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SAPONINS ISOLATED FROM THE VIETNAMESE SEA CUCUMBER *STICHOPUS CHLORONOTUS*

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Abstract

Using various chromatographic methods, three triterpene saponins neothyonidioside (**1**), stichoposide D (**2**), and holothurin B (**3**), were isolated from the methanol extract of the sea cucumber *Stichopus chloronotus*. Their structures were elucidated by 1D and 2D-NMR experiments and comparison of their NMR data with reported values. Compound **1** was isolated from *S. chloronotus* for the first time.

Keywords. *Stichopus chloronotus*, Stichopodidae, sea cucumber, triterpene saponin.

1. INTRODUCTION

Sea cucumbers belonging to the family Stichopodidae (phylum Echinodermata, class Holothurioidea, order Aspidochirotida) are usually served as a culinary delicacy and traditional tonic. Among the members of this family, *Stichopus chloronotus* Brandt is a marine invertebrate found in benthic areas and deep seas in the Pacific, Indo-Pacific, and Atlantic oceans [1]. Triterpene saponins are main constituents of this species [2-4].

As a part of our ongoing investigations on Vietnamese echinoderms, we address herein the isolation and structure identification of three triterpene saponins (figure 1) from *S. chloronotus*.

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

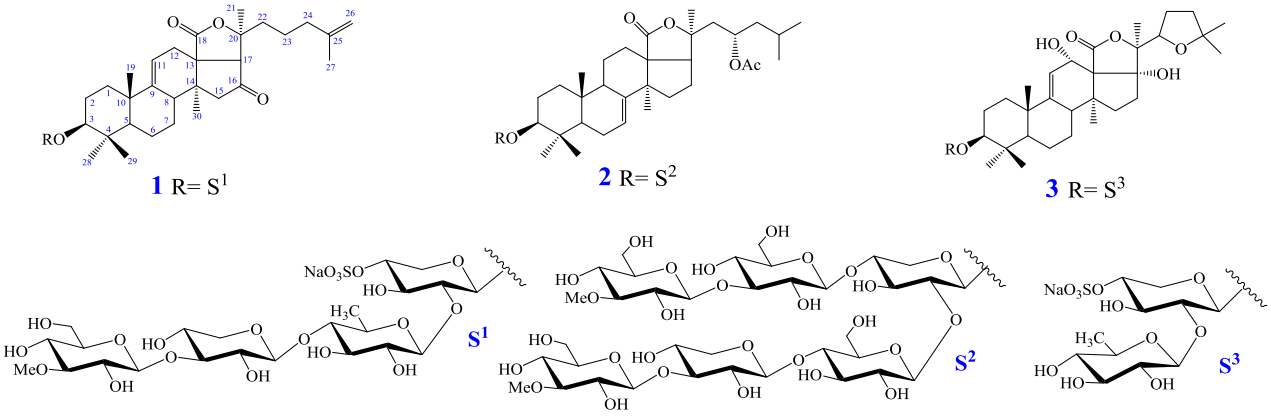

Figure 1: Chemical structures of **1-3**

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 sea cucumber *S. chloronotus* Brandt was collected at Cat Ba, Haiphong, Vietnam, in November 2011, and identified by Professor Do Cong Thung (Institute of Marine Environment and Resources, VAST). A voucher specimen (SC-11-2011-01) was deposited at the Institute of Marine Biochemistry and Institute of Marine Environment and Resources, VAST, Vietnam.

2.3. Isolation

The fresh body walls of *S. chloronotus* (6 kg) were cut into small pieces and immersed in hot methanol (3 times for 6 h each) to afford a MeOH extract (10.45 g, A) after removal of the solvent under reduced pressure. This extract was partitioned between H_2O and *n*-butanol, 3 times (0.7 L each). The *n*-butanol soluble portion (2.42 g, B) was subjected to CC over silica gel (230−400 mesh) eluting with a gradient (dichloromethane−methanol 10:1, 3:1, 1:1, v/v.).

