Synthesis, Characterization, and Antipeptic Activity of a Novel Surfactant Having an Azulene Moiety

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A novel surfactant having an azulene moiety, sodium 3-decyl-1-azulenesulfonate (C10AS), has been synthesized. The surface-activity of C10AS is lower than that of its structural isomer, sodium 4-decyl-1-naphthalenesulfonate (C10NS), probably caused by the higher hydrophilicity of C10AS due to the dipole moment of azulene moiety. C10AS exhibits the higher antipeptic activity than the propyl derivative (C3AS) below its critical micelle concentration.

Azulene is a non-benzenoid aromatic compound and, in spite of being a structural isomer of naphthalene, has a dipole moment of ca. 0.8 D[1] and absorption bands in the visible region.[2] In addition, it has attractive spectroscopic properties: the main emission from the second excited singlet state[3] and substantial self-quenching of fluorescence.[4] Azulene interests scientists not only physicochemically but also pharmacologically because its sulfonated derivatives have pharmacological effects such as anti-inflammatory and anti-ulcer activities and have been clinically used.[5] Yanagisawa et al. synthesized five sodium 3-alkyl-1-azulenesulfonates (R = CH$_3$–C$_5$H$_{11}$) and many other derivatives and investigated the relationship between their inhibitory potency against Shay ulcer and their lipophilicity, log P (the logarithm of octanol–water partition coefficient), concluding that the optimal value of log P was ca. −1.2.[6] Therefore, the propyl derivative (C3AS) shows the highest inhibitory potency among the above 3-alkyl-1-azulenesulfonates.

C10AS was synthesized by the modified method of Yanagisawa et al.[6] (Scheme 1). Sodium methoxide was slowly added to an ethanol solution of 2-tosyloxytropone (**1**) 5.0 g and dimethyl malonate 4.8 g with stirring in an ice-water bath. After 6 h the reaction mixture was diluted with water to give 2.5 g of 3-methoxycarbonyl-2*H*-cyclohepta[*b*]furan-2-one (**2**) as yellow crystals. An ethanol solution containing the compound **2**, *n*-dodecanal (6.8 g) and morpholine (3.2 g) was heated under reflux for 4 h. After the solvent was removed, the residue was dissolved in benzene, washed with water, and dried over Na$_2$SO$_4$. The solvent was removed again and the residue was purified by a column chromatography with a silica-gel column and benzene
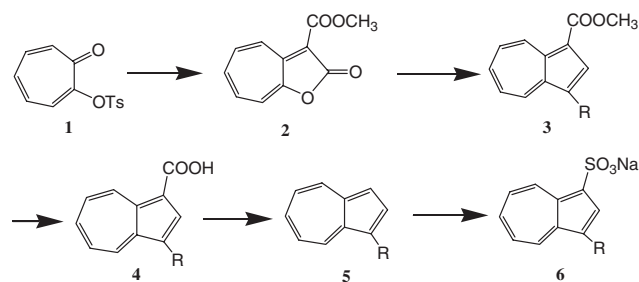
as an eluent to give 8.1 g of violet oil, which was a mixture of methyl 3-decylazulene-1-carboxylate (**3**) and the unchanged *n*-dodecanal but was not purified further. Twenty mL of 9.5 M KOH aqueous solution was poured into an ethanol solution of the oil and the mixture was heated under reflux for 2 h. The reaction mixture was diluted with water and its pH was adjusted to 3 by adding hydrochloric acid. Seven point one g of 3-decyl-azulene-1-carboxylic acid (**4**) was obtained as violet crystals. A benzene solution containing the compound **4** and trichloroacetic acid (0.3 g) was heated under reflux for 23 h. The solvent was removed and the residue was purified by a column chromatography with an alumina column and toluene to give 3.4 g of 1-decyl-azulene (**5**) as blue oil. A mixture of **5** (3.0 g) and pyridine-sulfur trioxide complex (3.5 g) in benzene was heated under reflux for 6 h. Precipitates were separated by filtration and dissolved in water. After 10 mL of aqueous solution containing 0.9 g of NaOH was poured into the solution, the reaction mixture was stirred at 30–35 °C for 1 h and extracted with *n*-butanol. The crude product was given by the removal of the extractant and the succeeding vacuum drying and recrystallized once from acetone and twice from ethanol to yield 0.7 g (12%) of C10AS (**6**; R = CH$_3$(CH$_2$)$_9$–) as violet needle-like crystals.[7]

