Minor asterosaponin archasteroside C from the starfish *Archaster typicus*

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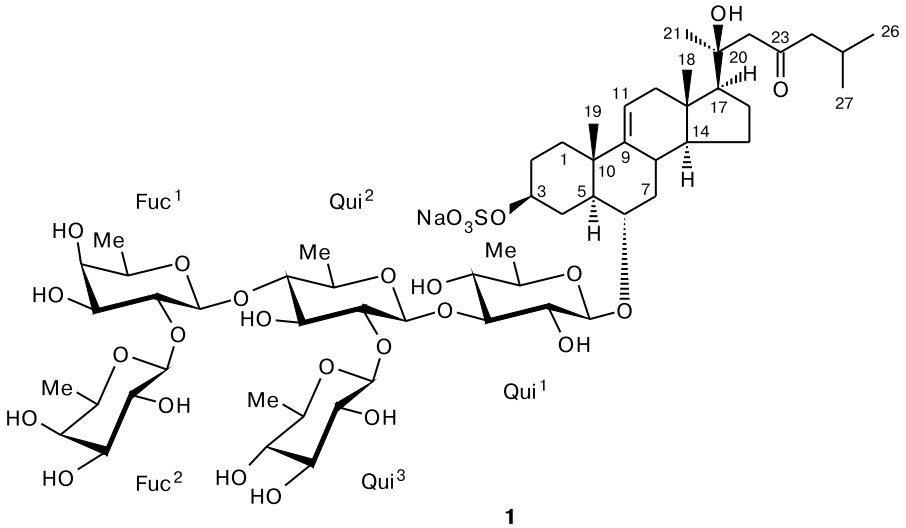
A new minor asterosaponin (20*S*)-6-*O*-{β-D-fucopyranosyl-(1→2)-β-D-fucopyranosyl-(1→4)-[β-D-quinovopyranosyl-(1→2)]-β-D-quinovopyranosyl-(1→3)-β-D-quinovopyranosyl}-3β,6α,20-trihydroxycholest-9(11)-en-23-one 3-sulfate (archasteroside C) was isolated from the starfish *Archaster typicus* collected in shallow coastal waters of Vietnam. The structure of archasteroside C was determined by 2D NMR spectroscopy and electrospray ionization (ESI) tandem mass spectrometry.

Key words: starfish, *Archaster typicus*, asterosaponin, glycoside, steroidal aglycone, NMR spectroscopy.

Starfishes contain chemically diverse polar steroids (polyhydroxysteroids and related biosides and monosides) and steroidal oligoglycosides called asterosaponins.[1] Generally, these compounds are present in animal extracts as complex mixtures, which are difficult to separate. Minor components of these mixtures mostly escape study although certain features of the biosynthesis of particular metabolites are associated with the formation of these minor compounds. Asterosaponins have a wide range of biological activities, including embryotoxic, hemolytic, antifungal, antiviral, antibiofouling, *etc.*[1] Previously,[2] we have investigated the starfish *Archaster typicus* (order Valvatida, family Archasteridae) collected in shallow coastal waters of Quang Ninh province (South China Sea, Vietnam) and isolated two new cytotoxic asterosaponins, *viz.*, archasterosides A and B, as well as the known glycoside regularoside C. In the present study, we isolated a new minor asterosaponin archasteroside C from this starfish and determined its structure.

An ethanolic extract of *A. typicus* was subjected to successive chromatographic separation on columns with Polychrome 1, silica gel, and Florisil. Reversed-phase ion-pair HPLC of the oligosaccharide fraction on Diasfer-110-C18 and Discovery C18 columns afforded glycoside **1** in a yield of $1.4 \cdot 10^{-5}$% based on the animal weight.



