Acknowledgment. We thank the Natural Sciences and Engineering Research Council of Canada for Financial support, Heather Schroeder for the 100-MHz ^1H NMR spectra, Doug Hairsine for the mass spectra, and Mary-Ellen Sturgeon and Professor J. B. Stothers for the ^{13}C NMR spectra.

Registry No. 1 (X = Cl), 2516-50-9; **3**, 14161-45-6; **4**, 71766-29-5; **5**, 1452-34-2; **6**, 2042-05-9; **6** bromo keto ester, 86118-83-4; **6** keto ester, 86118-84-5; **7**, 51014-33-6; **7** bromo keto ester, 86118-85-6; **7** keto ester, 86118-86-7; **8**, 4657-43-6; **8** bromo keto ester, 86161-64-0; **8** keto ester (isomer 1), 86161-65-1; **8** keto ester (isomer 2), 86161-66-2; **9**, 86118-87-8; **9** Cl derivative, 86118-88-9; **9** CN derivative, 86118-89-0; **10**, 86118-90-3; **10** CN derivative, 86118-91-4; **11**, 13914-51-7; **12**, 5885-22-3; **13**, 71557-24-9; **14**, 71557-26-1; **16**, 86118-92-5; **17**, 86118-93-6; **18**, 86118-94-7; **20**, 86118-95-8; **21**, 19347-07-0; **22**, 86118-96-9; **24**, 86118-97-0; EDA, 623-73-4; $BF_3{\cdot}Et_2O$, 109-63-7; 2α,4α-dibromo-5α-cholestan-3-one, 2239-57-8; 2β,4β-dibromo-5β-cholestan-3-one, 4575-78-4; 2-carbethoxy-*A*-nor-5α-cholestane, 86118-98-1; *A*-nor-5α-cholestan-2-carboxylic acid, 86161-67-3; ethyl 2-phenylacetoacetate, 5413-05-8; ethyl 2-(*p*-chlorophenyl)acetoacetate, 30186-24-4; ethyl 2-benzylacetoacetate, 620-79-1; *cis*-6-carbethoxy-2-chloro-2-methylcycloheptanone, 86118-99-2; *trans*-6-carbethoxy-2-chloro-2-methylcycloheptanone, 86119-00-8; 6-carbethoxy-2-methylcycloheptanone, 86119-01-9; 2-acetoxycyclohexanone, 17472-04-7; diazoacetonitrile, 13138-21-1; diazomethane, 334-88-3; 5α-cholestan-3-one, 566-88-1; phenacyl bromide, 70-11-1; phenylacetone, 103-79-7; *p*-chlorophenacyl bromide, 536-38-9; (4-chlorophenyl)acetone, 5586-88-9; 3-chloro-1-phenylacetone, 937-38-2; 4-phenylbutan-2-one, 2550-26-7; 2-chloro-2-methylcyclohexanone, 10409-46-8; 2-methylcycloheptanone, 932-56-9.

Notes

Thiol–Disulfide Interchange Reaction between Ellman's Reagent (5,5′-Dithiobis(2-nitrobenzoic acid)) and Functionalized Thiol Vesicles

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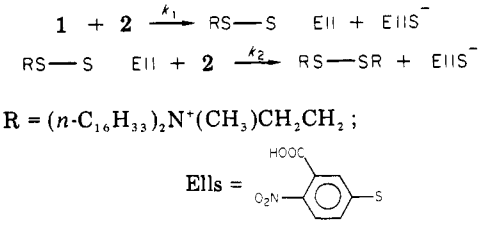
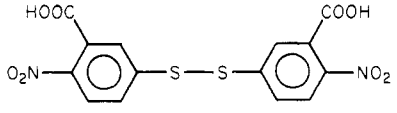
There has recently been much attention focused on the behavior of chemical reactions in synthetic surfactant vesicles.[2] Studies of reactions of fully functionalized thiol vesicles[3] and of organic thiols noncovalently bound to "inert" surfactant vesicles[4] have been particularly interesting. Large rate enhancements have been observed in the thiolyses of activated esters,[3a,4a,b] and the diastereoselectivity of peptide ester cleavages was lower in thiol-functionalized vesicles than in comparable micelles.[3b] Perhaps the most interesting were observations of kinetically distinct exovesicular, endovesicular, and transvesicular reactions.[3a,c,4c] This very interesting and unusual pattern of reactivity was a direct result of the vesicle structure.[3c]

