mol) in dichloromethane (30 mL, distilled from calcium hydride) at 5 °C under a positive pressure of argon in the dark. The reaction was stirred at 25 °C for 3 h and then concentrated in vacuo to a dark oil. Flash chromatography (230–400 mesh, 7 × 30 cm, eluted with hexane–ethyl acetate 8:1) gave **8b** having R_f = 0.31 (2.3 g, 83%) as a light-sensitive, pale yellow oil: IR (neat) 2943, 2869, 1736, 1494, 1454, 1202, 1131, 1078, 1036 cm^{-1}; ^1H NMR δ 7.4 (sharp m, 5 H), 5.8 (s, 1 H), 4.4 (m, 3 H), 3.65 (m, 2 H), 1.6 (m, 6 H); HRMS (EI) for $C_{14}H_{17}BrO_3$ calcd 311.02824, found 311.02704.

1-Methyl-2-phenyl-3-[[((2-propyl)carbamoyl)oxy]-methyl]-3-pyrroline (10). 2-Propyl isocyanate (0.32 mL, 0.0033 mol) was added in one portion to a stirred solution of **1** (0.25 g, 0.00132 mol) and di-*n*-butyltin diacetate (2 drops) in dichloromethane (10 mL, distilled from calcium hydride) at 25 °C under a positive pressure of argon. The reaction was stirred for 1.5 h and then concentrated in vacuo to give an oil that was flash chromatographed (230–400 mesh, 5 × 25 cm, eluted with 3:2 ethyl acetate–dichloromethane) to give a pale yellow solid. The solid was recrystallized from dichloromethane–hexane to give **10** (0.29 g, 81%) as a white fluffy solid: mp 66–67 °C; IR ($CHCl_3$) 3338, 2968, 2773, 1719, 1453, 1247, 1168 cm^{-1}; ^1H NMR δ 7.3 (s, 5 H), 5.85 (m, 1 H), 4.6–3.25 (complex m, 7 H), 2.4 (s, 3 H), 1.15 (d, J = 6 Hz, 6 H). Anal. Calcd for $C_{16}H_{22}N_2O_2$: C, 70.04; H, 8.08; N, 10.21. Found: C, 70.05; H, 8.13; N, 10.20.

2,3,5,7a-Tetrahydro-5-phenyl-6-[[((2-propyl)carbamoyl)-oxy]methyl]-1*H*-pyrrolizine (12). Compound **2** was converted to **12** by using the procedure described for **10**. Compound **12** was obtained as a highly air sensitive pale yellow gum (70%): IR ($CHCl_3$) 3443, 2967, 2873, 1715, 1509, 1455, 1229, 1084 cm^{-1}; ^1H NMR δ 7.25 (s, 5 H), 5.80 (s, 1 H), 4.40 (m, 4 H), 3.75 (m, 2 H), 3.20 (m, 1 H), 2.75 (m, 1 H), 1.73 (m, 4 H), 1.10 (d, J = 6 Hz, 6 H); CIMS, m/e 197 (53), 198 (64), 301 (M + 1, 100). Anal. Calcd for $C_{18}H_{24}N_2O_2$: C, 71.97; H, 8.05; N, 9.32. Found C, 72.19; H, 7.74; N, 9.38.

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Registry No. 1, 135733-62-9; 2, 135733-63-0; 3, 109-83-1; 4, 34477-69-5; 5, 23356-96-9; 6, 135733-64-1; 7a, 4870-65-9; 7b, 19078-72-9; 7c, 135733-67-4; 8a, 116204-25-2; 8b, 135733-69-6; 9, 135733-65-2; 10, 135733-66-3; 11, 135733-70-9; 12, 135733-68-5; H-Pro-OH, 147-85-3; *i*-PrNCO, 1795-48-8; 3,4-dihydro-2*H*-pyran, 110-87-2.

Supplementary Material Available: ^1H NMR spectra (90 MHz) for compounds **8a**, **8b**, and **12** (3 pages). Ordering information is given on any current masthead page.