The synthesis procedure of C3AS (**6**; R = CH$_3$(CH$_2$)$_2$–) was similar to that of C10AS, except that pentanal was used in place of dodecanal. C3AS was obtained as violet crystals with the 24% yield.[8]

The surface-activity of the synthesized C10AS was confirmed by surface tension measurements (drop weight method), as shown in Figure 1. The figure includes the data for its structural isomer, C10NS,[9] for comparison. The cmc, surface tension
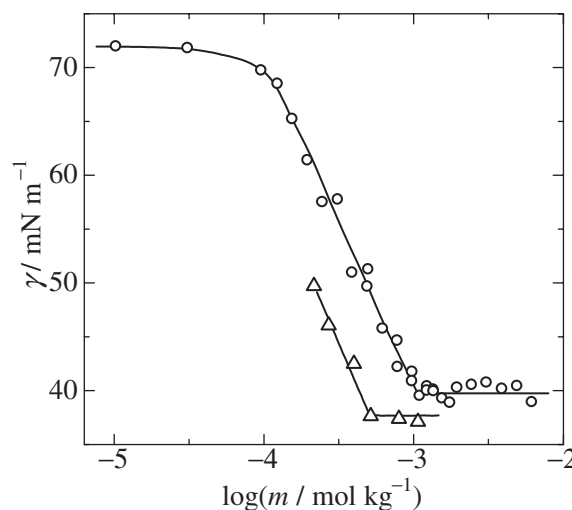
Scheme 1. Synthetic procedures of C10AS and C3AS.

Figure 1. Concentration dependence of surface tension of C10AS (○) and C10NS[9] (△) aqueous solutions at 25 and 30 °C, respectively.

Table 1. Surface properties of C10AS and C10NS at air–water interface

| Compounds	cmc /mmol kg^{-1}	γ_{cmc} /mN m^{-1}	pC_{20}	A_{cmc} /nm^2
C10AS(25 °C)	1.06	39.8	3.69	0.620
C10NS(30 °C)[9]	0.52	37.4	3.98	0.646

at cmc (γ_{cmc}), efficiency in surface tension reduction (pC_{20}: the negative logarithm of the surfactant concentration required to reduce the surface tension of solvent by 20 mN m^{-1}), and surface area per surfactant molecule (A_{cmc}) obtained from Figure 1 were summarized in Table 1. These data show that C10AS exhibits the weaker surface-activity than C10NS. This would be attributed to the higher hydrophilicity of C10AS due to the dipole moment of azulene ring. The smaller A_{cmc} may be caused by the attractive interaction between the azulene ring dipole moments.

The antipeptic activity of C10AS was measured with the same method as Yanagisawa et al.[10] A mixture of 1.0 mL of 5 mg/mL bovine serum albumin (BSA), 0.3 mL of 1 M HCl and 2.0 mL of C10AS solution with various concentrations was preincubated at 37 °C for 5 min. Zero point five mL of pepsin derived from porcine stomach mucosa (10 μg/mL of 0.5 M HCl) was added to the mixture and incubated at 37 °C for 10 min. Two point zero mL of 10% trichloroacetic acid was then added to stop the enzyme reaction. Zero point one mL of a supernatant obtained after centrifugation at 3000–3500 rpm for 10 min was added to 2.0 mL of 0.2 M borate buffer solution (pH 9.0). To determine the amount of digested BSA (that is, hydrolysed BSA) fluorescence intensity at 475 nm was measured 5 min after 1.0 mL of fluorescamine solution (0.3 mg/mL of acetone) was added to the supernatant solution. The percentage inhibition was calculated by

$$\%\text{inhibition} = 100 \times (A - B)/A \qquad (1)$$

where A and B is the amount of digested BSA without and with C10AS, respectively.

