Electronic Supplementary Materials

Self-Assembly of the Bio-Surfactant Aescin in Solution: A Small-Angle X-Ray Scattering and Fluorescence Study

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Fractionation of Aescin (Sigma Aldrich, CAS: 6805-41-0) by Reversed-Phase High-Pressure Liquid Chromatography (RP-HPLC)

Aescin (30 mg) was dissolved in a mixture of acetonitrile/Millipore water (MPW) (20:80) and purified *via* reversed phase high pressure liquid chromatography (RP-HPLC, from Hitachi, Tokyo, Japan). Fraction 1 (t_R = 16 min, 2.8 mg) and 2 (t_R = 18 min, 4.8 mg) were obtained after freeze-drying as colourless solids. NMR spectra of fractions 1 and 2 were recorded in deuterated pyridine (C_5D_5N) on a Bruker Avance III 500 spectrometer (^1H: 500 MHz, from Bruker, Billerica, USA). Chemical shifts are given in parts per million (ppm) relative to tetramethylsilane, residual solvent peaks for ^1H were used as internal standard.

Analytical liquid chromatography coupled with mass spectrometry (LC-MS) and determination of ESI-HRMS was performed on an Agilent 6220 TOF-MS (Agilent Technologies, Santa Clara, USA) with a dual ESI-source operating with a spray voltage of 2.5 kV, 1200 HPLC system with autosampler, degasser, binary pump, column oven, diode array detector and a Phenomenex Luna C_{18} column (3 µm, 100 × 2 mm). Nitrogen was generated by a nitrogen generator NGM 11 and served as nebulizer and dry gas. External calibration was performed with ESI-L Tuning Mix (Agilent Technologies, Santa Clara, USA).

Gradient for preparative RP-HPLC:

Flow: 10 mL/min (Hypersil Gold C_{18} column, 50 × 21.2 mm, 1.9 µM particles)

Eluent A: MPW:Acetonitrile:TFA, 94.9:5:0.1

Eluent B: Acetonitrile:MPW:TFA, 94.9:5:0.1

min	Eluent A [%]	Eluent B [%]
0	80	20
80	40	60

Gradient for LC-MS:

Flow: 0.3 mL/min, 40 °C (Phenomenex Luna C_{18} column, 3 µm, 100 × 2 mm)

Eluent A: MPW:Acetonitrile:HCOOH, 94.9:5:0.1

Eluent B: Acetonitrile:MPW:HCOOH, 94.9:5:0.1

min	Eluent A [%]	Eluent B [%]
0	100	0
11	2	98
11.5	100	0
15	100	0

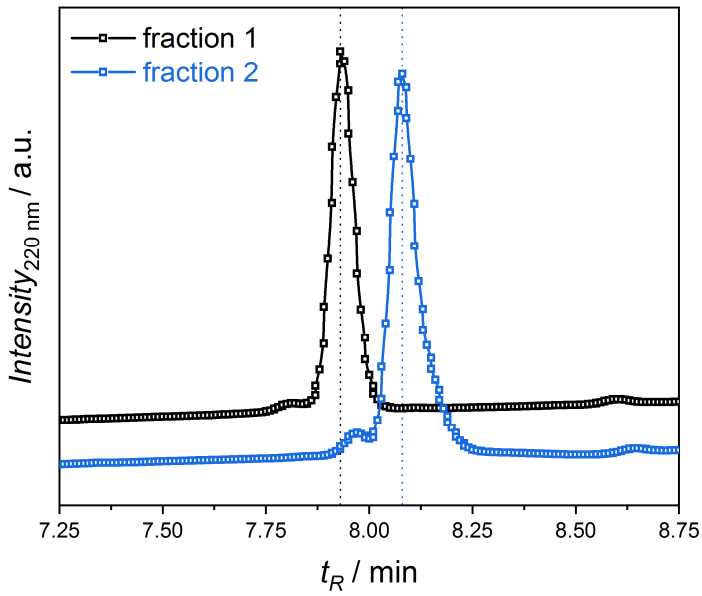

Figure S1: UV-intensities (220 nm) of fractions 1 and 2 recorded as function of the retention time t_R by analytical liquid chromatography coupled with mass spectrometry (LC-MS). Fractions 1 and 2 were successfully separated.