Ellman's reagent,[5] 5,5′-dithiobis(2-nitrobenzoic acid) (1), reacts with a variety of thiols and is widely used in their analysis.[6] Several groups have carefully examined the thiol–disulfide interchange reactions of 1.[7] Other investigations have characterized the reactivity of 1 toward other nucleophiles such as aqueous hydroxide ion,[8] cyanide ion,[9] and organic amines.[10] More recently, the reaction of 1 with poly(ethylenimine),[11] the reaction of cyanide ion and 1 in polysoaps,[12] and the hydroxide ion cleavage of 1 in micellar[13] and vesicular[13b] solutions have been studied. This paper describes the thiol–disulfide interchange re-

Scheme I





1

$(n\text{-}C_{16}H_{33})_2N^+(CH_3)X,Y^-$

2, X = CH_2CH_2SH; Y = Cl
3, X = CH_3; Y = Br

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Table I. Rate Constants and Maximum Product Concentrations for the Vesicular Reaction of 1 and 2[a]

$10^4[2]$,[b] M	$k_{\psi 1}$,[c] s^{-1}	β_{max}[d]	$k_{\psi 2}^{rel}$[e]
10.0	36 ± 3	0.099	1.00
5.02	17 ± 3	0.062	0.56
3.33	6.0 ± 0.6	0.036	0.30
2.01	2.6 ± 0.1	<0.025	<0.20
1.00	1.56 ± 0.03	<0.025	<0.20

[a] Conditions: 0.001 M total vesicular surfactant, pH 7.5 ± 0.2, 0.01 M Tris buffer, μ = 0.01 ± 0.003 (KCl), 25 °C, $[1]_0 = 1.0 \times 10^{-5}$ M. [b] [3] = 0.001 M − [2]. [c] Pseudo-first-order rate constant for the first step in Scheme I. [d] $\beta_{max} = ([EllS^-]_{max} - 1 \times 10^{-5}\ M)/1 \times 10^{-5}$ M. [e] Relative pseudo-first-order rate constants for the second step in Scheme I, calculated from β_{max} values.

action between 1 and functionalized thiol vesicles prepared from thiol surfactant 2 and "inert" surfactant 3. This work represents the first attempt at observing the thiol–disulfide interchange reaction in vesicles.[14]

Thiol-functionalized vesicles were prepared by the alcohol injection technique.[15] In all cases, the reacting solution had a total surfactant concentration of 0.001 M, a thiol concentration between 10^{-3} and 10^{-4} M, and an initial concentration of 1 of 1×10^{-5} M. The reaction between 1 and 2 proceeded in two steps as outlined in Scheme I,[16] and was followed spectroscopically by monitoring the appearance of 5-thio-2-nitrobenzoate anion ($EllS^-$) at 445 nm. In these experiments, a third process which slowly consumed the product $EllS^-$ was observed in addition to those shown in Scheme I. This process, which was assumed to be oxidation of $EllS^-$ back to 1, occuring despite the use of nitrogen-purged solvents and a nitrogen atmosphere for the injection, complicated the interpretation of the reaction kinetics. Pseudo-first-order rate constants for the first step in Scheme I were obtained directly from the initial portion of each absorption vs. time reaction trace. The second step of Scheme I was slower; this process occured at a rate comparable to that of product oxidation. As a result, the concentration of the product $EllS^-$ passed through a maximum, which was measured in several of the experiments. From these maximum concentration values, *relative* pseudo-first-order rate constants were calculated for the second step. Maximum relative product concentrations and pseudo-first-order rate constants for both steps of Scheme I are given in Table I.