A Reconsideration of the Cleavage of Ellman's Reagent (5,5′-Dithiobis(2-nitrobenzoic acid)) in the Presence of Dioctadecyldimethylammonium Chloride Surfactant Vesicles

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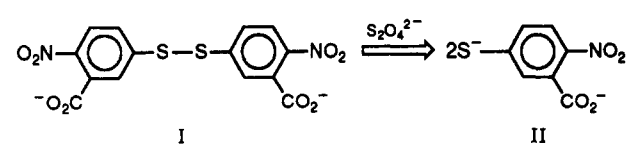
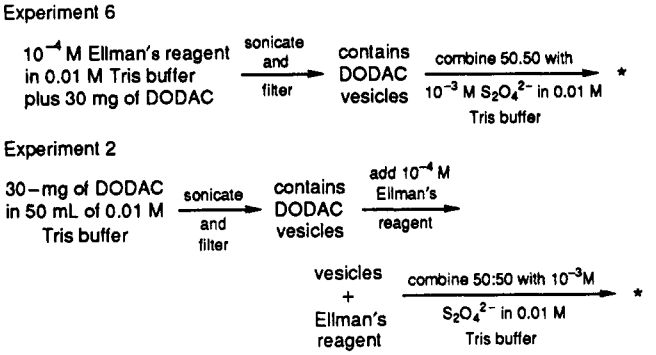
During the process of attempting to find a reaction system that could be used to measure the occurrence of structural modifications of surfactant vesicular bilayers in aqueous solution by chemical kinetics, we repeated experiments 2–6 of Table III of a previous study by Moss and co-workers on the cleavage of Ellman's reagent (I) by dithionite (Scheme I) in the presence of dioctadecyldimethylammonium chloride (DODAC) vesicles.[2]

Scheme I



I II

Scheme II[a]



[a]* Follow rate of appearance of absorbance (λ_{max} = 440 nm) in a spectrophotometer.

Experiment 2 of this table involved the cosonication of 10^{-4} M Ellman's reagent with 10^{-3} M DODAC in pH 8, 0.01 M aqueous Tris buffer μ = 0.01 (KCl) followed by combination of the resulting solution 50:50 with 10^{-3} M ($S_2O_4^{2-}$) also in Tris buffer, μ = 0.01 (KCl) inside a UV–vis spectrophotometer thermostated at 25 °C[2] (see Scheme II). A biphasic kinetic appearance of yellow color (λ_{max} = 450 nm) was observed. The fast kinetic phase was over in a few seconds and was interpreted to involve a bimolecular reaction between dithionite and that portion of the substrate (Ellman's reagent) that is adsorbed onto the outer DODAC vesicular surface and remains in contact with the outer vesicular–aqueous interface.[3] The 450-nm absorbance peak is attributed to the formation of Ellman's anion (II) (Scheme I; λ_{max} = 440 nm (ϵ = 13,600)).[2] The slow kinetic phase lasted about 1 h and was interpreted to involve cleavage of the remaining portion of the substrate, which was encapsulated within the vesicles formed by the sonication process by buffer supplied hydroxide since the $S_2O_4^{2-}$ was believed to be unable to pass through the DODAC vesicular bilayer. The reported pseudo first-order rate constant[2] at pH 8 for this slow phase ($k = 5.09 \times 10^{-4}$ s^{-1}) is within experimental error equal to that reported earlier[4] for the reaction of Ellman's reagent with hydroxide at pH 8 ($k = 7.8 \times 10^{-4}$ s^{-1}). Experiment 2 of this same table was identical with that of 6 except that the substrate was added after sonication of the DODAC in the buffer but prior to combination with the $S_2O_4^{2-}$ solution in the spectrometer (see Scheme II). In this case only a single rapid kinetic phase was reported to take place at 450 nm

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(2) Moss, R. A.; Swarup, S.; Zhang, H. *J. Am. Chem. Soc.* **1988**, *110*, 2914.

(3) The cleavage of Ellman's reagent is significantly faster in the presence of DODAC vesicles than alone in aqueous solution. Also, Ellman's reagent experiences a 12-nm hypsochromic shift in its major aqueous absorption band in the presence of DODAC vesicles. These results were earlier reported to be due to substrate and reactant electrostatic adsorption onto the anionic outer vesicular surface prior to reaction.

(4) Fendler, J. H.; Hinze, W. L. *J. Am. Chem. Soc.*, **1981**, *103*, 5439.