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The high-resolution negative ion electrospray ionization (ESI) mass spectrum of archasteroside C (**1**) has a peak of the decationized molecule at *m/z* 1241.5648 $[M - Na]^-$. The positive ion ESI mass spectrum has a peak at *m/z* 1287 $[M + Na]^+$. According to the mass-spectrometric and NMR spectroscopic data, compound **1** has the molecular formula $C_{57}H_{93}O_{27}NaS$. The fragmentation peaks at *m/z* 1167 $[(M + Na) - NaHSO_4]^+$ in the positive ion ESI mass spectrum and at *m/z* 97 $[HSO_4]^-$ in the negative ion ESI mass spectrum are indicative of the presence of the sulfate group in compound **1**. The ^1H and ^{13}C NMR spectra of the aglycone moiety of glycoside **1** show signals for the protons and the carbon atoms of two angular methyl groups $H_3C(18)$ and $H_3C(19)$ (δ_H 0.77, 0.98; δ_C 13.6, 19.7), the 9(11) double bond (δ_H 5.33; δ_C 146.4, 117.8), one methine group HC(3) (δ_H 4.17; δ_C 79.7) bound to sulfate, and one methine group HC(6) (δ_H 3.57; δ_C 81.2) bound to the oligosaccharide chain, which are characteristic of the steroid core of asterosaponins.[3] The spectroscopic data provide evidence that the side chain of the aglycone contains the methyl group $H_3C(21)$ (a singlet in the ^1H NMR spectrum), the methyl groups $H_3C(26)$ and $H_3C(27)$ (a doublet in the ^1H NMR spectrum), the methylene groups $H_2C(22)$ and $H_2C(24)$, and the 23-keto group. The NMR spectroscopic data are in good agreement with the corresponding data for the known aglycone 3β,6α,20-trihydroxycholest-9(11)-en-23-one (thornasterol A) with a glycosidic bond at C(6) and the sulfate group at C(3), which was found in thornasteroside A from *Acanthaster planci* and, more recently, in some other starfishes.[3,4] The chemical shift of the singlet for the protons $H_3C(21)$ at δ_H 1.33 is indicative of the 20*S* configuration of the chiral center (*cf.* δ_H 1.28 for 20*S*- and δ_H 1.13 for 20*R*-hydroxycholesterol).[3] The structure of the aglycone of compound **1** was confirmed by 2D NMR spectroscopy, in particular, by $^1H—^1H$ COSY-45, HSQC, HMBC, and ROESY experiments.

The ^1H NMR spectrum of glycoside **1** (Table 1) shows signals for five anomeric protons at δ_H 4.40, 4.53, 4.45, 4.41, and 4.52 (spin-spin coupling constants vary from 7.6

Table 1. NMR spectroscopic data for the carbohydrate moiety of compound **1**[a] (700 MHz, methanol-d_4, δ, *J*/Hz)