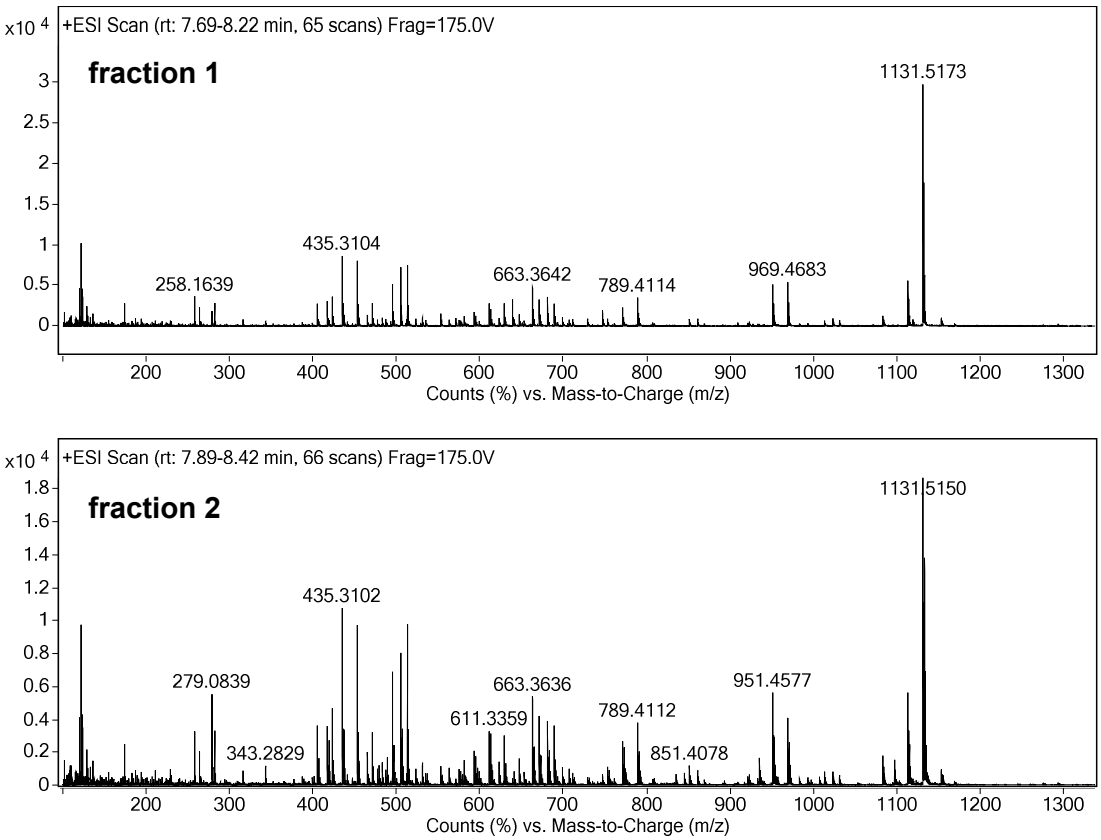

Figure S2: Mass spectra of fractions 1 and 2 recorded by ESI-HRMS (while LC-MS run). Spectra of both fractions show the molecular signal of aescin at m/z = 1331.5 (exact mass of aescin = 1330.5 g/mol) and the fragmentation patterns looks very similar. This indicates the presence of isomers and the substances contained in each fraction cannot be distinguished only by mass spectrometry.

