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HIGHLY OXYGENATED STEROLS FROM THE STARFISH *ARCHASTER TYPICUS*

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SUMMARY

Three highly oxygenated sterols named ergost-22-ene-3β,4β,5α,6α,8β,14α,15α,25R,26-nonol, 27-norcholestane-3β,4β,5α,6α,7β,8β,14α,15α,24R-nonol, 27-norcholestane-3β,4β,5α,6α,8β,14α, 15α,24R-octol were isolated from the methanolic extract of the starfish Archaster typicus. Their structures were determined by the physicochemical and spectral data in comparison with the reported literature. This is the first report of these compounds from the Vietnamese starfish Archaster typicus.

I - INTRODUCTION

In recent years, research in the marine sterol field has progressed at an impressive pace. Sterols are required compounds in all eukaryotes, but the nature of sterols in a particular organism are varies considerably. The basic role of sterols is the maintenance of optimal fluidity of cell membranes, although these compounds also serve as precursors for the production of diverse steroid classes such as the polyhydroxylated marine sterols.

Echinoderms are rich sources of free sterols as well as their sulfates. In which, sea cucumbers (class: Holothuridea) and starfishes (class: Asteroidea) produce stanols (E) and Δ^7 and Δ^9 sterols [1]. The first nonol, 27-nor-5α-cholestan-3β, 4β, 5, 6α,7β,8,14,15α,24α-nonol as well as its 6-sulphate and related polyols [1-3], from the starfish *Archaster typicus*, are the most highly oxygenated sterols isolated to date. Later, various chromatographies led to the isolation of sterols as cholest-24-ene-3,4,6,8,14,15,26-heptol-15-sulfate, cholest-24-ene-3,6,8,14,15,26-hexol-15-sulfate, cholest-24-ene-3,4,5,6,7,8,14,15,26-nonol-6-sulfate [2], ergost-22-ene-3,4,5,6,8,14, 15,25,26-nonol [3], campest-22-ene-3,4,5,6,8,14,15,25-octol [3], 27-norcholestane-3β,4β,5α,6α,7β,8β,14α,15α, 24*R*-nonol [2], 27-norcholestane-3,4,5,6,7,8,14, 15,24-nonol-6- sulfate [2], 27-norcholestane-3β,4β,5α,6α,8β, 14α,15α,24*R*-octol [2], 3β,4β,5α,6α,8β, 14α,15α-heptahydroxy-27-norcholestan-24-one [2].

As a part of our study to find bioactive compounds from marine organism, hundreds marine organisms were screened for their *in vitro* bioactivities in which starfish *Archaster typicus* exhibited potential cytoxic activity. Therefore, it was chosen to study on chemical constituents. Various chromatographies led to the isolation of the sterols ergost-22-ene-3β,4β,5α,6α,8β,14α, 15α,25*R*,26-nonol, (**1**), 27-norcholestane-3β,4β, 5α,6α,7β,8β,14α,15α, 24*R*-nonol (**2**), 27-norcholestane-3β,4β,5α,6α, 8β,14α,15α,24*R*-octol (**3**) from the methanolic extract of the starfish *Archaster typicus*. Their structures were determined from the physicochemical and spectral data in

comparison with the reported literature.

II - EXPERIMENTAL

1. General experimental procedures

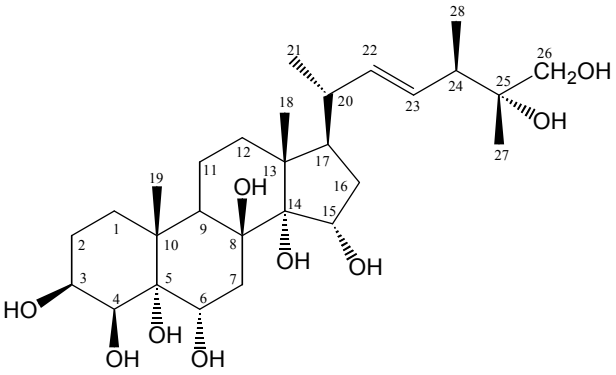
The Electrospray Ionization (ESI) mass spectrum was obtained using AGILENT 1100 LC-MSD Trap spectrometer. The ^{1}H-NMR (500MHz) and ^{13}C-NMR (125MHz) spectra were recorded on Bruker AM500 FT-NMR spectrometer. Chemical shifts are referenced to δ using tetramethylsilan (TMS) as an internal standard. Column chromatography (CC) was performed on silicagel 230 - 400 mesh (0.040 - 0.063 mm, Merck) or YMC RP-18 resins (30 - 50 μm, FuJisilisa Chemical Ltd.). Thin layer chromatography (TLC) was performed on DC-Alufolien 60 F_{254} (Merck 1.05715) or RP_{18} F_{254s} (Merck) plates.

