_T = 78.8 kcal mol^{-1}), Z = 74.0 by comparison with **4**]. This smaller reduction of E_T may reflect stronger hydration of the head group of **2** as compared with **1** and/or less close packing of the head groups for the more hydrophilic surfactant **2**.

Table I. Absorption Maxima, E_T Values, and Z Values for Vesicles and Mixed Vesicles

vesicles prepared from[a]	λ_m, nm	E_T, kcal mol^{-1}	Z
1	358	80.1	64.2
1:1 **1**–PC	357	80.4	64.6
1:5 **1**–PC	358	80.1	64.2
1:20 **1**–PC	*b*		
2	364	78.8	74.0
1:1 **2**–PC	366	78.4	73.6
1:2 **2**–PC	359	79.9	75.2
1:5 **2**–PC	358	80.1	75.3
1:20 **2**–PC	354	81.0	76.2

[a] Molar ratio is given. [b] Not observed.

No change in micropolarity is observed upon formation of mixed vesicles from **1** with PC regardless of the molar ratio. By contrast, vesicle formation from **2** with PC leads to a blue shift of λ_m and an increase in Z (Table I).

We like to emphasize that the micropolarity in the electrical double layer of vesicles is much less than that in the Stern layer of micelles formed from structurally related amphiphiles.[14] As argued by Menger,[30] the micellar surface shows definite roughness allowing for some penetration of water molecules between the hydrocarbon chains. For structural reasons, no such deep "fjords" will be present at the surface of vesicles and, consequently, head-group hydration will be less complete. The low micropolarity in the electrical double layer of vesicles might well mimic the microenvironmental properties in similar regions of biological membranes. This question, as well as the properties and aggregation behavior of other pyridinium salt surfactants, is the subject of further studies in this laboratory.

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$C_{40}H_{72}NO_4I$: C, 63.39; H, 9.58; N, 1.85; I, 16.74. Found: C, 63.50; H, 9.62; N, 1.67; I, 16.41.

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(16) A typical procedure is as follows. Amphiphile (10 mg) is dissolved in ~1 mL of chloroform (P.A. quality, distilled from P_2O_5) in a glass tube. The chloroform is evaporated in a stream of pure nitrogen gas and 1.5 mL of Tris•NaCl buffer solution (5 mM Tris•HCl + 0.15 M NaCl, pH 7.4) is added. The thin film of amphiphile on the glass vessel is dispersed by ultrasonic irradiation using a Bransonic 220 water bath during 5 min. The turbid solution is poured into a plastic tube and is sonicated for another 30 min at 0 °C by means of a pulsed high-energy probe (Branson Sonifier Cell Disruptor B 15). After centrifugation (Hettich rotafix II, ~1000 g), a transparant supernatant is obtained which was used in all experiments.

(17) The two-step droplet method was used to prepare the specimens on Formvar and carbon coated grids. A 1% (by weight) solution of uranyl acetate was used as negative stain. Micrographs were obtained with a Philips EM 300 electron microscope (operating at 80-kV accelerating voltage) on Kodak 4463 sheets. Electron magnification was 25.000 and 50.000 times.

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(21) 0.01 M Hepes + 0.135 M NaCl, pH 7.4.

(22) Perkin-Elmer MPF-43 fluorescence spectrophotometer, excitation wavelength 490 nm, emission wavelength 520 nm.

(23) The total amount of entrapped CF was determined after the experiment via destruction of the vesicles with an aqueous solution of Triton X-100 (final concentration: 1% by weight).