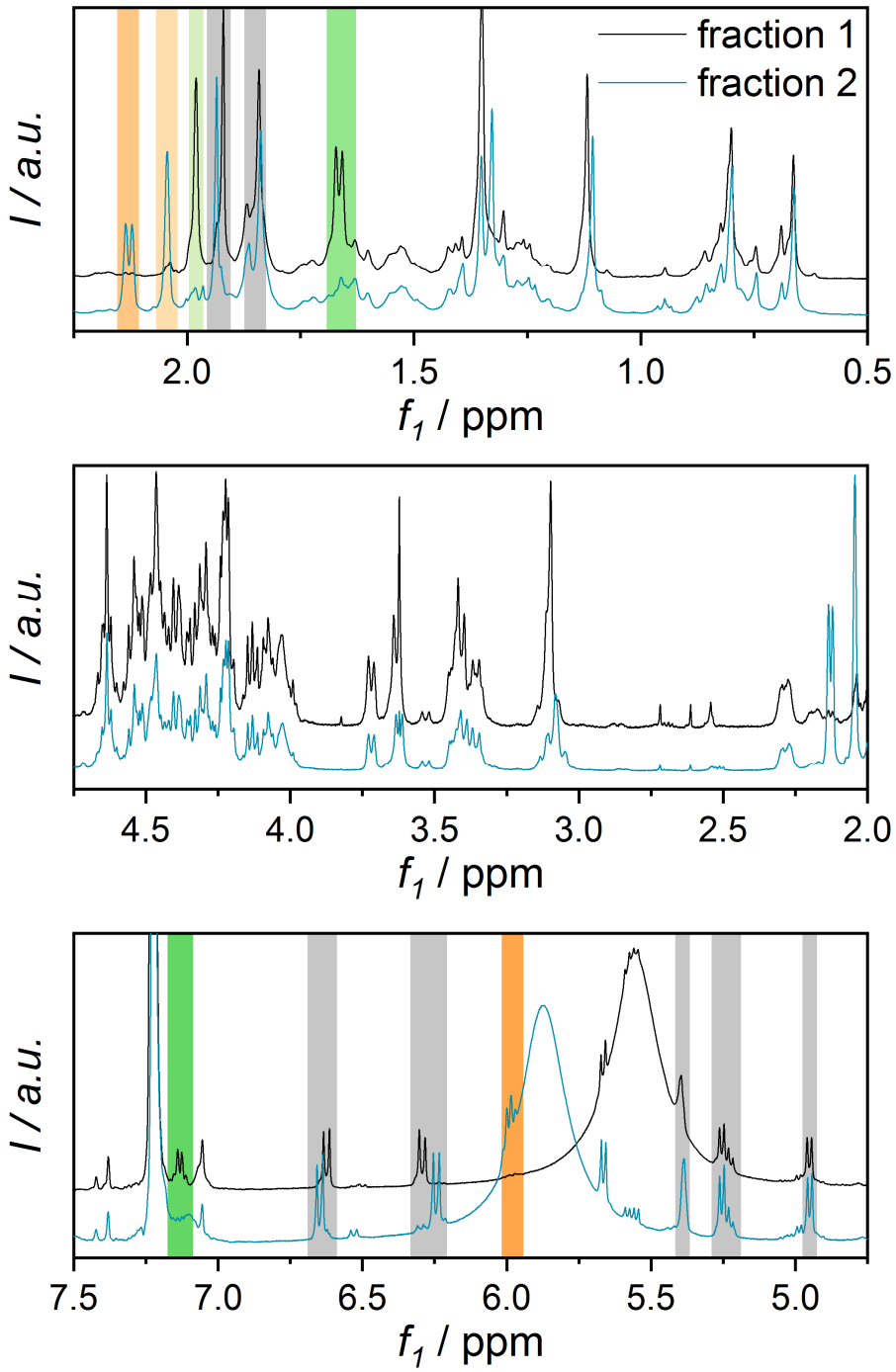

Figure S3: ^{1}H-NMR spectra of aescin fractions in deuterated pyridine obtained from preparative liquid chromatography (RP-HPLC). Signals assigned to specific protons of possible aescin structures are marked with different colours (see Figure S4 for assignment). Positions of the signals are moreover listed in Table S1. The assignment of the signals was done on the basis of works of Yoshikawa et al.[1,2].