From the $k_{\psi 1}$ data, it was calculated that in 0.001 M vesicular surfactant solution, the second-order rate constant for the first step in Scheme I (k_1) was 23 000 ± 2000 L mol^{-1} s^{-1}. On the assumption that the rate of product oxidation is constant in all of our experiments, the pseudo-first-order rate constants for the second step ($k_{\psi 2}^{rel}$) were linearly related to the thiol concentration; k_2 was therefore a constant value. Additionally, it was observed that in the reaction of 0.001 M fully functionalized 2 the maximum product concentration occurs at least 10 s after the initiation of the reaction. This value allows us to estimate conservatively that the second step of Scheme I occurs *at least* 700 times more slowly than the first; e.g., $k_2 < 32$ L mol^{-1} s^{-1}.

From these results, several conclusions can be drawn. The two surfactants used to form the vesicles differ only slightly in structure; 2 has a 2-thioethyl group where 3 has a methyl group. In the event that these two surfactants form "domains" on the surface of the mixed vesicles, and the individual surfactant molecules move only slowly between "domains", we would expect a nonlinear dependence of $k_{\psi 2}$ on the thiol concentration. We believe that the observed linearity of the pseudo-first-order rate constants for the second step indicates either that the two surfactants in the mixed vesicles are uniformly distributed over the vesicle surface or that movement of the individual surfactant molecules over the surface is relatively rapid.

More importantly, the second step in Scheme I occurs at least 700 times more slowly than the first step of the vesicular reactions. In previous investigations of thiol–disulfide interchange reactions between 1 and a variety or organic thiols in homogeneous aqueous solution, the largest observed ratio between k_1 and k_2 was 86, as found for 2-thioacetic acid;[7a] most thiols afforded considerably smaller ratios.[7] Our measured k_1 value is "normal", because it conforms very well to the Brønsted correlation found for many thiol–disulfide interchange reactions of 1;[17] we therefore conclude that the second step of this vesicular reaction is *unusually retarded*. This observation and the large ratio between k_1 and k_2 are very interesting, because the two reactants in the slower second step are both *integral parts* of the vesicular structure. Rate retardation in such an event is consistent with a previous observation that the reactions of two molecules tightly bound to a vesicle surface are unusually slow.[19] In the present case, moreover, the mixed disulfide is surrounded by vesicular thiol groups; there is no need for a second reactant to diffuse to the site of the mixed disulfide. Our result thus suggests that restricted diffusional mobility is not the only cause of such rate retardations. Conformational restrictions of the surfactant head groups[20] may perhaps play a role in determining the rates of vesicular reactions.

Experimental Section

Materials. Compound 1 was obtained from Aldrich Chemical Co. and was used without further purification; 3 was a gift from Professor T. Kunitake. The preparation of 2 has been summarized[3a] and will be described in detail in a forthcoming paper. Buffer solutions were prepared from ACS reagent grade materials and steam-distilled water and purged with nitrogen immediately prior to use.

Vesicle Preparation. Vesicles were prepared by the alcohol injection technique.[15] Soft glass capillary tubing (0.86-mm outside diameter) was pulled on an automatic micropipet puller to give tips with a very gentle taper; the inside diameter of the tips increased from 50 to 2000 nm. The tips were broken along this portion so that the exit aperture was on the order of 100 nm.[21] The capillaries were connected by a 2-cm length of polyethylene tubing (0.86-mm inside diameter) to a 20-cm^3 syringe equipped with a plastic Luer stub adapter.

In a typical vesicle preparation, 0.150 mL of an ethanolic solution which contained 0.0241 M 2 and 0.0240 M 3 was placed

(14) The thiol–disulfide interchange between 1 and bovine plasma albumin has been investigated in micellar solutions. This has been the only previous work dealing with the thiol–disulfide interchange of 1 in surfactant solution (cf. ref 13a).

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(16) This scheme generally applies to thiol–disulfide interchange reactions (cf. ref 7).