The percentage inhibition of C10AS increases linearly with its concentration below 0.55 mmol kg^{-1}, but it decreases
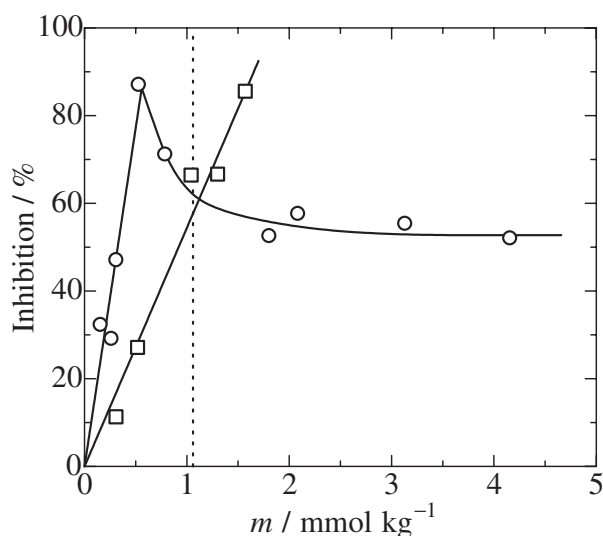
Figure 2. Antipeptic activity of C10AS (○) and C3AS (□). The dashed line represents the cmc of C10AS at 25 °C.

exponentially at the higher concentrations and approaches to 53% inhibition, as shown in Figure 2. C10AS exhibits the higher antipeptic activity than a reference compound C3AS below the cmc and the IC$_{50}$ (concentration which inhibits peptic activity by 50%) values are 0.31 and 0.91 mmol kg^{-1}, respectively. This would be caused by a hydrophobic binding of amphiphilic C10AS to the hydrophobic active pocket of pepsin.[11] It is known that native BSA is unfolded by a cooperative binding of sodium dodecyl sulfate around a half of its cmc.[12] C10AS can also unfold BSA around 0.55 mmol kg^{-1}. The unfolded BSA can be digested more easily by pepsin than native BSA. This may be the cause of decrease in percentage inhibition at the higher concentrations.

In summary, the surfactant having an azulene moiety, C10AS, newly synthesized are slightly less surface-active than its structural isomer, C10NS, probably due to the dipole moment of azulene moiety. C10AS exhibits the higher antipeptic activity than its propyl derivative, C3AS, below a half of the cmc, above which the antipeptic activity of C10AS are weaken probably by the unfolding of BSA which promotes its digestion by pepsin.

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7 Mp 140–142 °C (dec.). ^{1}H NMR (D$_2$O, 400 MHz) δ 0.85 (3H, t, CH$_2$CH$_2$(CH$_2$)$_7$C<u>H</u>$_3$), 1.09 (14H, brs, CH$_2$CH$_2$(C<u>H</u>$_2$)$_7$CH$_3$), 1.40 (2H, quint, CH$_2$C<u>H</u>$_2$(CH$_2$)$_7$CH$_3$), 2.58 (2H, t, C<u>H</u>$_2$CH$_2$-(CH$_2$)$_7$CH$_3$), 6.43 (1H, dd, H-5), 6.96–7.06 (2H, m, H-6 and H-7), 7.68 (1H, d, H-4), 7.90 (1H, s, H-2), 8.68 (1H, d, H-8). ^{13}C NMR (D$_2$O, 100 MHz) δ 16.8, 25.5, 29.6, 32.3, 32.6, 34.1, 34.8, 126.4, 128.1, 129.3, 131.1, 137.1, 137.7, 138.6, 139.2, 140.5, 141.7. IR (KBr) 2921, 2850, 1573, 1390, 1180, 1064, 887, 744, 730 cm^{-1}. MS *m/z* (relative intensity) 268 (8.6), 141 (100), 43 (23.5).
8 Mp 160–162 °C (dec.). ^{1}H NMR (D$_2$O, 400 MHz) δ 0.91 (3H, t, CH$_2$CH$_2$C<u>H</u>$_3$), 1.72 (2H, sext, CH$_2$C<u>H</u>$_2$CH$_3$), 2.97 (2H, t, C<u>H</u>$_2$CH$_2$CH$_3$), 7.36 (1H, dd, H-5), 7.43 (1H, dd, H-7), 7.81 (1H, dd, H-6), 8.02 (1H, s, H-2), 8.49 (1H, d, H-4), 8.92 (1H, d, H-8). ^{13}C NMR (D$_2$O, 100 MHz) δ 16.1, 26.8, 31.1, 127.9, 128.3, 132.3, 137.7, 138.6, 139.0, 139.2, 141.2, 142.7. IR (KBr) 2950, 2923, 2865, 1573, 1463, 1450, 1419, 1409, 1388, 1178, 1145, 1089, 1035, 877, 728 cm^{-1}.
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