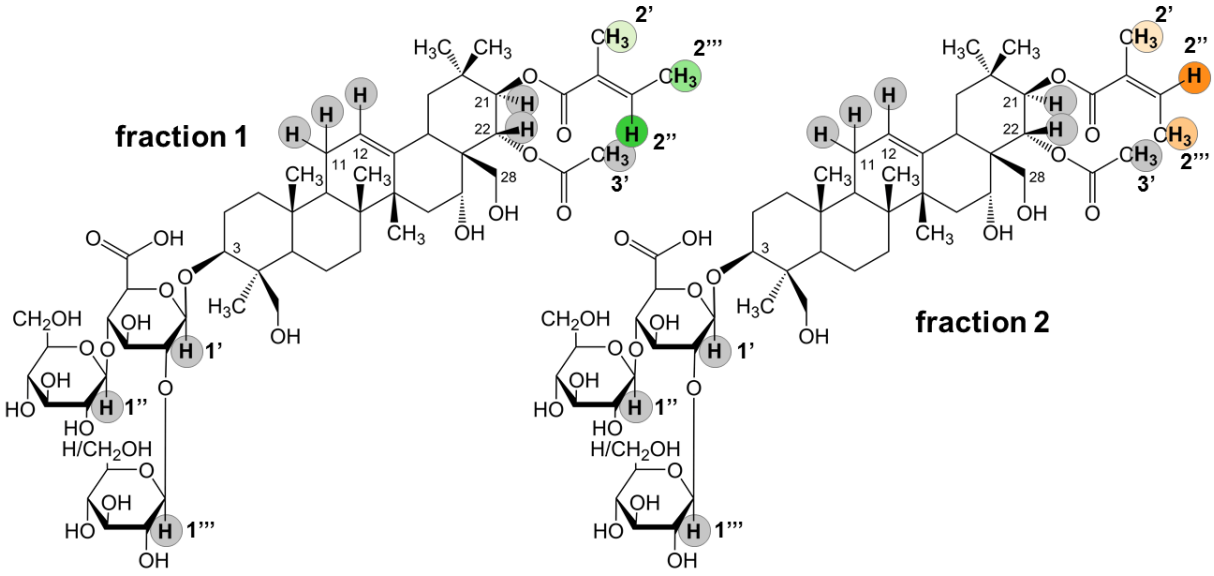

Figure S4: Molecular structures of *β*-aescin structures present in fractions 1 and 2 obtained by RP-HPLC. Signals belonging to the colour encoded protons can be found in Figure S3 as well as in Table S1. The main difference between both structures in the presence of a tiglic acid residue on C-21 in fraction 1 and an angelic acid residue in fraction 2. The presence of D-xylose instead of D-glucose on the position of the sugar marked with 1''' could not be confirmed.

Table S1: ^{1}H-NMR signals of protons in the possible molecular structures of *β*-aescin shown in Figure S4. The assignment of the signals was done on the basis of works of Yoshikawa et al.[1,2].

	fraction 1		***fraction 2***	
proton	**f_1 / ppm**	**multiplicity**	**f_1 / ppm**	**multiplicity**
1'	4.95	doublet	4.95	doublet
1''	5.22	doublet	5.22	doublet
1'''	5.26	doublet	5.26	doublet
C11-H	1.84	singlet	1.84	singlet
	1.87	singlet	1.86	singlet
C12-H	5.40	broad singlet	5.39	broad singlet
C21-H	6.62	doublet	6.65	doublet
C22-H	6.29	doublet	6.25	doublet
2'	1.98	singlet	2.05	singlet
2''	7.13	quartet	5.99	quartet
2'''	1.66	doublet	2.13	doublet
3'	1.92	singlet	1.94	singlet

