VIETNAM JOURNAL OF CHEMISTRY VOL. 53(2e) 33-36 APRIL 2015
DOI: 10.15625/0866-7144.2015-2e-008

CHEMICAL CONSTITUENTS FROM THE STARFISH *PROTOREASTER NODOSUS*

Nguyen Phuong Thao[1], Tran Thi Hong Hanh[1], Vu Anh Tu[1], Pham Thi Mai Huong[1], Nguyen Van Thanh[1], Nguyen Xuan Cuong[1*], Nguyen Hoai Nam[1], Do Cong Thung[2], Phan Van Kiem[1], Young Ho Kim[3], Chau Van Minh[1]

[1]*Institute of Marine Biochemistry, Vietnam Academy of Science and Technology*

[2]*Institute of Marine Environment and Resources, Vietnam Academy of Science and Technology*

[3]*College of Pharmacy, Chungnam National University, Korea*

Received 23 January 2015; Accepted for Publication 18 March 2015

Abstract

Using various chromatographic methods, a glycosylated polyhydroxysterol nodososide (**1**) and three polyhydroxylated sterols (25*S*),5α-cholestane-3β,6α,8β,15α,16β,26-hexol (**2**), (25*S*),5α-cholestane-3β,6α,7α,8β,15α,16β,26-heptol (**3**), (25*S*),5α-cholestane-3β,4β,6α,7α,8β,15α,16β,26-octol (**4**), were isolated from the methanol extract of the starfish *Protoreaster nodosus*. The structural elucidations were done using 1D and 2D-NMR experiments and comparison of the NMR data with reported values.

Keywords. *Protoreaster nodosus*, Oreasteridae, starfish, glycosylated polyhydroxysterol, polyhydroxylated sterol.

1. INTRODUCTION

Starfish are found in all oceans. There are more than 1,500 known species, and many remain undiscovered. Forcipulatida, Paxillosida, Platyasterida, Spinulosida, and Valvatida are the main subclasses of Asteroidea. Starfish have been investigated by organic chemists, biochemists, and pharmacologists as a potential source of bioactive marine natural products. Various secondary metabolites, including steroids, steroidal glycosides, anthraquinones, alkaloids, phospholipids, peptides, and fatty acids, have been reported in starfish [1].

As a part of our ongoing investigations on Vietnamese starfish, we address herein the isolation and structure identification of a glycosylated polyhydroxysterol and three polyhydroxylated sterols from the starfish *Protoreaster nodosus*.

2. EXPERIMENTAL

2.1. General experimental procedures

The ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded on a Bruker AM500 FT-NMR spectrometer, TMS was used as an internal standard. The electrospray ionization mass spectra (ESI-MS) were obtained on an Agilent 1260 series single quadrupole LC/MS system. Medium pressure liquid chromatography (MPLC) was carried out on a Biotage - Isolera One system (SE-751 03 Uppsala, Sweden). Column chromatography (CC) was performed on silica gel (Kieselgel 60, 70-230 mesh and 230-400 mesh, Merck) and YMC RP-18 resins (30-50 μm, Fuji Silysia Chemical Ltd.). Thin layer chromatography (TLC) used pre-coated silica gel 60 F_{254} (1.05554.0001, Merck) and RP-18 F_{254S} plates (1.15685.0001, Merck). Compounds were visualized by spraying with aqueous 10 % H_2SO_4 and heating for 3–5 minutes.

2.2. Marine materials

The sample of the starfish *P. nodosus* was collected at Ha Long, Quang Ninh, Vietnam, in October 2012 and identified by Prof. Do Cong Thung. A voucher specimen (PN-VHS-2012) was deposited at the Institute of Marine Resources and Environment and Institute of Marine Biochemistry, VAST, Vietnam.

2.3. Isolation

Fresh frozen samples of the starfish *P. nodosus* (5.0 kg) were ground and extracted three times with hot MeOH (at 50 °C for 3 h each time). The obtained

solutions were filtered, combined, and concentrated under reduced pressure to yield a dark viscous residue (56.0 g, A). This residue was suspended in water (1.0 L) and partitioned in turn with CH_2Cl_2 (3×1.0 L). The combined dichloromethane-soluble portions were evaporated under reduced pressure to afford CH_2Cl_2 fraction (27.5 g, B) and water layer (C).