(17) Szajewski, R. P.; Whitesides, G. M. *J. Am. Chem. Soc.* **1980**, *102*, 2011. Our k_1 is very close to that predicted for an aliphatic thiol with pK_a = 7.3[18] by the Brønsted correlation displayed in Figure 4 of this reference.

(18) The homologue of 2 which forms micelles has pK_a = 7.32 in its aggregated form. Cf.: Moss, R. A.; Bizzigotti, G. O.; Huang, C.-W. *J. Am. Chem. Soc.* **1980**, *102*, 754.

(19) See: Lim, Y. Y.; Fendler, J. H. *J. Am. Chem. Soc.* **1979**, *101*, 4023. These authors report unusually slow rates for a reaction between two anions bound to a cationic vesicular surface.

(20) In phospholipid dispersions, the head-group conformations are confined to more or less restrictive ranges. Cf.: Skarjune, R.; Oldfield, E. *Biochemistry* **1979**, *18*, 5903 and references cited therein. It seems reasonable to expect similar conformational restrictions on vesicular surfactants.

(21) On the order of the vesicle diameter (cf. ref 3a and 15b, pp 43–45).

in the syringe and injected into 3.00 mL of an aqueous solution containing 0.0091 N HCl and 0.017 M KCl. The temperature of the solution was maintained at 60 °C by using a water bath, and the procedure was performed inside an enclosure containing a nitrogen atmosphere. Another aqueous solution was prepared which contained 0.02 M Tris in the free base form, and 2×10^{-5} M 1. After equal volumes of these two solutions were mixed in the stopped-flow spectrophotometer, the final pH was measured as 7.50. After correction for a 0.025-mL holdup in the injection syringe, the final concentration of 2 was calculated to be 5.02×10^{-4} M and that of 3 to be 5.00×10^{-4} M. In all experiments, the ionic strength of the mixed solution was calculated to be 0.01 ± 0.003, disregarding the contribution of the surfactant to the ionic strength.

Kinetic Studies. Reactions were monitored with a Durrum Model D-130 stopped-flow spectrophotometer equipped with a Beckmann Model DU-2 monochromater and a Tektronix Model 5103N/D15 storage oscilloscope. The oscilloscope readout was photographed by using a Polaroid camera. A constant-temperature circulating bath maintained the temperature at 25 ± 0.2 °C. The appearance of the EllS$^-$ product was followed at 445 nm.[13b] All reactions were monitored with two oscilloscope time bases, one appropriate to the faster reactions and one appropriate to the slower reactions. Rate constants were calculated from the appropriate traces. First-order kinetic behavior was observed for the fastest reaction, with correlation coefficients greater than 0.999 for computer-generated correlations of log $(A_1^\infty - A^t)$ with time. The fastest reaction was rapid enough so that product oxidation could be ignored.

The rate of the second reaction in Scheme I was in all cases comparable to the rate of product oxidation. The relative rate constants for the second reaction were therefore calculated from the maximum product concentrations observed. The relative maximum concentration from the second step, β_{max}, was obtained by subtracting from the maximum observed concentration that amount of product that was due to the first step (1×10^{-5} M) and then dividing the result by the theoretical total product yield from the second step (also 1×10^{-5} M). If $k_{\psi 2}$ is the pseudo-first-order rate constant for the second step in Scheme I and k_{ox} is the pseudo-first-order rate constant for product oxidation, then β_{max} can be approximated by $(k_{ox}/k_{\psi 2})$ raised to the power $k_{ox}/(k_{\psi 2} - k_{ox})$.[22] The ratio between $k_{\psi 2}$ and k_{ox} was then calculated for β_{max} in each case. On the assumption that k_{ox} was the same in each case, the "ratio of ratios" thus gave the relative rate constants for the second step at different thiol concentrations.[23]