2. Animal material

The samples of starfish *Archaster typicus* were collected from Do Son, Hai Phong in January 2006 and were identified by Dr. Nguyen Cong Thung, The Hai Phong Institute of Sea Research. An authentic sample No 20060102 was deposited at the Institute of Natural Products Chemistry, VAST, Vietnam.

3. Extraction and isolation

The dried and powdered starfish *Archaster typicus* (5.0 kg) was extracted with hot methanol to give the methanol extract (120 g), which was suspended in water and then desalted by DIANION-HP20 column eluting by water. After desalting, the methanol was used as eluted solvent to give 85.0 g methanol extract, which was then resuspended in water and extracted in turn with *n*-hexane, chloroform and ethyl acetate to give the *n*-hexane (20.0 g), chloroform (35 g) and ethyl acetate extract (15.0 g). The chloroform extract (35.0 g) was then chromatographed on a silica gel column eluted with chloroform/methanol as eluent containing increasing concentrations of methanol (from 0.0% to 100%) to give five fractions SB1 (5.0 g), SB2 (8.0 g); SB3 (10.0 g), SB4 (4.5 g) and SB5 (2 g). The SB3 fraction (10.0 g) was then repeatedly chromatographed on silica gel or YMC RP-18 column to give **1**, **2** and **3** as white crystals.



1

2 R = OH; **3** R = H

Figure 1: The structures of **1** - **3**

Ergost-22-ene-3β,4β,5α,6α,8β,14α,15α,25*R*,26-nonol (1)

White crystals, mp. 288 - 290°C, $[\alpha]_D^{25}$ + 33.5° (c, 1.0 MeOH).

ESI-MS m/z: (positive) 551.3 $[M+Na]^+$, (negative) 527.4 $[M-H]^+$ ($C_{28}H_{48}O_9$).

^{1}H-NMR (500 MHz, DMSO) and ^{13}C-NMR (500 MHz, DMSO), see tables 1 and 2.

Table 1: The ^{1}H-NMR data for **1** - **3**

C	**1**		**2**	**3**
	$\delta_H^{a,b}$ (J, Hz)	ROESY	$\delta_H^{a,b}$ (J, Hz)	$\delta_H^{a,b}$ (J, Hz)
1	1.17*; 1.42*		1.16 *; 1.42 *	1.11*; 1.41*
2	1.39*; 1.56 (m)		1.38*; 1.54*	1.35*, 1.53*
3	3.73 (1H, br t, 9,0)	5-OH	3.87 (1H, br t, 9,0)	3.70*
4	4.30 *	14-OH	3.81*	3.78*
6	4.13 (dd, 4.5, 11,5)	H-19	3.80 (1H, d, 7.0)	4.08 (1H, m)
7	1.65*; 1.47 (br t, 12.5)		3.78 (1H, d, 7.0)	1.66*, 1.47*
9	2.12 (1H, dd, 2.5, 13.0)		2.18 (1H, dd, 2.5, 13.0)	2.11 (1H, dd, 2.5, 13.0)
11	1.08*; 1.50*		1.13*; 1.56*	1.03*; 1.46*
12	1.38*; 1.67*		1.15*; 1.38*	1.38*, 1.62*
15	3.80 (1H, dd, 4.0, 9.0)		4.27 (1H, dd, 4.0, 9.0)	4.29 (1H, dd, 4.0, 9.0)
16	1.65*; 1.89 (t, 12.0)		1.57*; 1.68*	1.64*; 1.93 (t, 12.0)
17	1.90 (1H, dd, 9.0, 18.0)		1.82 (1H, dd, 9,0, 18,0)	1.85 (1H, dd, 9.0, 18.0)
18	1.02 (3H, s)	8-OH	1.03 (3H, s)	0.98 (3H, s)
19	1.18 (3H, s)	8-OH	1.18 (3H, s)	1.06 (3H, s)
20	2.12 (m)		1.26 *	1.20 (m)
21	0.84 (3H, d, 6.0 Hz)		0.77 (3H, 6.5 Hz)	0.73 (3H, 6.5 Hz)
22	5.12 (1H, dd, 8.0, 15.5)		0.88 (m); 1.45 *	0.90 (m); 1.42 *
23	5.35 (1H, dd, 8.0, 15.5)		1.15*; 1.38*	1.15*; 1.40*
24	2.11 (1H, m)		3.24 (1H, m)	3.20 (1H, m)
25	-		1.26*; 1.37*	1.22*; 1.36*
26	3.21 (2H, dt, 5.0, 17.0)		0.84 (3H, t, 7.0)	0.82 (3H, t, 7.0)
27	0.95 (3H, s)			
28	0.88 (3H, t, 7.0)			
3-OH				3.91 (1H, d, 4.0)
5-OH	3.05 (s)	H-3	3.43 (s)	
6-OH				3.01 (1H, s)
8-OH	3.62 (s)	H-18, H-19	3.53 (s)	3.58 (1H, s)
14-OH	3.30 (s)	H-4, 14-OH	3.36 (s)	3.23 (1H, s)
24-OH			4.18 (d, 1,0)	4.15 (1H, 5.0)