Determination of pKa-Value of Aescin by Titration

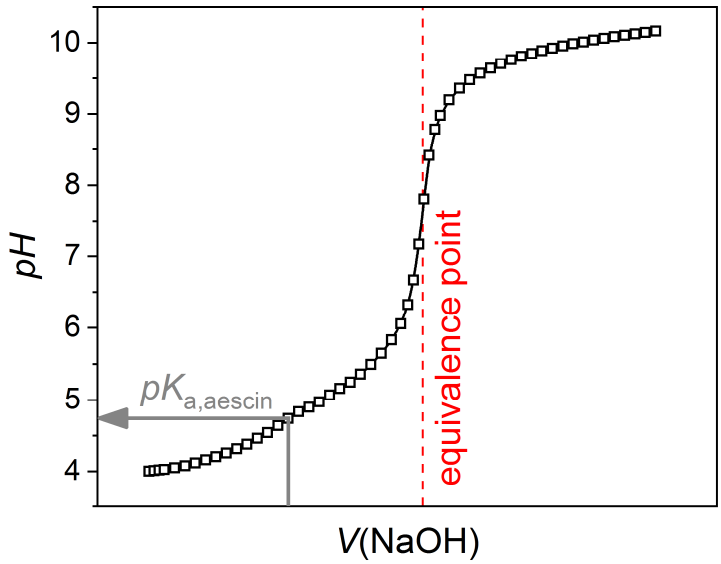

Figure S5: Titration curve of an aqueous aescin solution with a concentration of 0.45 mM. This concentration is near the solubility limit of aescin in pure water. As base a 10 mM sodium hydroxide solution was used. Measurements were performed with a 905 Titrando (Methrom, Filderstadt, Germany). The pK_a value of aescin was determined from the buffer region to a value of 4.7 ± 0.2.

cmc-Determination of Aescin by Fluorescence Spectroscopy

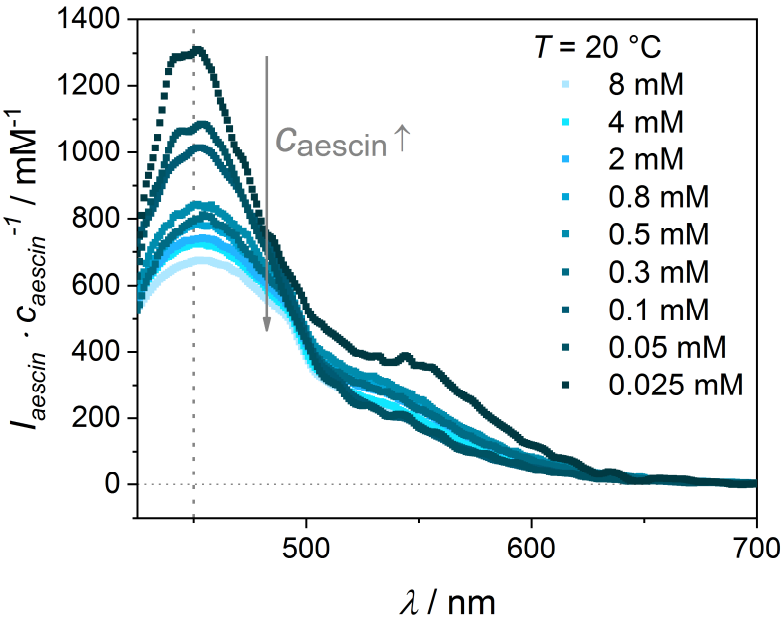

Figure S6: Concentration-normalized autofluorescence of aescin at different concentrations. The fluorescence intensity of aescin was normalized to the aescin concentration in solution. Consequently, the value $I_{aescin} \cdot c_{aescin}^{-1}$ describes the fluorescence ability of a defined number of aescin molecules. This ability strongly decreases with increasing aescin concentration. Measurements were performed in a buffer solution with constant pH value of 7.4.