Extract B was crudely separated by silica gel MPLC using gradient concentrations of ethyl acetate (EtOAc) in *n*-hexane from 0 to 100 % to yield four fractions (B-1 to B-4). Fraction B-4 (2.31 g) was further separated by silica gel CC using CH_2Cl_2-MeOH-H_2O (5:1:0.1) as eluents, to give three subfractions (B-1.1 to B-1.4). Subfraction B-1.3 (1.35 g) was then subjected to silica gel CC using eluent of CH_2Cl_2-MeOH (3.5:1), and further purified by reversed-phase flash CC (YMC Gel ODS-A, 60 Å, 400/500 mesh) and eluting with MeOH-H_2O (2:1) to afford **2** (2.1 mg). Next, **3** (1.8 mg) was purified via the subfraction of B-1.2 (0.21 g) by silica gel CC and eluting with CH_2Cl_2-MeOH (4.5:1). Similarly, subfraction B-1.1 (4.16 g) was subjected to YMC RP-18 CC, using MeOH-acetone-H_2O (80:10:10) to yield **4** (2.3 mg).

The water layer was desalted by Diaion™ HP-20 CC and eluted first with water and then with MeOH. The desalted water residue (6.2 g, C) was separated by silica gel CC and eluted with CH_2Cl_2-MeOH (from 25:10:1, v/v/v) to yield five fractions (C-1 to C-5). Fraction C-3 (1.2 g) was separated by silica gel CC, using CH_2Cl_2-MeOH (5:1) as the eluent to yield three subfractions (C-3.1 to C-3.3).

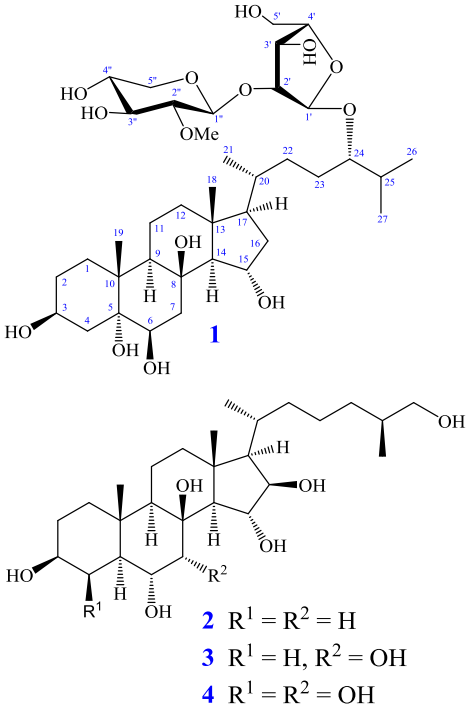

Figure 1: Chemical structures of **1**–**4**

Finally, subfraction C-3.3 (0.15 g) was further separated by Sephadex® LH-20 CC using acetone-MeOH (1:2) as the mobile phase, followed by YMC RP-18 CC using MeOH-H_2O (1:1) to afford **1** (3.5 mg).

Nodososide (**1**): White powder; ^{1}H-NMR (500 MHz, CD_3OD) and ^{13}C-NMR (125 MHz, CD_3OD) see table 1; ESI-MS: *m/z* 769 $[M+Na]^+$ ($C_{38}H_{66}O_{14}$, M = 746).

(25*S*),5α-cholestane-3β,6α,8β,15α,16β,26-hexol (**2**): White powder; ^{1}H-NMR (500 MHz, CD_3OD) and ^{13}C-NMR (125 MHz, CD_3OD) see table 2; ESI-MS: *m/z* 491 $[M+Na]^+$ ($C_{27}H_{48}O_6$, M = 468).

(25*S*),5α-cholestane-3β,6α,7α,8β,15α,16β,26-heptol (**3**): White powder; ^{1}H-NMR (500 MHz, CD_3OD) and ^{13}C-NMR (125 MHz, CD_3OD) see table 2; ESI-MS: *m/z* 507 $[M+Na]^+$ ($C_{27}H_{48}O_7$, M = 484).

