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Highly hydroxylated steroids of the starfish *Archaster typicus* from the Vietnamese waters

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ABSTRACT

Five new steroidal compounds, including an unusual glucoside, along with several known steroids were isolated from the starfish *Archaster typicus* collected in shallow waters of Quang Ninh province (Vietnam). Three new compounds are 27-nor-cholestane derivatives and the other two are 24,26-dihydroxycholestane derivatives. A biogenesis pathway for the unusual side chain of 27-nor-cholestane derivatives is proposed. Isolated compounds presented moderate toxic effects in the sperm- and 8-blastomere tests on embryonal development of the sea urchin *Strongylocentrotus intermedius*.

1. Introduction

Some echinoderms, especially sea cucumbers and starfish, are characterized by steroids with unique structural features that are products of unusual biosyntheses. For example, radiolabelling experiments have indicated an unusual steroid biosynthetic pathway in the sea cucumber *Eupentacta fraudatrix*, involving transformation of squalene to parkeol instead of lanosterol followed by subsequent demethylation from C-4 [1]. As result unusual sterols such as 4α,14α-dimethylcholest-9(11)-en-3β-ol and 14α-methylcholest-9(11)-en-3β-ol are formed [2]. Unusual biosynthetic transformations were also detected in the studies of polar steroids from starfish. In fact, starfish contain a great number of polar steroids, including polyhydroxylated derivatives, many of which have no counterpart within other animals [3–5]. Earlier, studies on the starfish *Archaster typicus* collected off Nouméa, New Caledonia by Minale's group have yielded nine new polar steroids [6,7], four of which had a 27-nor-cholestane skeleton. Detection of polyhydroxysteroids with the 27-nor-cholestane side chain was very noteworthy because similar natural products have not been found in the animal kingdom except a few rare findings, including a minor steroid from the sponge *Axinella cannabina* [8] and 27-nor-5β-cholestane-3α,7α,12α,24*S*,25ξ-pentaol, isolated as a major steroid constituent from the urine and feces of patients with liver diseases [9]. We decided to study polar steroids from this species hoping to isolate new compounds of this rare series and extract new structural information concerning unusual steroid biosynthesis in this species. The starfish *A. typicus* was collected near coast-line of Quang Ninh province (Vietnam). Separation of ethanol extract, using different chromatographic techniques, led to five new polar steroids (**1**–**5**), including four new highly hydroxylated steroids (**1**, **3**–**5**) and a glycoside named as typicusoside A (**2**), along with the six previously known compounds (**6**–**11**).

2. Experimental

2.1. General methods

Optical rotations were determined on a PerkinElmer polarimeter Model 343. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DRX 500 spectrometer at 500 and 125.8 MHz, respectively, using tetramethylsilane as an internal standard. HR ESI mass spectra were recorded on an Agilent 6510 Q-TOF LC/MS mass spectrometer; samples were dissolved in MeOH (c 0.001 mg/mL).

HPLC separations were carried out on an Agilent 1100 Series chromatograph equipped with a differential refractometer. Diasfer-110-C18 (10 μm, 250 mm × 15 mm) and Diasfer-110-C18 (5 μm, 250 mm × 4.6 mm) columns were used. Low pressure column liquid chromatography was performed using Polychrome 1 (powdered Teflon, Biolar, Latvia), Si gel KSK (50–160 μm, Sorbpolimer, Krasnodar, Russia), and Florisil (200–300 mesh, Aldrich Chemical Co.). Sorbfil Si gel plates (4.5 × 6.0 cm,

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Table 1
^{1}H NMR data of **1**–**5** (500 MHz, *J* in Hz)[a].

Position	**1**[b]	**2**[c,d]	**3**[b]	**4**[b]	**5**[b]
1	1.72 m	2.17 m	1.59 m	1.72 m	1.72 m
	1.01 m	1.68 m	1.33 m	1.00 m	0.99 m
2	1.81 m	2.32 m	1.75 m	1.72 m	1.72 m
	1.53 m	2.04 m	1.56 m	1.46 m	1.47 m
3	3.42 m	4.78 m	4.02 m	3.47 m	3.47 m
4	4.23 brs	4.92 d (3.9)	3.92 d (3.9)	2.17 m	2.17 m
				1.18 m	1.19 m
5	0.91 m			1.01 m	1.02 m
6	4.07 td (4.2, 10.1)	4.95 d (11.3)	4.32 dd (4.9, 11.5)	3.63 td (4.3, 10.9)	3.63 td (4.3, 10.8)
7	2.15 dd (4.4, 13.1)	4.97 d (11.4)	2.18 dd (11.6, 13.1)	2.29 dd (4.3, 13.2)	2.28 dd (4.3, 13.3)
	1.70 dd (11.0, 13.1)		1.82 dd (4.9, 13.1)	1.67 m	1.67 m
8					
9	1.55 m	3.15 dd (3.0, 13.0)	2.22 dd (3.6, 13.0)	1.57 m	1.57 m
10					
11	1.61 m	2.15 m	1.63 m	1.65 m	1.65 m
	1.34 m	1.62 m	1.22 m	1.40 m	1.41 m
12	1.72 m	2.25 m	1.75 m	1.76 m	1.78 m
	1.54 m	1.80 m	1.55 m	1.54 m	1.53 m
13					
14					
15	4.41 dd (4.2, 9.5)	4.81 m	4.42 dd (4.1, 9.4)	4.92 m	4.95 dd (4.3, 9.1)
16	1.87 m	2.11 m	1.89 dt (9.5, 13.6)	2.07 m	2.10 m
	1.62 m	2.01 m	1.62 m	1.96 m	2.00 m
17	1.97 q (9.6)	2.32 m	1.99 q (9.6)	2.02 m	2.06 m
18	1.10 s	1.44 s	1.10 s	1.15 s	1.18 s
19	1.18 s	2.01 s	1.29 s	1.02 s	1.02 s
20	1.34 m	1.41 m	1.36 m	1.39 m	1.63 m
21	0.84 d (6.5)	0.92 d (6.5)	0.84 d (6.5)	0.85 d (6.5)	0.87 d (6.5)
22	1.54 m	1.64 m,	1.56 m	1.52 m	2.35 m
	0.97 m	1.06 m	0.95 m	0.98 m	1.99 m
23	1.53 m	1.65 m,	1.54 m	1.68 m/1.62 m	6.91 ddd (6.3, 8.7, 15.5)
	1.25 m	1.29 m	1.32 m	1.42 m/1.48 m	
24	3.36 m	3.76 m	3.58 m	4.04 t (6.9)/4.06 m	6.07 d (15.5)
25	1.48 m	1.69 m,	1.69 m		
	1.37 m	1.60 m			
26	0.92 t (7.5)	0.95 t (7.5)	3.57 dd (6.6, 10.6)	4.09 dm (14.5)	2.23 s
			3.43 m	4.12 td (1.3, 14.5)/4.11 td (1.3, 14.5)	
27			0.87 d (7.0)	5.05 m/5.06 m	
				5.11 q (1.5)/5.09 q (1.5)	