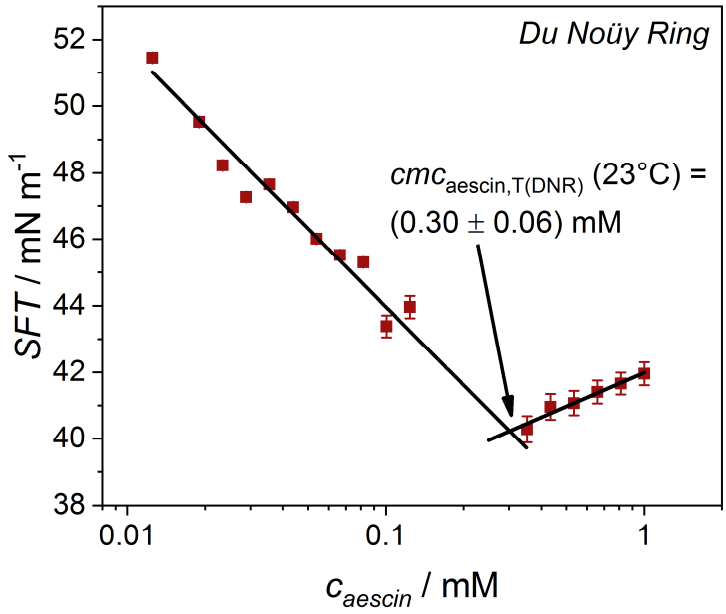

Figure S7: Surface tension isotherm of aescin in aqueous phosphate buffer at a temperature of 23 °C. Data were recorded with the Du Noüy Ring (DNR) method. The intersection of the two linear regressions defines the $cmc_{aescin,T(DNR)}$ value. The error results from error propagation of the regression parameters. The cmc-value obtained here by the DNR method equals the value obtained from the Wilhelmy plate method. The increase of SFT above $cmc_{aescin,T(DNR)}$ probably results from impurities of the aescin powder[3–5].

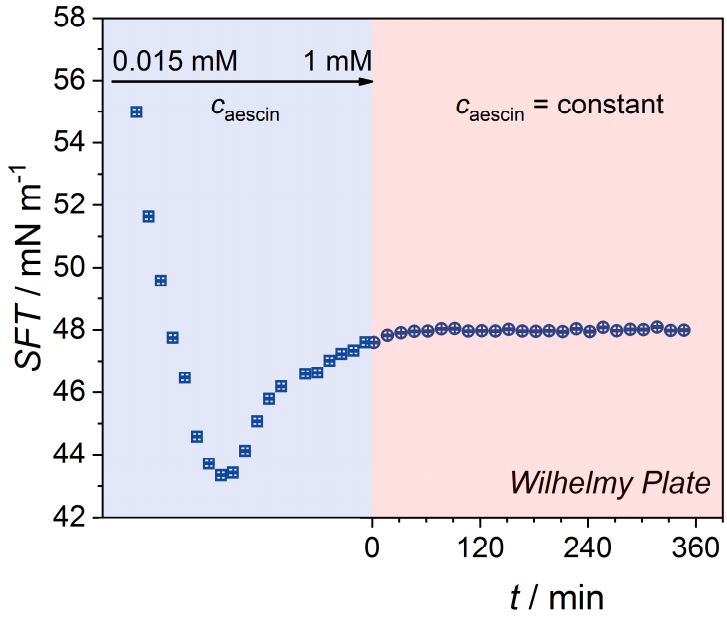

Figure S8: Surface tension (SFT) isotherm of aescin in aqueous phosphate buffer at a temperature of 23 °C (blue area) followed by time dependent measurement of SFT at constant aescin concentration (1 mM, red area). Data were recorded with the Wilhelmy Plate (WP) method. After reaching $cmc_{aescin,T(WP)}$ SFT increases reproducibly with increasing aescin concentration. At constant aescin concentration SFT stays constant over time already after a few minutes. This indicates that the comparably strong increase in SFT after reaching $cmc_{aescin,T(WP)}$ cannot be solely attributed to a nonattainment of an equilibrium state.

References

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