[a]Measured in DMSO, [b]500 MHz, *Overlap signals.

Table 2: The ^{13}C-NMR data for **1** - **3**

C	**1**		**2**		**3**	
	δ_C [a,c]	HMBC C to H	δ_C [a,c]	HMBC C to H	δ_C [a,c]	HMBC C to H
1	31.98 (t)	H-19	31.88 (t)		31.97 (t)	H-19
2	25.77 (t)	H-3	25.60 (t)		25.77 (t)	
3	70.95 (d)		71.83 (d)	H-4	70.95 (d)	
4	67.51 (d)		69.13 (d)	H-3, 5-OH	66.94 (d)	H-6
5	76.78 (s)	H-3, H-19, 5-OH	76.94 (s)	H-19, 5-OH	76.77 (s)	H-19, 6-OH
6	64.40 (d)	5-OH	66.62 (d)		64.40 (d)	6-OH
7	38.05 (t)	8-OH	70.83 (d)		38.99 (t)	6-OH
8	77.28 (s)	8-OH	78.41 (s)	8-OH	77.26 (s)	8-OH
9	39.49 (d)	8-OH, H-19	39.49 (d)	H-19, 8-OH	39.49 (d)	H-19, 8-OH
10	38.50 (s)	5-OH, H-19	38.35 (s)	H-9, H-19, 5-OH	38.53 (s)	H-19
11	16.90 (t)		16.85 (t)	H-9	16.89 (t)	
12	33.88 (t)	H-18	33.92 (t)		33.95 (t)	H-18
13	46.61 (s)	H-18, 14-OH	47.33 (s)	H-18, 14-OH	46.70 (s)	H-18, 14-OH
14	83.22 (s)	H-18, 14-OH	82.39 (s)	H-18, 14-OH	83.06 (s)	H-18, 8-OH, 14-OH
15	66.94 (d)	14-OH	67.67 (d)	14-OH	67.47 (d)	14-OH
16	39.35 (t)		36.41 (t)		38.05 (t)	
17	49.84 (d)	H-18, H-21	49.65 (d)	H-18, H-21	49.89 (d)	H-18, H-21
18	16.73 (q)		16.23 (q)		16.54 (q)	H-17
19	16.55 (q)		16.53 (q)		16.54 (q)	
20	39.50 (d)	H-21, H-23	34.49 (d)	H-17, H-21	34.46 (d)	H-21
21	20.41 (q)	H-22	18.36 (q)	H-17	18.29 (q)	H-17
22	136.19 (d)	H-21, H-25	31.61 (t)	H-21	31.63 (t)	H-17, H-21
23	127.78 (d)	H-20, H-28	33.16 (t)	24-OH	33.22 (t)	24-OH
24	43.12 (d)	H-28, H-22 H-27	71.56 (d)	H-26, 24-OH	71.55 (d)	24-OH, H-26
25	73.14 (s)	H-27, H-28	29.52 (t)	H-26, 24-OH	29.50 (t)	24-OH
26	67.51 (t)	H-24, H-27	9.86 (q)		9.89 (q)	
27	22.31 (q)	H-24				
28	15.05 (q)	H-24				

[a]Measured in DMSO, [b]125 MHz.

27-Norcholestane-3β,4β,5α,6α,7β,8β,14α, 15α,24*R*-nonol (**2**)

White crystals, mp. 288 - 290°C, $[\alpha]_D^{25}$ +37.5° (c, 1.0 MeOH).

ESI-MS m/z: (positive) 525.2 $[M+Na]^+$, (negative) 501.3 $[M-H]^+$ ($C_{26}H_{46}O_9$).

^{1}H-NMR (500 MHz, DMSO) and ^{13}C-NMR (500 MHz, DMSO), see tables 1 and 2.

27-Norcholestane-3β,4β,5α,6α,8β,14α,15α, 24*R*-octol (**3**)

White crystals, mp. 293 - 294°C, $[\alpha]_D^{25}$ +43.5° (c, 1.0 MeOH).