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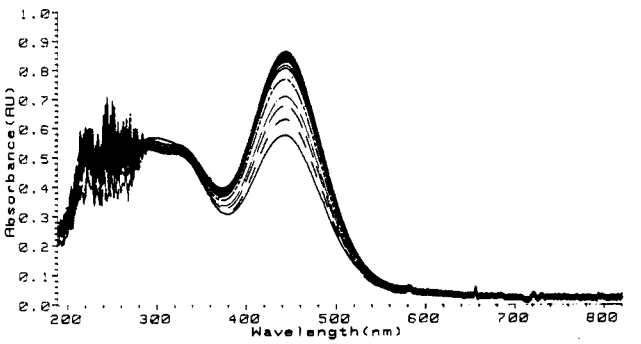

Figure 1. A series of spectral recordings at 0.5-min intervals for the cleavage of Ellman's reagent (I) with dithionite in the presence of DODAC vesicles in pH 8 aqueous Tris buffer, 25 °C.

whether the time of incubation of the substrate with vesicles was 10 s or 30 min prior to mixing with dithionite. This result of experiment 2 was interpreted to mean that since substrate was added after vesicle formation was complete, none of it was encapsulated and all reacted at the fast phase rate of experiment 6. Rate constants were reported to substantiate this result.

This paper reports and interprets our results, which are in disagreement with those of Moss and co-workers, in particular with respect to experiment 2 of their Table III. Figure 1 shows our result when 10^{-4} M Ellman's reagent[5] containing 10^{-3} M sonicated DODAC[5,6] in N_2 sparged pH 8 0.01 M aqueous Tris buffer was mixed 50:50 with 10^{-3} M dithionite ($S_2O_4^{2-}$)[5] also in Tris buffer at 25 °C, μ = 0.007. The sample was placed in a 1-cm quartz cuvette, and the spectra were taken on a Hewlettt-Packard Model 8452 diode array UV–vis spectrophotometer. The resulting reaction mixture was thermostated for 15 min while spectral recordings were registered every 0.5 min from 200 to 800 nm. This same result was observed many times whether the Ellman's reagent was cosonicated with the DODAC in the buffer (Experiment 6, Table III)[2] or it was added after sonication of the DODAC was complete (Experiment 2, Table III)[2] (see Scheme II). DODAC vesicles were prepared by sonication 15 min in buffer at 70 W (21-mm Horn–Branson Model 450 sonifier) at 60 °C followed by cooling and filtering through a 0.8-μm millipore filter. A mean particle diameter of 90–95 nm was measured by laser light scattering (NiComp Model 200 particle sizer).[7] To the best of our knowledge, with the exception of an ionic strength change from 0.01 to 0.007 (we did not add KCl) our experiments were identical with those numbered 6 and 2 of Table III.[2]

Inspection of Figure 1 clearly indicates a biphasic kinetic process involving a rapid, initial burst of absorbance due to Ellman's anion followed by a slower first-order phase (k = 0.0103 ± 0.0005 s^{-1}).[7] Kinetic measurement of the fast reaction also showed it to be first order by stopped-flow spectrophotometric techniques affording k = 33.4 ± 0.6 s^{-1} (Dionex Model D-100 spectrometer thermostated at 25 °C).[7] Identical experiments using L-cysteine as reactant also gave biphasic results (fast phase, k = 28.0 ± 1.4 s^{-1}; slow phase k = 0.0033 ± 0.00017 s^{-1}).[7] Within experimental error[2] the plot shown in Figure 1 and the values obtained for both rate constants for cleavage by dithionite (also for L-cysteine) were entirely independent of whether substrate is cosonicated with DODAC in the buffer or is added after sonication is complete. These results differ from the data already reported in two respects. The addition of Ellman's reagent to previously sonicated DODAC also results in a biphasic kinetic reaction upon subsequent combination with dithionite and not a single fast kinetic phase and that the slow-phase kinetic process observed with dithionite is some 20 times faster than earlier reported.[2]

In our experiments, the fact that the same kinetic biphasic result of Figure 1 occurs whether Ellman's reagent is cosonicated with the DODAC or is added after vesicle preparation is complete and the fact that it is independent of the time of contact between preformed vesicles and combination with dithionite (i.e., 15 s or 0.5 h) renders the earlier interpretation of the cause of the slow kinetic phase incorrect although we have no quarrel with the earlier interpretation of the cause of the fast kinetic phase. Our measurement of the slow rate of Figure 1 makes it some 20 times faster than the previously measured hydroxide reaction rate. These results also prohibit the slow rate being due to reestablishment of substrate distribution between the inner and outer vesicular bilayer surface by transport through the vesicular bilayer after outer surface depletion by reaction with added dithionite. The results of these experiments therefore make the system considerably more complicated than the earlier report suggests.

