Synthesis of Two Bicyclic Surfactants Which Form Reversed Micelles Capable of Selective Protein Extraction

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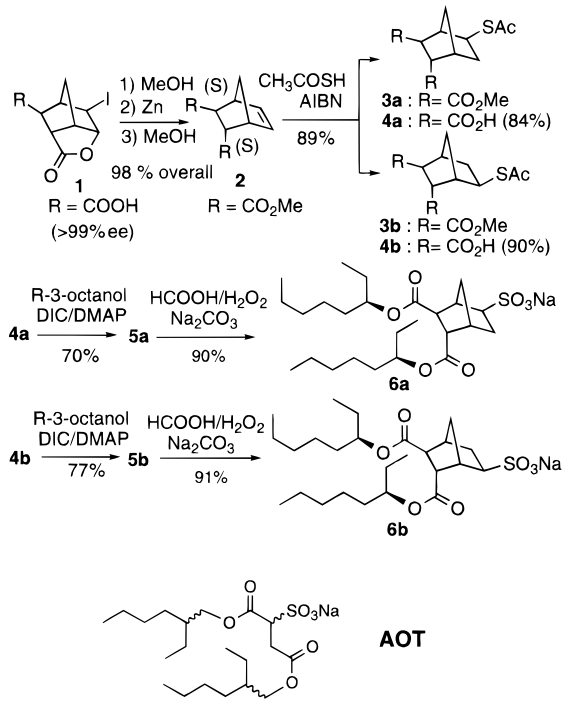
Certain surfactants self-assemble in organic solvents to form reversed micelles that are capable of solubilizing hydrophilic molecules (e.g., proteins) in their aqueous interior.[1−3] It is clear that there is a relationship between the structure of the monomeric surfactant and the structure of the micelle.[4] However, the details of this relationship, important for the purposes of design, are unclear due, in part, to the fact that enantiomerically pure, conformationally restrained surfactants are not available.[5] For example, the widely utilized surfactant AOT (di-2-ethylhexyl sulfosuccinate, Scheme 1) is a mixture of eight conformationally flexible diastereomers[5] that would be expected to produce a complex mixture of reversed micelles which differ with respect to the ratio of the constituent AOT optical and conformational isomers.[6] We report herein on the synthesis and self-assembly of two new conformationally-restrained surfactants. Reversed micelles comprising these surfactants were more homogeneous than those comprising AOT and extracted proteins from aqueous media in a molecular weight-dependent manner.

Scheme 1. Synthesis of Bicyclic Surfactants 6a and 6b



Bicyclic diester **2**[7] (prepared from iodolactone **1**, >99% ee[8]), was treated with thiolacetic acid in the presence of AIBN to afford the regioisomeric acetylthio diesters **3a** and **3b** in 40% and 49% yield, respectively. The methyl ester groups in **3a** and **3b** were selectively cleaved with trimethylsilyl iodide in refluxing chloroform to produce the corresponding diacids **4a** and **4b**. Subsequent esterification of diacids **4a** and **4b** with (*R*)-3-octanol (98% ee[9]) using *N*,*N*-diisopropylcarbodiimide and DMAP in methylene chloride afforded the di-3-octyl esters **5a** (70%) and **5b** (77%). The acetylthio groups in **5a** and **5b**[10] were directly oxidized to sulfonic acids with performic acid[11] to afford surfactants **6a** (90%) and **6b** (91%) (Scheme 1). Although the *R* stereoisomer of 3-octanol was chosen in

(1) (a) Luisi, P. L.; Straub, B. E. *Reverse Micelles*; Plenum Press: New York, 1984. (b) Hatton, T. A. *Reversed Micellar Extraction of Proteins*; Marcel Dekker: New York, 1989. (c) Luisi, P. L.; Giomini, M.; Pileni, M. P.; Robinson, B. H. *Biochim. Biophys. Acta* **1988**, *947*, 209. (d) Menger, F. M. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1086. (e) Castro, M. J. M.; Cabral, J. M. S. *Biotech. Adv.* **1988,** *6*, 151. (f) Eicke, H.-F.; Gauthier, M.; Hilfiker, R.; Struis, R. P. W.; Xu, G. *J. Phys. Chem.* **1992**, *96*, 5175. (g) Adachi, M.; Harada, M. *J. Phys. Chem.* **1993**, *97*, 3631. (h) Brochette, P.; Petit, C.; Pileni, M. P. *J. Phys. Chem.* **1988**, *92*, 3505. (i) Petit, C.; Brochette, P.; Pileni, M. P. *J. Phys. Chem.* **1986,** *90*, 6517. (j) Chang, Q.; Liu, H.; Chen, J. *Enzyme Microb. Technol.* **1994**, *16*, 970. (k) Andrews, B. A.; Pyle, D.; Asenjo, J. A. *Biotechnol. Bioeng.* **1994**, *43*, 1052. Formation of reversed micelle is not an absolute requisite for protein solubilization. For nonmicellar extraction of proteins, see: Paradkar, V. M.; Dordick, J. S. *Biotechnol. Bioeng.* **1994**, *43*, 529 and ref 4g.