The estimate of k_2 was obtained from measurement of the time at which β_{max} was observed, τ_{max} in the experiment with 0.001 M 2. By use of the above rate constants, τ_{max} can be approximated by $(1/(k_{ox} - k_{\psi 2}))\ \ln\ (k_{ox}/k_{\psi 2})$.[22] The nature of the absorption vs. time reaction trace was such that a precise τ_{max} value was not obtained; nevertheless τ_{max} lay between 10 and 100 s. Using these two boundary τ_{max} values and the relation $k_{ox} = 0.138\ k_{\psi 2}$ (from the β_{max} value), we calculated that $0.0032\ s^{-1} < k_{\psi 2} < 0.032\ s^{-1}$; we thus estimate that $k_2 < 32\ L\ mol^{-1}\ s^{-1}$.

The occurrence of the oxidation reaction, which forces us to resort to the foregoing analysis, cannot be easily prevented. The vesicles were prepared in a nitrogen atmosphere, but oxygen is apparently introduced during the transfer of the solutions to the stopped-flow spectrophotometer. We suspect that only be enclosing that instrument in a nitrogen atmosphere, and thus performing the *entire* experiment under an inert atmosphere, could we completely eliminate the complication of product oxidation.

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Registry No. 1, 69-78-3; 2, 79246-00-7; 3, 70755-47-4; $(n\text{-}C_{16}H_{33})_2N^+(CH_3)CH_2CH_2SSEll$, 85908-82-3.

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(23) It must be noted that this is a crude calculation; the relations used are exactly true only for two consecutive first-order reactions. The system we observed involved two parallel pseudo-first-order reactions, followed by a third, consecutive pseudo-first-order reaction. There is no simple kinetic solution which can analyze completely such a system. Therefore, the initial reaction has been ignored, because it is much more rapid than the two reactions which follow.

Diels–Alder Reactions of 2-Alkynoyl Chlorides with Cyclopentadiene: A Reinvestigation

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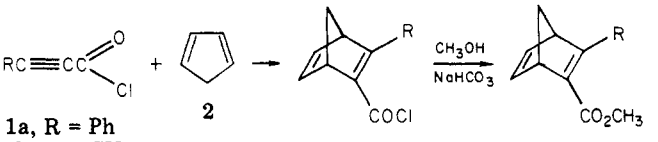
Diels–Alder reactions of cyclopentadiene represent a simple access to the bicyclo[2.2.1] skeleton.[1a] While electron-deficient alkenes react readily with cyclopentadiene,[1b,c] cycloadditions to triple bonds appear to be problematic.

While alkenoyl chlorides are among the most reactive dienophiles,[1b,c] phenylpropynoyl chloride has been reported "not to undergo appreciable reaction with cyclopentadiene at room temperature even after 24 h".[2] Therefore, Baum and Viehe used a route via acetylenic iminium salts for synthesizing compounds of type 4.[3] In earlier papers, however, Diels–Alder reactions of **1a** with cyclopentadiene have been reported.[4,5] Forty-one percent of cycloadduct was obtained when a solution of **1a** and 2 in toluene was heated at reflux for 48 h.[4] Other workers describe a spontaneous reaction of **1a** with 2 at room temperature in the presence of a "few crystals of picric acid".[5] The inconsistency of these reports prompted us to reexamine the reactions of alkynoyl chlorides with cyclopentadiene.

When **1a** and 2 were mixed at 0 °C, warmed up to room temperature, and poured into a suspension of sodium bicarbonate in methanol, 84% of ester **4a** was obtained. Combining these cycloaddends without external cooling resulted in a rapid exothermic cycloaddition reaction accompanied by partial polymerization of cyclopentadiene. Alkynoyl chlorides **1b** and **1c** reacted with cyclopentadiene at room temperature to give good yields of norbornadienes **4b** and **4c**, respectively. Because of the mild reaction



1a, R = Ph
b, R = CH_3
c, R = $C(CH_3)_3$

4a, 84%
b, 78%
c, 82%

conditions employed for these cycloadditions[6] and the versatile reactivity of acid chlorides 3, we consider the title reaction as the method of choice for the synthesis of any

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