It is conceivable that a rapid favorable equilibrium is established to insert a portion of the substrate into the vesicular bilayer[3] with the majority remaining at the outer aqueous surface. The slow reaction phase then is due to an unfavorable slow leakage of the subsurface bilayer bound substrate back onto the depleted outer vesicular surface due to the initial fast reaction with dithionite or L-cysteine. A rapid equilibrium favoring bilayer bound substrate over that on the outer vesicular surface is not precluded by the fact that the actual distribution of the substrate within the two areas of equilibrium is about one-third bilayer bound, two-thirds outer vesicular surface (see relative absorbance changes of two kinetic phases in Figure 1). The relative volume ratio of the two areas in these experiments favors the outer vesicular surface. Preference for bilayer insertion by the substrate is also not precluded by its ionized carboxyl groups (pH 8 buffer). The remainder of the molecule is neutral, and a lateral mode of insertion could leave both ionized carboxyl groups in contact with the aqueous bilayer interface.

If this revised model is correct, then there is no actual encapsulation of the substrate by the DODAC bilayer but rather a bilayer temporarily diluted with substrate molecules. For an alternative explanation, please see the following private communication of April 15, 1991 to Dr. R. W. Huffman from Professor R. A. Moss and Mr. S. Bhattacharya.

> We have also been unable to repeat the results originally reported for Experiment 2 of Table III in reference 2. The reaction between aqueous dithionite, and Ellman's reagent that had been *subsequently* added to sonicated DODAC vesicles, gave *two* distinct kinetic phases, not one as had previously been reported. The final concentrations in the repeated experiments were [$S_2O_4^{2-}$] = 5 × 10^{-4} M, [Ellman's] = 5 × 10^{-5} M, and [DODAC] = 5 × 10^{-4} M, all in N_2-purged, 0.01 M, pH 8 aqueous Tris buffer, μ = 0.01 (KCl) at 25 °C. The rate constant of the slow kinetic process was ~5 × 10^{-3} s^{-1}. Similar

(5) Ellman's reagent, mp = 232 °C, was purchased from the Aldrich Chemical Co., dioctadesyldimethylammonium chloride (greater than 95% by chloride titration) was purchased from American Tokyo Kasei Inc., and sodium dithionite was purchased from J. T. Baker Chemical Co. All three reagents were used without further purification. DODAC: NMR ($CDCl_3$) δ 0.88 (t, 6 P), 1.3 (m, 60 P), 1.7 (b, 4 P), 3.42 (s, 10 P). Anal. Calcd for $C_{38}H_{80}NCl \cdot H_2O$:[6] C, 75.50; H, 13.67; N, 2.32; Cl, 5.86. Found: C, 75.40; H, 13.82; N, 2.32; Cl, 5.91.

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(7) Rate constants and particle size distributions were determined from standard computational procedures using software packages designed to be used with the instruments affording the primary data.

biphasic kinetic results were observed when analogous experiments were carried out in vesicles of $(n\text{-}C_{20}H_{41})_2N^+Me_2Br^-$, instead of DODAC.

In the preceeding experiments, the molar ratio of Ellman's reagent to surfactant was 1:10. It is important to note that the original observation of a single, fast cleavage of Ellman's reagent (after subsequent addition of this reagent to the performed vesicles) *can* be recreated if the Ellman's/surfactant ratio is adjusted to 1:100. In these experiments, the final concentrations are $[S_2O_4^{2-}] = 5 \times 10^{-4}$ M, [Ellman's] = 2.5×10^{-5} M, and [surfactant] = 2.5×10^{-3} M. With both DODAC and the di-C_{20} vesicles, only a single rapid reaction is observed upon addition of dithionite, even after aging the Ellman's/vesicle solution for 15–30 min. In contrast, when the Ellman's reagent is *cosonicated* with the surfactant before the dithionite addition, both fast and slow ($k < 1 \times 10^{-3}$ s^{-1}) kinetic phases are observed, with the slow phase accounting for ~70% of the total reaction. These phenomena are in general agreement with those reported in reference 2.