[a] Assignments from ^{1}H-^{1}H COSY, HSQC, and HMBC data.
[b] Measured in CD_3OD.
[c] Measured in C_5D_5N.
[d] The ^{1}H NMR data of the α-D-glucopyranosyl unit of compound **2** (500 MHz, *J* in Hz, C_5D_5N): 5.40 d (1H, d, *J* = 3.9, H-1′), 4.10 (1H, dd, *J* = 3.9, 9.7 Hz, H-2′), 4.55 (1H, t, *J* = 9.1, H-3′), 4.19 (1H, t, *J* = 9.0, H-4′), 4.43 (1H, m, H-5′), 4.39 (1H, dd, *J* = 5.3, 11.2, H-6′), 4.51 dd (1H, dd, *J* = 2.3, 11.2, H′-6′).

5–17 μm, Sorbpolimer, Krasnodar, Russia) in the eluent system BuOH/EtOH/H_2O (4:1:2) were used for thin-layer chromatography. GLC analysis was carried out on an Agilent 6580 Series apparatus, carried gas He (1.7 mL/min) at 100 °C (0.5 min) → 250 (5 °C/min, 10 min), capillary column HP-5 MS (30 m × 0.25 mm). Temperatures of injector and detector were 150 and 280 °C, respectively.

2.2. Animal material

Specimens of *A. typicus* (order Valvatida, family Archasteridae) were collected from shallow waters of Quang Ninh province (Vietnam) in August 2008 and were identified by Dr. Do Cong Thung, the Institute of Marine Resources and Environment. A voucher specimen [No. SB 08-2008] is deposited at the Institute of Natural Products Chemistry, VAST, Vietnam.

2.3. Extraction and isolation

Freshly collected animals (7 kg) were minced and extracted three times with EtOH at room temperature. The combined extract was evaporated, and the residue dissolved in H_2O (1 L). The H_2O-soluble fraction was passed through a Polychrome-1 column (7 cm × 26 cm) and eluted with distilled H_2O until a negative chloride ion reaction was obtained. This was followed by elution with EtOH. The combined EtOH eluate was evaporated to give a brownish material (29.7 g). The resulting total fraction of steroidal compounds was chromatographed on a Si gel column (6.5 cm × 20 cm) using $CHCl_3$/EtOH (stepwise gradient, 4:1 → 1:6) and the fractions obtained were purified on a Florisil column (4 cm × 17 cm) using $CHCl_3$/EtOH (stepwise gradient, 4:1 → 1:2). HPLC separation of the collected subfractions on a Diasfer-110-C18 column (10 μm, 250 mm × 15 mm, 2.5 mL/min) with EtOH/H_2O (55:45) as an eluent system yielded pure **6** (1 mg, R_f 0.68), **7** (1.5 mg, R_f 0.71), **8** (1 mg, R_f 0.70), **9** (37 mg, R_f 0.85), **10** (64 mg, R_f 0.75), **11** (20.5 mg, R_f 0.65), and several additional subfractions of polyhydroxysteroidal mixtures. Further HPLC separation of the subfractions on a Diasfer-110-C18 column (5 μm, 250 mm × 4.6 mm, 0.5 mL/min) with MeOH/H_2O/1 M NH_4OAc (80:19:1) as the eluent system gave **1** (3.0 mg, R_f 0.75), with MeOH/H_2O/1 M NH_4OAc (70:29:1) led to **2** (2.0 mg, R_f 0.61) and **5** (2.2 mg, R_f 0.70). MeOH/H_2O/1 M NH_4OAc (65:34:1) as the eluent system yielded **3** (1.4 mg, R_f 0.71) and **4** (2.8 mg, R_f 0.63).

2.4. Spectral data of new compounds

2.4.1. (24R)-27-Nor-5α-cholestane-3 β,4β,6α,8,14,15α,24-heptaol (*1*)

$C_{26}H_{46}O_7$, amorphous powder; $[\alpha]_D^{25}$ + 13.3° (c 0.1, EtOH); ^{1}H and ^{13}C NMR data, see Tables 1 and 2; (−)ESIMS *m*/*z* 469 $[M-H]^-$; (−)ESIMS/MS of the ion $[M-H]^-$ at *m*/*z* 469: 451 $[(M-H)-H_2O]^-$,