(2) (a) Bommarius, A. S.; Hatton, T. A.; Wang, D. I. I. *J. Am. Chem. Soc.* **1995**, *117*, 4515. (b) Candau, F.; Zekhnini, Z.; Durand, J.-P. *J. Colloid Interface Sci.* **1986**, *114*, 398. (c) Gauduel, Y.; Migus, A.; Martin, J. L.; Antonetti, X. *Chem. Phys. Lett.* **1984**, *108*, 319.

(3) (a) Schmitt, L.; Dietrich, C.; Tampe, R. *J. Am. Chem. Soc.* **1994**, *116*, 8485. (b) Regalado, C.; Asenjo, J. A.; Pyle, D. L. *Biotechnol. Bioeng.* **1994**, *44*, 674. (c) Kelly, B. D.; Wang, D. I. C.; Hatton, T. A. *Biotechnol. Bioeng.* **1993**, *42*, 1199. (d) Paradkar, V. M.; Dordick, J. S. *Biotechnol. Prog.* **1993**, *9*, 199. (e) Leser, M. E.; Luisi, P. L. *Chimia* **1990**, *44*, 270. (f) Woll, J. M.; Hatton, T. A.; Yarmush, M. L. *Biotechnol. Prog.* **1989**, *5*, 57.

(4) (a) Hilhorst, R.; Sergeeva, M.; Heering, D.; Rietveld, P.; Fijneman, P.; Wolbert, R. B. G.; Dekker, M.; Bijsterbosch, B. H. *Biotechnol. Bioeng.* **1995**, *46*, 375. (b) Leydet, A.; Boyer, B.; Lamaty, G.; Roque, J. P.; Catlin, K.; Menger, F. M. *Langmuir* **1994**, *10*, 1000. (c) Yu, Z.-J.; Neuman, R. D. *J. Am. Chem. Soc.* **1994**, *116*, 4075. (d) Wittouck, N.; Negri, R. M.; Ameloot, M.; De Schryver, F. C. *J. Am. Chem. Soc.* **1994**, *116*, 10601. (e) Chang, Q.-I.; Chen, J.-Y. *Biotechnol. Bioeng.* **1995**, *46*, 172. (f) Kelly, B. D.; Wang, D. I. C.; Hatton, T. A. *Biotechnol. Bioeng.* **1993**, *42*, 1199. (g) Matsuura, J.; Powers, M. E.; Manning, M. C.; Shefter, E. *J. Am. Chem. Soc.* **1993**, *115*, 1261. (h) Luisi, P. L.; Bonner, F. J.; Pellegrini, A.; Wiget, D.; Wolf, R. *Helv. Chim. Acta* **1979**, *62*, 740.

(5) Enantiomerically pure AOT has not been previously reported. A mixture of two AOT diastereoisomers has been prepared in which the lipophilic side chains are derived from enantiomerically pure alcohols but the stereochemistry at the polar head group (carbon−sulfonate bond) is not defined; see: (a) Larpent, C.; Chasseray, X. *Tetrahedron* **1992**, *48*, 3903. (b) Andriamanampisoa, R.; Boyer, B; Lamaty, G.; Roque, J. P. *Ibid.* **1987**, *43*, 77. For other classes of enantiomeric surfactants: see references cited in ref 5a.

(6) AOT micelle polymorphism might be responsible for the discrepancy in reported AOT micelle sizes; see ref 4e and references cited therein. Polymorphism in normal micelles has been discussed in: (a) Smit, B.; Esselink, K.; Hilbers, P. A. J.; van Os, N. M.; Rupert, L. A. M.; Szleifer, I. *Langmuir* **1993**, *9*, 9. (b) Smit, B.; Hilbers, P. A. J.; Esselink, K.; Rupert, L. A. M.; van Os, N. M.; Schlijper, A. G. *Nature* **1990**, *348*, 624.