Moiety	Atom	^{13}C	^1H	HMBC	ROESY
Qui[1]	1	104.6	4.40 (br.d, *J* = 7.6)	—	6 (aglycone); 3, 5 (Qui[1])
	2	74.4	3.33 (m)	—	—
	3	91.1	3.33 (m)	—	1 (Qui[1])
	4	75.2[b]	3.03 (t, *J* = 8.8)	3 (Qui[1])	6 (Qui[1])
	5	72.6	3.35 (m)		1 (Qui[1])
	6	18.2	1.26 (d, *J* = 6.2)	4, 5 (Qui[1])	4 (Qui[1])
Qui[2]	1	104.1	4.53 (d, *J* = 7.7)	3 (Qui[1])	3, 5 (Qui[2])
	2	85.1	3.48 (t, *J* = 8.4)	1, 3 (Qui[2]); 1 (Qui[3])	1 (Qui[3])
	3	75.4	3.70 (t, *J* = 9.2)	4 (Qui[2])	1, 5 (Qui[2])
	4	85.6	3.24 (t, *J* = 9.3)	3, 5 (Qui[2])	6 (Qui[2]), 1 (Fuc[1])
	5	72.4	3.56 (m)	—	1, 3 (Qui[2])
	6	18.3	1.43 (d, *J* = 6.1)	4, 5 (Qui[2])	4 (Qui[2])
Fuc[1]	1	103.0	4.45 (br.d, *J* = 7.6)	—	3, 5 (Fuc[1]); 4 (Qui[2])
	2	82.3	3.67 (m)	3 (Fuc[1]), 1 (Fuc[2])	—
	3	75.1[b]	3.67 (m)	—	1 (Fuc[1])
	4	72.5	3.62 (br.s)	—	6 (Fuc[1])
	5	72.1	3.72 (m)	4 (Fuc[1])	1 (Fuc[1])
	6	16.5	1.26 (d, 6.2)	4, 5 (Fuc[1])	4 (Fuc[1])
Fuc[2]	1	107.0	4.41 (d, *J* = 8.0)	2 (Fuc[1])	3, 5 (Fuc[2])
	2	73.8	3.53 (dd, *J* = 7.7, *J* = 10.1)	1, 3 (Fuc[2])	—
	3	75.0	3.47 (dd, *J* = 9.4, *J* = 3.5)	—	1 (Fuc[2])
	4	73.0	3.56 (d, *J* = 2.9)	3 (Fuc[2])	6 (Fuc[2])
	5	72.6	3.62 (m)	1, 4 (Fuc[2])	1 (Fuc[2])
	6	16.8	1.24 (d, *J* = 6.4)	4, 5 (Fuc[2])	4 (Fuc[2])
Qui[3]	1	107.0	4.52 (d, *J* = 7.7)	2 (Qui[2])	5 (Qui[3]); 2 (Qui[2])
	2	76.7	3.28 (dd, *J* = 7.3, *J* = 9.5)	—	—
	3	77.0	3.32 (t, *J* = 9.1)	—	—
	4	76.2	3.10 (t, *J* = 9.2)	3 (Qui[3])	6 (Qui[3])
	5	74.7	3.34 (m)	—	1 (Qui[3])
	6	18.0	1.36 (d, *J* = 6.3)	4, 5 (Qui[3])	4 (Qui[3])

[a] The assignment of the signals was made based on 2D $^1H—^1H$ COSY-45, HSQC, 1D TOCSY, HMQC-TOCSY, HMBC, ROESY, and H2BC NMR spectra.

[b] The assignment of the signals was ambiguous.

to 8.0 Hz) related to five signals for the carbon atoms at δ_C 104.6, 104.1, 103.0, and 107.0 (×2), respectively, in the HSQC spectrum. Along with the mass spectra, these data indicate that molecule **1** contains the carbohydrate chain consisting of five monosaccharide residues linked to each other and to the aglycone by β-glycosidic bonds (H_{1a}, H_{2a}). The doublets of the methyl groups at δ_H 1.26 (×2), 1.43, 1.24, and 1.36 in the ^{1}H NMR spectrum are indicative of the presence of five 6-deoxyhexose residues. This fact is confirmed also by mass spectrometric data. Thus the electrospray ionization ion trap tandem mass spectrum of the ion $[M - Na]^-$ at *m/z* 1241 has fragment peaks at *m/z* 1141 $[(M - Na) - 100]^-$, 995 $[(M - Na) - 100 - 146]^-$, 849 $[(M - Na) - 100 - 2\times146]^-$, 703 $[(M - Na) - 100 - 3\times146]^-$, 557 $[(M - Na) - 100 - 4\times146]^-$, 411 $[(M - Na) - 100 - 5\times146]^-$, and 393 $[(M - Na) - 100 - 4\times146 - 164]^-$ corresponding to the cleavage of the side chain of the aglycone and the successive elimination of one, two, three, four, and five 6-deoxyhexose residues, respectively. The ESI tandem mass spectrum of the ion $[M + Na]^+$ at *m/z* 1287 has fragment peaks at *m/z* 921 $[(M + Na) - NaHSO_4 - 100 - 146]^+$ and 775 $[(M + Na) - NaHSO_4 - 100 - 2\times146]^+$ corresponding to the successive elimination of the sulfate group, the side chain of the aglycone, and one and two 6-deoxyhexose residues, as well as peaks at *m/z* 607 $[4\times146 + Na]^+$, 461 $[3\times146 + Na]^+$, and 315 $[2\times146 + Na]^+$ corresponding to fragments consisting of four, three, and two 6-deoxyhexose residues, respectively.