ESI-MS m/z: (positive) 509.2 $[M+Na]^+$, (negative) 485.4 $[M-H]^+$ ($C_{26}H_{46}O_8$).

^{1}H-NMR (500 MHz, DMSO) and ^{13}C-NMR (500 MHz, DMSO), see tables 1 and 2.

III - RESULTS AND DISCUSSION

Compounds **1** - **3** were obtained as white crystals. The ^{1}H-NMR spectrum of **1** showed singlets of three methyl groups at δ 0.95 (3H), 1.02 (3H) and 1.18 (3H), two doublets at δ 0.84 (3H, J = 6.0 Hz) and 0.88 (3H, J = 7.0 Hz). The double bond with *trans* configuration was assigned at δ 5.12 (1H, dd, 8.0, 15.5) and 5.35 (1H, dd, 8.0, 15.5). Four protons on the oxymethine carbons were at δ 3.73, 4.30, 4.13, 3.80 and two protons of the oxymethylene carbon were at δ 3.21 as a doublet of triplet (2H, J = 5.0, 17.0 Hz). The ^{13}C-NMR and DEPT spectra of **1** exhibited signals of 28 carbon including 2 quaternary, 10 methine, 7 methylene, five methyl carbons and 4 tertiary carbon bearing oxygen atom. The double bond was confirmed at δ 136.19 and 129.78, the oxymethylene carbon was at 67.51, four oxymethine carbons were at δ 64.40, 66.94, 67.51, 70.95, four tertiary carbons bearing oxygen atom were at δ 73.14, 76.78, 77.28 and 83.22, and five methyl carbons were evident at δ 15.05, 16.55, 16.73, 20.41, and 22.31. All the NMR data of **1** (tables 1 and 2) were in good agreement with those of ergost-22-ene-3β,4β,5α,6α,8β,14α,15α,25*R*,26-nonol [3] isolated from the starfish *Archaster typicus*. To further confirm the structure and stereochemistry of this compound, the HMBC and ROESY spectra were measured and detail analysis of C-H long-range correlations in the HMBC spectrum were listed in table 2, and NOEs effects in the ROESY spectrum were shown in table 1. In addition, the ESI-MS spectrum of **1** exhibited the quasi molecular ion peaks at *m/z* (positive) 551.3 $[M+Na]^+$, (negative) 527.4 $[M-H]^+$ corresponding to the molecular formula $C_{28}H_{48}O_9$. Consequently, the structure of **1** was determined to be ergost-22-ene-3β,4β,5α,6α,8β,14α,15α,25*R*,26-nonol, which was first isolated from Vietnamese starfish *Archaster typicus*.

Compounds **2** and **3** were also obtained as white crystals. The ^{1}H- and ^{13}C-NMR spectra of 2 and 3 were similar to those of **1**, except for the absence of the double bond and oxymethylene signals. The similarly of the NMR data of the basis sterol skeleton of **3** with **1** (tables 1 and 2) suggested that they had the same substituted hydroxyl groups, in which no hydroxyl group was at C-7. The additional presence of a hydroxyl group at δ_C 70.83/δ_H 3.78 (1H, d, J = 7.0 Hz) instead of the absence of the methylene group at C-7 in the NMR spectra of **2** comparing with those of **1** and **3** suggesting that the hydroxyl group attached to C-7 of **2**. The side chains of **2** and **3** were determined by comparing the ^{1}H-, ^{13}C-NMR spectra of **2** and **3** with those of 27-norcholestane-3β,4β,5α,6α,7β,8β,14α,15α,24*R*-nonol, 27-norcholestane-3β,4β,5α,6α,8β,14α,15α,24*R*-octol [2], respectively. The above data led to the molecular formula of **2** and **3** as $C_{26}H_{46}O_9$ and $C_{26}H_{46}O_8$, respectively, which were confirmed by the exhibition of the quasi ion peaks at *m/z* 501.3 $[M-H]^+$ (negative) for **2** and 509.2 $[M+Na]^+$ (positive), 485.4 $[M-H]^+$ (negative) for **3** in the ESI-MS spectra. All the NMR assignments of **2** and **3** were also further confirmed by detail analysis of HSQC and HMBC spectra as shown in table 2. Thus, compounds **2** and **3** were identified as 27-norcholestane-3β,4β,5α,6α,7β,8β,14α,15α,24*R*-nonol and 27-norcholestane-3β,4β,5α,6α,8β,14α,15α,24*R*-octol, respectively, which were previously isolated from the starfish *Archaster typicus*. However, these sterols were first isolated from the Vietnamese starfish *Archaster typicus*.

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