(7) The absolute stereochemistry of diester **2** was assigned by reducing to the known diol, 5-norbornenyl-2,3-dimethanol, and its enantiomeric purity was calculated by comparing the experimental and literature optical rotations. Maruoka, K.; Akakura, M.; Saito, S.; Ooi, T.; Yamamoto, H. *J. Am. Chem. Soc.* **1994**, *116*, 6153.

(8) (a) Hamanaka, N.; Seko, T.; Miyazaki, T.; Naka, M. *Tetrahedron Lett.* **1989**, *18*, 2399. (b) Janssen, A. J. M.; Klunder, A. J. H.; Zwanenburg, B. *Tetrahedron* **1991**, *47*, 5513.

(9) (a) Federici, C.; Righi, G.; Rossi, L.; Bonini, C.; Chiummiento, L.; Funicello, M. *Tetrahedron Lett.* **1994**, *35*, 797. (b) Bonini, C.; Righi, G.; Sotgiu, G. *J. Org. Chem.* **1991**, *56*, 6206. (c) Fujiwhara, M.; Mori, K. *Agric. Biol. Chem.* **1986**, *50*, 2925. The chirality of hydroxy group was acquired by asymmetric Sharpless epoxidation of (*E*)-crotyl alcohol, followed by *in situ* tosylation. The enantiomeric purity of the 2,3-epoxy crotyl sulfonate (initially 90% ee) was enhanced by single recrystallization from hexane to 98% ee.

(10) Thiol esters **5a** and **5b** were also synthesized via an asymmetric Diels−Alder cycloaddition, followed by the addition of thiolacetic acid. This route was abandoned since we were unable to separate **5a** and **5b**.

(11) Higashiura, K.; Ienaga, K. *J. Org. Chem.* **1992**, *57*, 764.

0022-3263/96/1961-1920$12.00/0

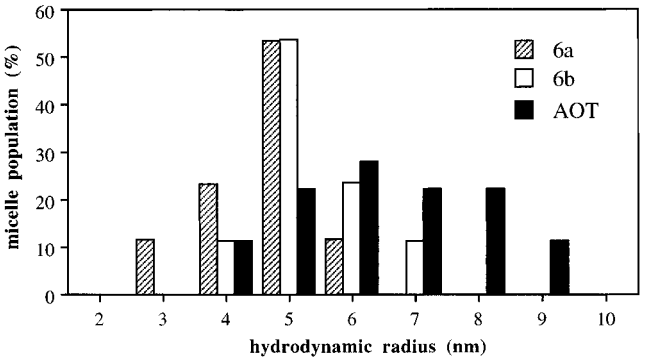


Figure 1. Size of reversed micelles and distributions of AOT, **6a** and **6b**. 2% (w/w) solutions of surfactants in isooctane.

our initial synthesis, the *S* isomer is also readily available, making two additional stereoisomers of **6** available.[12]

Both **6a** and **6b** self-assembled in isooctane to form reversed micelles. Dynamic light scattering indicated that the **6a** and **6b** micelle populations were more homogeneous and, on average, smaller than AOT micelles (Figure 1).[13] As expected, the **6a** and **6b** micelles had smaller aqueous cores than did the AOT micelles (w_{max} = $[H_2O]_{max}$/[surfactant];[14] **6a**, 23; **6b**, 24; AOT, 64).[15]

Reversed micelles are capable of extracting water-soluble proteins into organic solvents by enveloping a single molecule in their internal aqueous cavity. Extraction of a series of globular proteins of various molecular weights was measured (Figure 2, top).[16] AOT,[17] a reference system, was moderately effective in extracting cytochrome c (MW = 12 500), lysozyme (MW = 14 400), trypsin (MW = 23 800), and α-chymotrypsin (MW = 25 000) but was unable to extract the larger proteins, pepsin (MW = 35 000) and bovine serum albumin (MW = 66 000). Surfactants **6a** and **6b** were more effective than AOT in extracting low MW proteins but significantly less effective in extracting the trypsin (23.8 kD) and α-chymotrypsin (25 kD). A competitive extraction experiment (1:1 lysozyme and α-chymotrypsin) showed that a single extraction with **6a** removed about 85% of lysozyme and less than 15% of α-chymotrypsin from the aqueous phase, whereas AOT showed no selectivity (Figure 2, bottom). These results are consistent with the difference in the micellar size distribution and the size of water pool encapsulated in the reversed micelles.[18]

The synthetic surfactants **6a** and **6b** form micelle populations with distinct physical and functional properties. These compounds allow a systematic study of the relationship between surfactant and reversed micelle structures. In addition, the behavior of these compounds suggests that it should be possible to synthesize a library of surfactants in which each member self-assembles into a distinct population of micelles. Furthermore, mixtures of surfactants may have altered properties, adding to the potential diversity of such a library. Preliminary experiments show that a 1:1 mixture of **6a** and **6b** has altered properties with respect to protein extraction relative to either of the pure surfactants. A self-assembling surfactant library could be filtered to remove those micelles with the desired properties or could be driven to self-assemble by a template.