Combination of similar fractions on the basis of TLC analysis afforded 3 fractions (Fr. B1−B3). Fraction B3 (0.45 g) was further separated by reverse-phase silica (75 μm) MPLC eluting with a H_2O–CH_3OH (35-65 %) gradient into two fractions (Fr. B3.1–B3.2). Subfraction B3.2 (0.27 g) was gel-filtered on Sephadex LH-20 (CH_3OH–H_2O, 4.5:1) followed by silica gel CC (CH_2Cl_2–CH_3OH–H_2O, 1.8:1:0.2) to yield **2** (34.11 mg). Subfraction B3.1 (0.18 g) was subjected to silica gel CC with CH_2Cl_2–CH_3OH–H_2O (2.5:1:0.15) and further separated by YMC RP-18 CC using CH_3OH–H_2O (3.5:1, v/v) as the eluent to afford **1** (12.25 mg). Next, fraction B2 (0.62 g) was further subjected to silica gel CC with a CH_2Cl_2–MeOH–H_2O (65:15:2−10:10:2) gradient to obtain 3 subfractions (Fr. B2.1−B2.3). Subfraction B2.1 (0.33 g) was further separated by YMC RP-18 CC using acetone−water (2:1, v/v) as eluent to give **3** (15.82 mg).

Neothyonidioside (**1**): White powder; $[\alpha]_D$: −70 (*c* 0.15, MeOH); ^{1}H-NMR (500 MHz, Pyridine-d_5) and ^{13}C-NMR (125 MHz, Pyridine-d_5) see tables 1 and 2; ESI-MS: *m/z* 1179 $[M+Na]^+$ ($C_{53}H_{81}NaO_{24}S$, M = 1156).

Stichoposide D (**2**): White powder; $[\alpha]_D$: −44 (*c* 0.15, MeOH); ^{1}H-NMR (500 MHz, Pyridine-d_5) and ^{13}C-NMR (125 MHz, Pyridine-d_5) see tables 1 and 2; ESI-MS: *m/z* 1477 $[M+Na]^+$ ($C_{68}H_{110}O_{33}$, M = 1454).

Holothurin B (**3**): White powder; $[\alpha]_D$: −11 (*c* 0.15, MeOH); ^{1}H-NMR (500 MHz, DMSO-d_6) and ^{13}C-NMR (125 MHz, DMSO-d_6) see tables 1 and 2; ESI-MS: *m/z* 905 $[M+Na]^+$ ($C_{41}H_{63}NaO_{17}S$, M = 882).

3. RESULTS AND DISCUSSION

Compound **1** was obtained as a white amorphous powder. The NMR features indicated a holostane-type saponin, one of the main constituents of sea cucumbers [9]. The ^{13}C-NMR spectrum exhibited 53 carbon signals, of which 30 are belonging to a triterpene aglycon and 23 of a tetrasaccharide chain. The aglycon part contained signals of an oxymethine group [δ_C 88.98 (C-3)/δ_H 3.19 (1H, m, H-3)], one oxygenated quaternary carbon [δ_C 83.11 (C-20)], two double bonds [δ_C 151.26 (s, C-9) and 111.46 (d, C-11)/δ_H 5.31 (1H, br s, H-11) and δ_C 145.58 (s, C-25) and 110.77 (t, C-27)/δ_H 4.76 (2H, s, H-27)], two carbonyl [δ_C 213.11 (C-16) and 175.99 (C-18)], and six tertiary methyl groups [δ_C 22.31 (C-19), 26.81 (C-21), 22.31 (C-27), 28.06 (C-28), 16.69 (C-29), and 20.82 (C-30)/δ_H 1.39 (H-19), 1.38 (H-21), 1.67 (H-27), 1.22 (H-28), 1.04 (H-29), and 0.89 (H-30), each 3H, s].

The HMBC cross-peaks of methyl protons H-28 (δ_H 1.22) and H-29 (δ_H 1.04) with C-3 (δ_C 88.98) confirmed the common position of the oxymethine group at C-3. The position of two double bonds at C-9/C-11 and C-25/C-26 was assigned by HMBC correlations of H-19 (δ_H 1.39) with C-9 (δ_C 151.26) and H-27 (δ_H 1.67) with C-25 (δ_C 145.58) and C-26 (δ_C 110.77). The cross-peak of H-12 (δ_H 2.50) with C-18 (δ_C 175.99), H-15 (δ_H 2.23 and 2.37) with C-16 (δ_C 213.11), and those of H-17 (δ_H 2.80) with C-18 (δ_C 175.99) and C-16 (δ_C 213.11) confirmed the positions of the two carbonyl groups at C-16 and C-18.