(25*S*),5α-cholestane-3β,4β,6α,7α,8β,15α,16β, 26-octol (**4**): White powder; ^{1}H-NMR (500 MHz, CD_3OD) and ^{13}C-NMR (125 MHz, CD_3OD) see table 2; ESI-MS: *m/z* 523 $[M+Na]^+$ ($C_{27}H_{48}O_8$, M = 500).

3. RESULTS AND DISCUSSION

Compound **1** was obtained as a white powder. The NMR features are typical for a glycosylated polyhydroxyterol, one main constituent from starfish [1]. The ^{1}H-NMR spectrum revealed signals of two tertiary methyl [δ_H 1.03 (H-18) and 1.00 (H-19), each 3H, s] and three secondary methyl groups [δ_H 0.94 (H-21), 0.93 (H-26), and 0.92 (H-27), each 3H, d, J = 6.5 Hz] suggesting for a presence of a cholestane-type sterol. In addition, the steroidal skeleton contained four oxymethine groups [δ_C 68.25 (C-3), 77.90 (C-6), 70.11 (C-15), and 84.41 (C-24)/δ_H 4.10 (1H, m, H-3), 3.60 (1H, H-6), 4.29 (1H, dt, J = 3.0, 9.5 Hz, H-15), and 3.33 (1H, m, H-25)] and two oxygenated quaternary carbon [δ_C 76.45 (C-5) and 77.40 (C-8)] as determined in the ^{1}H and ^{13}C-NMR spectra of **1**. The HMBC cross-peaks of H-26 (δ_H 0.93) and H-27 (δ_H 0.92) with C-24 (δ_C 84.41) and C-25 (δ_C 31.48) confirmed position of one oxymethine group at C-24. Detailed analysis of other COSY and HMBC correlations (figure 2) confirmed the structure of the aglycon part of **1**.

In addition, analysis of the NMR spectra of **1** revealed four anomeric carbon signals at δ_C 107.77 (C-1′) and 105.40 (C-1″) which correlated with corresponding anomeric protons at δ_H 5.10 (1H, br s, H-1′) and 4.44 (1H, d, J = 7.5 Hz, H-1″) in the HSQC spectrum, confirming the presence of two sugar moieties. The first anomeric carbon and proton

signals were strongly shifted downfield suggesting for the presence of a furanose moiety. In addition, a methoxy group was identified at δ_C 61.15/δ_H 3.60 (3H, s). The structure of disaccharide chain was identified by a good agreement of the ^{1}H and ^{13}C-NMR data of **1** with reported values [2]. The attached position of the disaccharide chain at C-24 was assigned by an HMBC cross-peak of H-1' (5.10) with C-24 (84.41). Thus, compound **1** was identified as nodososide [2].

Table 1: NMR data of **1** and nodososide [2]

C	[a]**δ$_C$**	**δ$_C$**[b,c]	**δ$_H$**[b,d] mult. (*J* = Hz)	**C**	[a]**δ$_C$**	**δ$_C$**[b,c]	**δ$_H$**[b,d] mult. (*J* = Hz)
1	34.3	34.44	1.40 m/1.70 m	21	18.9	19.02	0.94 d (6.5)
2	31.8	30.95	1.32 m/1.80 m	22	31.9	32.85	1.02 m/1.61 m
3	67.3	68.25	4.10 m	23	27.8	28.45	1.33 m/1.80 m
4	42.4	41.10	1.59/2.15[e]	24	83.5	84.41	3.33 m
5	75.7	76.45	-	25	30.6	31.48	1.88 m
6	77.9	77.90	3.60[e]	26	18.2	18.38	0.93 d (6.5)
7	41.8	40.42	2.12[e]/2.22 dd (3.5, 15.0)	27	18.2	18.18	0.92 d (6.5)
8	76.7	77.40	-	*Ara(f)*			
9	48.7	49.15	1.58[e]	1′	107.6	107.77	5.10 br s
10	39.1	39.18	-	2′	93.1	92.86	4.07 dd (1.0, 3.5)
11	19.4	19.71	1.42 m/1.80 m	3′	77.6	77.77	4.01 dd (3.5, 7.5)
12	42.4	42.92	1.28 m/1.99 m	4′	85.0	83.70	3.97 m
13	44.8	45.53	-	5′	62.4	62.46	3.66 dd (5.0, 12.0)
							3.80 dd (3.5, 12.0)
14	66.3	66.52	1.27[e]	*MeXyl*			
15	69.2	70.11	4.29 dt (3.0, 9.5)	1″	105.2	105.40	4.44 d (7.5)
16	40.9	41.70	1.75 m/1.94 m	2″	84.1	84.84	2.87 dd (7.5, 9.0)
17	55.0	55.89	1.37 m	3″	77.8	77.28	3.32 t (9.0)
18	15.6	15.39	0.97 s	4″	71.0	71.15	3.50 m
19	18.2	18.06	1.33 s	5″	67.1	66.95	3.16 dd (10.5, 12.0)
							3.84 dd (5.5, 12.0)
20	35.4	36.35	1.37 m	OMe	60.7	61.15	3.60 s