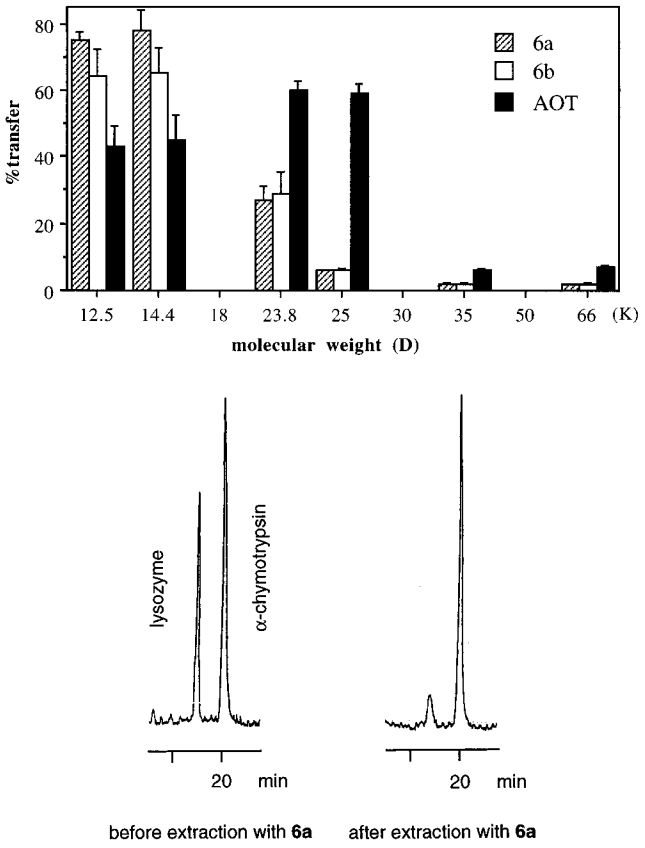


Figure 2. (Top) liquid−liquid phase-transfer of enzymes. Organic layer: 50 mM solution of surfactants in 2 mL of isooctane. Aqueous layer: 16 μM solution of enzymes in 2 mL of acetate buffer (20 mM sodium acetate, 100 mM $CaCl_2$, pH 5.0). (Bottom) analytical HPLC trace of aqueous enzyme solution.

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Supporting Information Available: The experimental procedures for the synthesis and spectral data of **6a**, **6b** as well as all the intermediates are available (27 pages).

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(12) We used 3-octanol in lieu of 2-ethylhexanol as in AOT for synthetic simplicity. For synthesis of optically pure 2-ethylhexanol; see ref 5a and: Rettinger, K.; Burschka, C.; Scheeben, P.; Fuchs, H.; Monsandl, A. *Tetrahedron Assymetry* **1991**, *2*, 965.

(13) Jean, Y.-C.; Ache, H. J. *J. Am. Chem. Soc.* **1978**, *100*, 984.

(14) w_{max} was measured by adding aliquots of water into a 50 mM solution of surfactants in isooctane until cloudiness or phase separation occurred. During this process, the mixture was periodically sonicated (ref 4b).

(15) The literature w_{max} value of AOT in isooctane is 60; see: Zulauf, M.; Eicke, H.-F. *J. Phys. Chem.* **1979**, *83*, 480.

(16) Physical properties of enzymes investigated, enzyme (molecular weight, Stoke's radius (Å), p*I*): cytochrome *c* (12 500, 15.3, 10.6), lysozyme (14 400, 15.8, 11.0), trypsin (23 800, n/a, 10.8), α-chymotrypsin (25 000, 19.4, 9.1), pepsin (35 000, 21.8, 1.0), bovine serum albumin (66 000, 26.8, 4.8).[3e]

(17) We purchased AOT from Aldrich (the same material as from Sigma) which was recommended for protein extraction experiments by the authors in ref 3e.

(18) Some cases have been reported where w_{max} and the size of extracted guest molecules are unrelated. Menger, F. M.; Yamada, K. *J. Chem. Soc.* **1979**, *101*, 6731.