Table 1: ^{1}H-NMR and ^{13}C-NMR data for the aglycon of **1**–**3** and reported compounds

C	[a]δ_C	**1**		[e]δ_C	**2**		[f]δ_C	**3**	
		δ_C[b,c]	δ_H[b,d] mult. (*J* in Hz)		δ_C[b,c]	δ_H[b,d] mult. (*J* in Hz)		δ_C[c,g]	δ_H[d,g] mult. (*J* in Hz)
1	36.7	36.22	1.40 m/1.83 m	35.9	36.27	1.40 m/1.45 m	36.5	35.63	1.68 m/1.73 m
2	27.3	27.11	1.93 m/2.15 m	27.0	27.13	1.89 m/2.09 m	27.0	26.17	1.65 m/1.85 m
3	89.1	88.98	3.19 m	88.8	88.87	3.21 m	88.6	87.80	3.02 m
4	40.2	39.96	-		39.45	-	40.0	39.20	-
5	53.3	52.89	0.92 m	47.8	47.98	0.95 m	52.8	51.91	0.86 m
6	21.5	21.18	1.50 m/1.70 m	22.5	23.19	1.92 m	21.3	20.34	1.45 m/1.65 m
7	28.8	28.56	1.28 m/1.60 m	119.4	119.95	5.61 br s	28.3	27.29	1.33 m/1.90 m
8	39.1	38.74	3.25 m	146.4	146.57	-	40.9	39.65	2.87 m
9	151.7	151.26	-	47.1	47.36	3.40 m	153.6	152.57	-
10	40.1	39.75	-	35.2	35.53	-	39.8	38.76	-
11	111.4	111.46	5.31 br s	22.8	22.91	1.43 m/1.71 m	115.4	114.46	5.24 d (4.0)
12	32.6	32.09	2.50 m	30.4	30.25	1.85 m/1.93 m	71.6	70.16	4.40 brs
13	56.1	55.72	-	58.2	58.43	-	58.9	57.43	-
14	42.4	42.08	-	51.0	51.21	-	46.0	44.82	-
15	52.3	52.00	2.23 d (15.5) 2.37 d (15.5)	33.9	34.22	1.60 m/1.75 m	36.8	35.82	1.00 m/1.38 m
16	213.9	213.11	-	24.6	24.80	1.89 m/2.05 m	35.6	34.34	1.95 m/2.64 m
17	61.8	61.36	2.80 s	54.3	54.17	2.30 m	89.6	88.39	-
18	176.8	175.99	-	180.0	179.77	-	174.1	173.42	-
19	20.9	22.31	1.39 s	23.9	24.00	1.16 s	20.3	21.81	1.03 s
20	83.3	83.11	-	83.3	83.17	-	86.5	85.95	-
21	**22.5**	**26.81**	1.38 s	26.7	26.97	1.47 s	19.0	18.34	1.36 s
22	38.8	38.50	1.63 m/1.80 m	44.0	44.06	1.85 m/2.15 m	80.7	79.41	4.10 t (7.0)
23	22.7	22.42	1.50 m/1.78 m	68.1	68.28	5.39 m	28.3	27.39	1.68 m/2.00 m
24	38.3	38.02	1.95 m	44.9	45.32	1.25 m/1.55 m	38.5	37.73	1.63 m/1.72 m
25	145.9	145.58	-	24.3	24.54	1.54 m	81.3	80.73	-
26	110.8	110.77	4.76 s	22.3	23.19	0.87 d (6.5)	28.6	28.40	1.21 s
27	**28.4**	**22.31**	1.67 s	23.2	22.16	0.92 d (6.5)	27.4	27.04	1.16 s
28	27.2	28.06	1.22 s	28.4	28.77	1.19 s	28.1	27.39	1.00 s
29	17.0	16.69	1.04 s	16.9	17.36	1.06 s	16.7	16.08	0.80 s
30	23.4	20.82	0.89 s	30.9	30.93	1.03 s	22.5	19.42	1.18 s
OAc				170.8	170.73	-			
OAc				21.4	21.34	2.12 s			

[a]δ_C of neothyonidioside [5], [b]recorded in pyridine-d_5, [c]125 MHz, [d]500 MHz, [e]δ_C of stichoposide E [6], [f]δ_C of holothurin B [7], [g]recorded in DMSO-d_6.