We are uncertain why we cannot precisely reproduce the reported phenomena[2] at the original concentrations. In the current 1:10 experiments, we believe that not all of the added Ellman's reagent can be bound at the exovesicular surface. Accordingly, the initial, fast, exovesicular reduction of bound Ellman's reagent is followed by a slower product–desorbtion rate-limiting cleavage of the excess (aqueous) Ellman's reagent as it diffuses to the vesicular surface. At the higher, 1:100 Ellman/surfactant dilution, added Ellman's reagent can be adsorbed at the exovesicular surface, and, upon dithionite addition, it cleaves in a single, fast, uniphasic process. When the Ellman's reagent is cosonicated with the surfactant, separate fast and slow reactions are seen for exo- and endovesicularly bound (or intercalated) Ellman's reagent.

Acknowledgment. Support for this work is gratefully acknowledged from the Research Corporation in the form of a High School–University partnership grant, and the Northern Arizona University Organized Research Fund.

Registry No. DODAC, 107-64-2; H-Cys-OH, 52-90-4; $Na_2S_2O_4$, 7775-14-6; Ellman's reagent, 69-78-3.

$Cr(ClO_4)_2$: An Effective Reagent for the Preparation of Mitomycin C Nucleophilic Substituted Compounds

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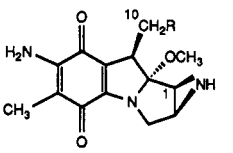
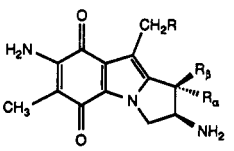
Advances in determining the mode of action of mitomycin C[1,2] have been achieved by the preparation and characterization of select mitomycin C-derived products.[1-10] Recently, we reported that addition of $Cr(ClO_4)_2$ to aqueous and methanolic solutions of **1a** led to the efficient activation of the C(1) and C(10) positions in the anticancer agent.[2i,j] In this paper, we describe the application of this novel reductive activation technique for the generation of mitomycin nucleophilic substituted products. The facility of these reactions provides promise that this method will find use in future mitomycin-based studies.



1a R = $OC(O)NH_2$

b R = OH



2a R_α = H, R_β = H, R = NHPh

b R_α = NHPh, R_β = H, R = NHPh

c R_α = OCH_3, R_β = H, R = OCH_3

d R_α = H, R_β = OCH_3, R = OCH_3

e R_α = H, R_β = H, R = OCH_3

f R_α = OH, R_β = H, R = $OC(O)NH_2$

g R_α = H, R_β = OH, R = $OC(O)NH_2$

h R_α = H, R_β = H, R = $OC(O)NH_2$

i R_α = OH, R_β = H, R = OH

j R_α = H, R_β = OH, R = OH

k R_α = H, R_β = H, R = OH

Results and Discussion

a. Use of Aniline. Addition of 2 equiv of $Cr(ClO_4)_2$ to a buffered methanolic solution (Tris·HCl, "pH" 7.00) containing **1a** and aniline (5 equiv) led to the production of **2a** and **2b**, along with the known adducts **2c–2e**,[2i,j,5e,11a] and several unidentified compounds. HPLC analysis of the reaction mixture indicated that the aniline-bound adducts **2a** and **2b** accounted for approximately 66% of the reaction mixture. Repetition of this experiment on a semipreparative scale permitted the isolation and structural characterization of the two new compounds as 10-decarbamoyl-10-anilino-2,7-diaminomitosene (**2a**) and *trans*-10-decarbamoyl-1,10-dianilino-2,7-diaminomitosene (**2b**). Key ^{1}H NMR resonances detected for **2a** included the diagnostic doublets of doublets located at δ 2.37 and 2.85 for the C(1) methylene protons, the doublet (J = 5.7 Hz) at δ 4.27 for the C(10) methylene hydrogens, the triplet (J = 5.7 Hz) at δ 5.86 for the aniline N–H proton, and the

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