[a]δ_C of nodososide in CD_3OD [2], [b]recorded in CD_3OD, [c]125 MHz, [d]500 MHz, [e]overlapped signals.

Table 2: ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) data of **2**–**4** and reported compounds in CD_3OD

C	[a]**δ$_C$**	**2**		[b]**δ$_C$**	**3**		[c]**δ$_C$**	**4**	
		δ$_C$	**δ$_H$** mult. (*J* = Hz)		**δ$_C$**	**δ$_H$** mult. (*J* = Hz)		**δ$_C$**	**δ$_H$** mult. (*J* = Hz)
1	39.6	39.53	1.01/1.73 m	39.6	39.37	1.06/1.72 m	39.7	39.70	0.98/1.71 m
2	31.5	31.44	1.50/1.76 m	31.5	31.41	1.49/1.74 m	26.1	26.16	1.58/1.82 m
3	72.2	72.14	3.50 m	72.3	72.20	3.51 m	73.6	73.68	3.45 m
4	32.4	32.34	1.23 m	32.3	32.24	1.26 m	69.5	69.47	4.18 br s
			2.20 dd (2.5, 10.0)			2.12 dd (2.5, 10.0)			
5	53.7	53.60	0.99 m	44.5	44.49	1.54 m	47.9	47.82	1.47 m
6	67.6	67.60	3.64 m	68.9	68.81	3.77 br d (3.5)	66.1	66.03	4.22 dd (3.0, 11.5)
7	*	50.03	1.36 m	76.5	76.37	3.79 br s	76.6	76.62	3.84 d (3.0)
			2.42 dd (4.0, 14.5)						
8	75.9	75.83	-	77.7	77.70	-	77.6	77.60	-
9	57.4	57.32	0.88 m	51.2	51.18	1.20 m	52.1	52.18	1.14 m
10	37.8	37.82	-	37.8	37.78	-	37.9	37.94	-
11	19.4	19.41	1.48/1.74 m	19.3	19.26	1.55/1.79 m	18.6	18.70	1.43/1.74 m
12	43.2	43.17	1.97/1.22 m	43.2	43.05	1.96/1.21 m	42.9	42.90	1.92/1.21 m
13	45.3	45.26	-	45.5	45.44	-	45.4	45.39	-
14	64.5	64.40	1.18 m	59.6	59.61	1.56 m	59.5	59.53	1.54 m
15	80.7	80.68	4.06 dd (2.5, 6.0)	79.3	79.72	4.15 dd (2.5, 6.0)	79.7	79.70	4.13 dd (2.0, 5.5)
16	83.0	82.94	4.00 dd (2.5, 7.5)	82.7	82.60	4.00 dd (2.5, 7.5)	82.6	82.57	4.00 dd (2.0, 7.0)