The chemical shifts and the spin-spin coupling constants of the protons HC(1)—HC(5) of the quinovose residues and the protons HC(1)—HC(4) of the fucose residues were determined based on the analysis of the 1D TOCSY spectra obtained by irradiation of the anomeric protons of monosaccharides; the spectroscopic parameters of the protons HC(5) of the fucose residues were determined by irradiation of the protons of the deoxy groups. The ^{1}H—^{1}H COSY, HSQC, HSQC-TOCSY, HMBC, and ROESY experiments allowed us to assign the signals for all protons and carbon atoms of the oligosaccharide chain of glycoside **1**, except for the carbon atoms (δ_C 82.3, 75.1), which are coupled in the HSQC spectrum with the two-proton multiplet at δ_H 3.67 belonging to the protons HC(2) and HC(3) of the residue Fuc^1. The position of the substituent in the residue Fuc^1 was refined with the use of the H2BC technique enabling the establishment of the correlation between the protons and adjacent carbon atoms.[5] Based on the H2BC corellations between the anomeric proton HC(1) (δ_H 4.45) and the adjacent atom C(2) (δ_C 82.3), the position of the substituent in the residue Fuc^1 was determined as C(2). The spectroscopic data for the monosaccharide residues are in good agreement with the corresponding data for the terminal β-fucopyranose and β-quinovopyranose residues and the internal 3-O-substituted β-quinovopyranose, 2,4-di-O-substituted β-quinovopyranose, and 2-O-substituted β-fucopyranose residues involved in known asterosaponins.[2,6] The hydrolysis of glycoside **1** with 2 *M* trifluoroacetic acid (TFA) followed by the treatment with (*R*)-(−)-octan-2-ol and acetylation afforded acetylated octyl fucosides and quinovosides. The D-configurations of the fucose and quinovose residues were determined by comparing the retention times (GLC) of the derivatives with those of the corresponding reference samples of D- and L-monosaccharides according to the known procedure.[7] The positions of the glycosidic bonds in the monosaccharide residues and the binding site of the oligosaccharide chain to the aglycone of glycoside **1** were confirmed by ROESY and HMBC correlations: HC(1) of Qui^1 with HC(6) of the aglycone, HC(1) of Qui^2 with C(3) of Qui^1, HC(1) of Qui^3 with HC(2) and C(2) of Qui^2, HC(1) of Fuc^1 with HC(4) of Qui^2, and HC(1) of Fuc^2 with C(2) of Fuc^1. Thus, based on the above-described data, we determined the structure of archasteroside C as (20*S*)-6-*O*-{β-D-fucopyranosyl-(1→2)-β-D-fucopyranosyl-(1→4)-[β-D-quinovopyranosyl-(1→2)]-β-D-quinovopyranosyl-(1→3)-β-D-quinovopyranosyl}-3β,6α,20-trihydroxycholest-9(11)-en-23-one 3-sulfate.

Archasteroside C is a rare asterosaponin, whose oligosaccharide chain consists only of 6-deoxyhexose residues, *viz.*, β-D-fucose and β-D-quinovose. Cabrohydrate chains consisting mainly of deoxy sugars are typical of the so-called cardiac glycosides. Previously,[2] only one asterosaponin of this type, *viz.*, archasteroside B, which has also been isolated from the starfish *A. typicus*, has been characterized.