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(29) E_T values have been measured for **3** and **4** in solvents CH_2Cl_2, DMF, MeCN, 2-PrOH, and EtOH and for **4** also in MeOH and H_2O. For **4** in H_2O λ_m was obtained by using Mukerjee's band-match method.[15]

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Ernst J. R. Sudhölter, Jan B. F. N. Engberts*
Department of Organic Chemistry, University of Groningen
Nijenborgh 16, 9747 AG Groningen, The Netherlands

Dick Hoekstra
Department of Physiological Chemistry
University of Groningen, Bloemsingel 10
9712 KZ Groningen, The Netherlands

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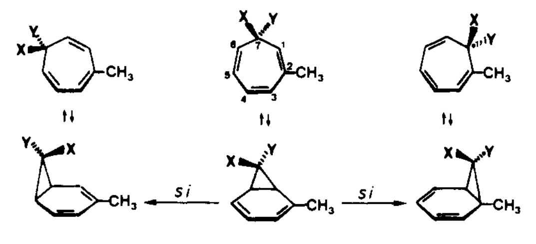
Stereochemistry of the Thermal Walk Rearrangement of Optically Active 2,7-Dimethyl-7-methoxymethyl-1,3,5-cycloheptatriene: 1,5-Carbon Migration with Inversion, *No* One-Center Epimerization at C-7

Sir:

The stereochemistry of the thermal norcaradiene walk rearrangement discovered by Berson and Willcott[1] is important for defining concerted and nonconcerted processes. Two experimental tests were reported giving conflicting results. The concern of this communication is to elucidate these discrepancies.

The same stereochemical course has been found for optically active ester **2** and nitrile **5** (Scheme I).[2] In both systems the thermal interconversion leading to **1** and **3**, respectively **4** and **6**, at 180 °C is highly stereoselective and proceeds with inversion at the migrating carbon, C-7 (*si*), contrary to the predictions of the Woodward-Hoffmann rules.[3]

Scheme I

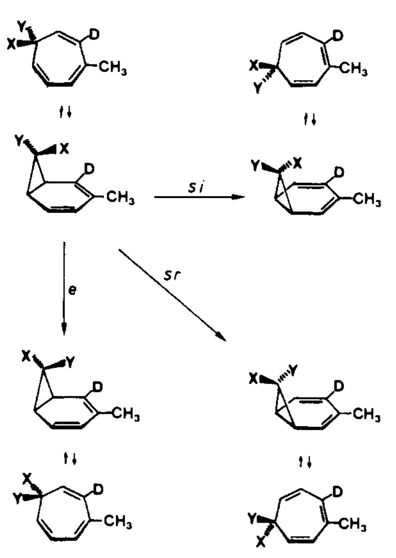


X,Y = CH_3, CO_2CH_3 **1** **2** **3**

X,Y = CH_3, CN **4** **5** **6**

Scheme II

7-2-D 7'-4-D



7'-2-D 7-4-D

X,Y = CH_3, CH_2OCH_3

Baldwin and Broline[4] carried out a stereochemical study for the 7-methoxymethyl-substituted derivative 7-*2*-*d*. In this case, however, the authors claimed three processes—one-center epimerization (*e*), rearrangement with retention (*sr*), and rearrangement with inversion (*si*)—to be responsible for racemization and deuterium scrambling in optically active 7-*2*-*d* (Scheme II). The rate constants $(\times 10^6$ s$)$ at 223.4 °C were determined to $k_e = 4.5$, $k_{sr} = 2.9$, and $k_{si} = 0.5$.

Surprisingly one-center epimerization is the dominating process. Neither in ester **2** nor in nitrile **5**[2] was a one-center epimerization detected. To clarify these discrepancies we repeated a stereochemical study for the 7-methoxymethyl-substituted system using optically active ether **8** (Scheme III). In the case of 7-*2*-*d* the one-center epimerization as well as the inversion process leads to racemization of starting material. The experimental distinction between these two processes depends critically on the NMR analysis of the deuterium distribution in the product (Scheme II).[4] In contrast the racemization of **8** at the beginning of reaction is only affected by the one-center epimerization (Scheme III). For that reason the experimental detection of the one-center epimerization of **8** should be unambiguous.

Optically active ether **8** was prepared by reduction of optically active ester **2** with lithium aluminum hydride followed by methylation using sodium hydride and methyl iodide. Optical purity (op) and relative configuration of **2** and **8** remained