C	[a]δ_C	2		[b]δ_C	3		[c]δ_C	4	
		δ_C	δ_H mult. (*J* = Hz)		δ_C	δ_H mult. (*J* = Hz)		δ_C	δ_H mult. (*J* = Hz)
17	60.6	60.59	1.25 m	61.4	61.40	1.28 m	61.3	61.35	1.28 m
18	16.9	16.88	1.13 s	16.6	16.83	1.14 s	16.8	16.84	1.17 s
19	14.2	14.19	1.04 s	13.9	13.83	1.02 s	16.8	16.81	1.11 s
20	30.6	30.60	1.88 m	30.6	30.56	1.48 m	30.5	30.54	1.85 m
21	18.4	18.40	0.95 d (6.5)	18.4	18.29	0.95 d (6.5)	18.3	18.30	0.93 d (6.5)
22	37.2	37.01	1.04 m	37.1	37.00	1.09 m	36.9	37.07	1.03 m
23	24.9	24.85	1.24/1.46 m	24.9	24.85	1.24/1.52 m	24.8	24.85	1.23/1.47 m
24	35.0	34.93	1.06/1.44 m	35.0	34.93	1.06/1.47 m	34.9	34.91	1.05/1.41 m
25	37.1	37.16	1.60 m	37.1	37.08	1.59 m	37.0	36.97	1.56 m
26	68.5	68.42	3.45 dd (5.5, 10.5) 3.34 m	68.5	68.41	3.45 dd (5.5, 10.5) 3.34 m	68.5	68.40	3.42 dd (6.5, 11.5) 3.30 m
27	17.4	17.29	0.92 d (6.5)	17.4	17.92	0.92 d (6.5)	17.3	17.31	0.90 d (6.5)

[a]δ_C of (25*S*),5α-cholestane-3β,6α,8β,15α,16β,26-hexol [3], [b]δ_C of (25*S*),5α-cholestane-3β,6α,7α,8β,15α,16β,26-heptol [3], [c]δ_C of (25*S*),5α-cholestane-3β,4β,6α,7α,8β,15α,16β,26-octol [3], *signal under solvent signal [3].

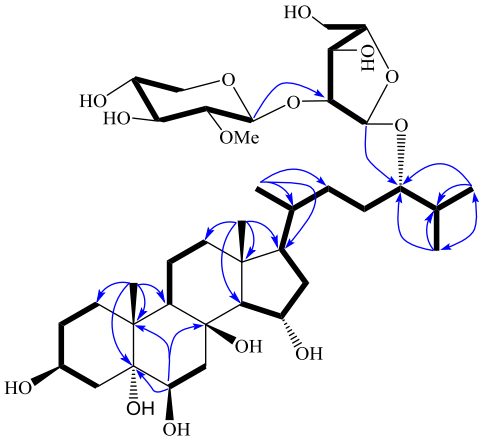

Figure 2: Key COSY (▬) and HMBC (→) correlations of **1**

Compounds **2**–**4** were elucidated as (25*S*),5α-cholestane-3β,6α,8β,15α,16β,26-hexol [3], (25*S*),5α-cholestane-3β,6α,7α,8β,15α,16β,26-heptol [3], and (25*S*),5α-cholestane-3β,4β,6α,7α,8β,15α,16β,26-octol [3], respectively, by detailed analysis of their 1D, 2D NMR data, and comparison of the ^{13}C-NMR data (Table 2) with the values reported in the literatures. Compounds **1**–**4** were previously isolated from *P. nodosus* confirming our taxonomic identification.

Acknowledgement. *This work was financially supported by MOST and Vietnam Academy of Science and Technology, code: VAST.TĐ.ĐAB.03/13–15.*

REFERENCES

1. G. Dong, T. Xu, B. Yang, X. Lin, X. Zhou, X. Yang, Y. Liu. *Chemical constituents and bioactivities of starfish*, Chemistry & Biodiversity, **8(5)**, 740-791 (2011).
2. R. Riccio, L. Minale, C. Pizza, F. Zollo, J. Pusset. *Starfish saponins, part 8. Structure of nodososide, a novel type of steroidal glycoside from the starfish Protoreaster nodosus*, Tetrahedron Letters, **23(28)**, 2899-2902 (1982).
3. R. Riccio, L. Minale, S. Pagonis, C. Pizza, F. Zollo, J. Pusset. *A novel group of highly hydroxylated steroids from the starfish Protoreaster nodosus*, Tetrahedron, **38(24)**, 3615-3622 (1982).

Corresponding author: **Nguyen Xuan Cuong**
Institute of Marine Biochemistry, Vietnam Academy of Science and Technology
18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam
E-mail: cuongnx@imbc.vast.vn.