Experimental

The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance III 700 spectrometer (700 and 176.0 MHz, respectively) with Me_4Si as the internal standard. The optical rotation was determined on a Perkin—Elmer 343 polarimeter (Germany). The high-resolution mass spectra were obtained on an Agilent 6510 LC-Q-TOF spectrometer (USA). High-performance liquid chromatography was performed on an Agilent 1100 Series (USA) chromatograph equipped with a refractometer as the detector and with Diasfer-110-C18 (10 μm, 250×15 mm) and Discovery C18 (5 μm, 250×10 mm) columns. Gas liquid chromatography was carried out on an Agilent 6580 Series chromatograph (USA) equipped with an HP-5 MS capillary column (30 m×0.25 mm) at 100 °C (0.5 min) → 270 °C (5 °C min^{-1}, 10 min) using He as the carrier gas (1.7 mL min^{-1}); the temperatures of the injector and the detector were 250 and 270 °C, respectively. Column chromatography was performed on Polychrome 1 (Teflon powder, Biolar, Latvia), silica gel KSK (50—160 μm, Sorbpolimer, Russia), and Florisil (200—300 mesh, Aldrich Chemical Co., USA). TLC was carried out on SiO_2-precoated Sorbfil plates (4.5×6.0 cm) (5—17 μm, Sorbpolimer, Russia).

Animals. Samples of the starfish *A. typicus* were collected in August 2008 from a depth of 5—10 m in shallow coastal waters of Quang Ninh province (South China Sea, Vietnam). The species

determination was carried out by Do Cong Thung (Institute of Natural Products Chemistry, Vietnam Academy of Science and Technology, Hanoi, Vietnam).

Isolation of compound 1. Starfishes (7 kg) were ground and twice extracted with ethanol (1.5 L kg^{-1}) at ~20 °C. The ethanolic extract was concentrated *in vacuo*. The residue was dissolved in water (1 L) and passed through a column (7×26 cm) with Polychrome 1. The column was washed with water until Cl^- ions disappeared from the eluate and then with ethanol. The ethanolic eluate was concentrated. The resulting total fraction of steroid compounds (29.7 g) was successively chromatographed on columns with silica gel (6.5×20 cm) using a chloroform—ethanol system (stepwise gradient, 4 : 1 → 1 : 6) and with Florisil (4×17 cm) using a chloroform—ethanol system (stepwise gradient, 4 : 1 → 1 : 2). The fraction containing asterosaponins (TLC data) was separated by HPLC on a Diasfer-110-C18 column (2.5 mL min^{-1}) in the EtOH—H_2O—1 *M* NH_4OAc system (55 : 44 : 1), resulting in the fraction rich in compound **1**. This fraction was additionally purified by HPLC on a Discovery C18 column (2.5 mL min^{-1}) in the MeOH—H_2O—1 *M* NH_4OAc system (72 : 27 : 1). Compound **1** was obtained in a yield of 1.1 mg.

Archasteroside C ((20*S*)-6-*O*-{β-D-fucopyranosyl-(1→2)-β-D-fucopyranosyl-(1→4)-[β-D-quinovopyranosyl-(1→2)]-β-D-quinovopyranosyl-(1→3)-β-D-quinovopyranosyl}-3β,6α,20-trihydroxycholest-9(11)-en-23-one 3-sulfate) (1). Amorphous compound, $[\alpha]_D$ −10.0 (*c* 0.1, MeOH). The ^1H NMR and ^{13}C NMR spectra of the carbohydrate moiety are given in Table 1. ^1H NMR of the aglycone (700 MHz, methanol-d_4), δ: 1.72 and 1.42 (both m, 1 H each, H(1), H(1′)); 2.25 and 1.62 (both m, 1 H each, H(2), H(2′)); 4.17 (m, 1 H, H(3)); 2.59 (br.d, 1 H, H(4), *J* = 11.9 Hz); 1.32 (m, 1 H, H(4′)); 1.25 (m, 1 H, H(5)); 3.57 (m, 1 H, H(6)); 2.38 and 0.96 (both m, 1 H each, H(7), H(7′)); 2.07 (m, 1 H, H(8)); 5.33 (m, 1 H, H(11)); 2.24 and 2.04 (both m, 1 H each, H(12), H(12′)); 1.27 (m, 1 H, H(14)); 1.77 and 1.22 (both m, 1 H each, H(15), H(15′)); 1.88 and 1.69 (both m, 1 H each, H(16), H(16′)); 1.64 (m, 1 H, H(17)); 0.77 (s, 3 H, C(18)Me); 0.98 (s, 3 H, C(19)Me); 1.33 (s, 3 H, C(21)Me); 2.61 (d, 1 H, H(22), *J* = 15.2 Hz); 2.51 (d, 1 H, H(22′), *J* = 15.3 Hz); 2.37 (dd, 2 H, H(24), H(24′), *J* = 3.6 Hz, *J* = 7.1 Hz); 2.08 (m, 1 H, H(25)); 0.90 (d, 6 H, C(26)Me, C(27)Me, *J* = 7.0 Hz). ^{13}C NMR of the aglycone (176.0 MHz, methanol-d_4), δ: 36.8 (C(1)), 29.6 (C(2)), 79.7 (C(3)), 31.0 (C(4)), 50.1 (C(5)), 81.2 (C(6)), 41.8 (C(7)), 36.4 (C(8)), 146.4 (C(9)), 39.2 (C(10)), 117.8 (C(11)), 43.4 (C(12)), 42.5 (C(13)), 55.1 (C(14)), 25.9 (C(15)), 23.7 (C(16)), 60.4 (C(17)), 13.6 (C(18)), 19.7 (C(19)), 75.0 (C(20)), 26.6 (C(21)), 55.3 (C(22)), 213.8 (C(23)), 54.8 (C(24)), 25.4 (C(25)), 22.9 (C(26)), 22.8 (C(27)). ESI MS (positive mode). Found: *m/z* 1287 $[M + Na]^+$. ESI MS/MS (positive mode), 1287. Found: *m/z* 1167 $[(M + Na) - NaHSO_4]^+$, 1067 $[(M + Na) - NaHSO_4 - 100]^+$, 921 $[(M + Na) - NaHSO_4 - 100 - 146]^+$, 775 $[(M + Na) - NaHSO_4 - 100 - 2\times146]^+$, 607 $[4\times146 + Na]^+$, 461 $[3\times146 + Na]^+$, 315 $[2\times146 + Na]^+$. ESI MS (negative mode). Found: *m/z* 1241 $[M - Na]^-$. ESI MS/MS (negative mode), 1241. Found: *m/z* 1141 $[(M - Na) - 100]^-$, 995 $[(M - Na) - 100 - 146]^-$, 849 $[(M - Na) - 100 - 2\times146]^-$, 703 $[(M - Na) - 100 - 3\times146]^-$, 557 $[(M - Na) - 100 - 4\times146]^-$, 411 $[(M - Na) - 100 - 5\times146]^-$, 393 $[(M - Na) - 100 - 4\times146 - 164]^-$, 97 $[HSO_4]^-$. High-resolution ESI MS (negative mode). Found: *m/z* 1241.5648 $[M - Na]^-$. $C_{57}H_{93}O_{27}S$. Calculated: 1241.5630.

Acid hydrolysis and the absolute configuration determination of monosaccharides in glycoside 1. The glycoside (0.6 mg) was dissolved in 2 *M* TFA (1 mL) and heated in a stoppered vessel at 100 °C for 2 h. The aglycone was extracted with chloroform (3×0.3 mL), and the aqueous solution was concentrated *in vacuo*. One drop of TFA and (−)-octan-2-ol (Aldrich) (0.5 mL) were added to the mixture of monosaccharides. The solution was heated in a stoppered vessel at 130 °C for 6 h. Then the solution was concentrated *in vacuo* and treated with a 1 : 1 Ac_2O—Py mixture (0.4 mL) at ~20 °C for 16 h. The resulting acetates of (−)-2-octyl glycosides of monosaccharides were analyzed by GLC using the corresponding derivatives of standard monosaccharides, *viz.*, D- and L-quinovose, D- and L-fucose. The peaks corresponding to D-quinovose derivatives (t_R: 24.03, 24.15, 24.52, and 24.83 min) and D-fucose derivatives (t_R: 24.03, 24.27, 24.58, and 25.00 min) were identified. The t_R values of the peaks belonging to the standard samples of the L series are as follows: L-quinovose, t_R = 23.89, 24.42, and 25.00 min; L-fucose, t_R = 24.30, 24.58, and 24.83 min.

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