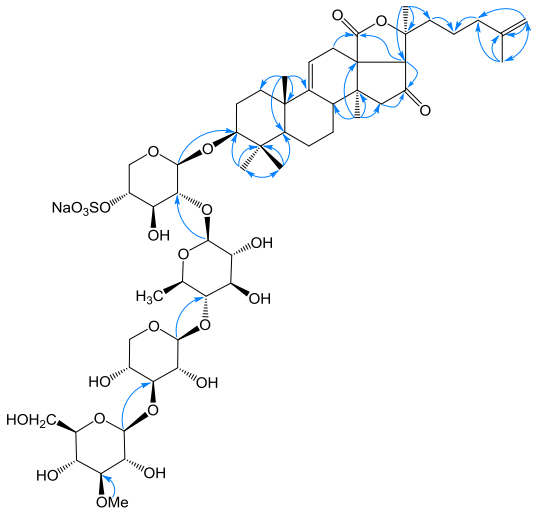

Figure 2: Key HMBC correlations of **1**

In addition, analysis of the NMR spectra of **1** revealed four anomeric carbon signals at δ_C 104.86 (C-1′), 104.00 (C-1″), 104.67 (C-1‴), and 105.59 (C-1⁗) which correlated with corresponding anomeric protons at δ_H 4.70 (1H, d, *J* = 7.0 Hz, H-1′), 5.03 (1H, d, *J* = 7.0 Hz, H-1″), 4.85 (1H, d, *J* = 7.0 Hz, H-1‴), and 5.27 (1H, d, *J* = 7.5 Hz, H-1⁗) in the HSQC spectrum, confirming the presence of four sugar moieties. The large coupling constants of the anomeric protons (*J* = 7.0 or 7.5 Hz) suggested the presence of *β*-glycosidic linkages. The ^{1}H and ^{13}C-NMR data for the sugar part of **1** (Table 2) was similar to those of neothyonidioside [5], which was further confirmed by HMBC experiment. The HMBC cross-peaks of H-1″ (δ_H 5.03) with C-2′ (δ_C

83.11), H-1‴ (δ_H 4.85) with C-4″ (δ_C 86.50), and those of H-1⁗ (δ_H 5.27) with C-3‴ (δ_C 87.09) confirmed the sequence of sugar units in **1**. Finally, the attached position of the tetrasaccharide chain at C-3 of the aglycon was assigned by HMBC correlation of H-1′ (δ_H 4.70) and C-3 (δ_C 88.98).

Table 2: ^{1}H-NMR and ^{13}C-NMR data for the sugar moieties of **1**-**3** and reported compounds

C	[a]δ_C	**1**		[e]δ_C	**2**		[f]δ_C	**3**	
		δ_C[b,c]	δ_H[b,d] mult. (*J* = Hz)		δ_C[b,c]	δ_H[b,d] mult. (*J* = Hz)		δ_C[c,g]	δ_H[d,g] mult. (*J* = Hz)
	Sulfo-Xyl			*Xyl I*			*Sulfo-Xyl*		
1′	105.6	104.86	4.70 d (7.0)	105.3	105.21	4.71 d (7.0)	104.8	103.97	4.32 d (7.5)
2′	83.6	83.11	3.95 [h]	83.8	83.05	4.05 [h]	83.0	81.52	3.35 [h]
3′	76.0	75.02	4.12 [h]	75.6	75.56	4.15 [h]	76.5	74.35	3.50 [h]
4′	73.8	73.45	3.87 [h]	78.05	77.51	4.20 [h]	74.7	74.28	3.95 [h]
5′	64.4	63.70	3.65/4.65 [h]	64.4	63.97	3.60/4.36 [h]	63.8	62.92	3.20/3.95 [h]
	Qui			*Glc I*			*Qui*		
1″	105.5	104.00	5.03 d (7.0)	105.3	105.35	5.17 d (7.0)	105.2	104.16	4.39 d (7.5)
2″	76.6	75.55	3.88 [h]	75.6	76.49	3.99 [h]	75.9	75.34	3.00 [h]
3″	75.1	75.02	3.95 [h]	76.3	76.54	3.76 [h]	77.5	75.79	3.11 [h]
4″	86.2	86.50	3.46 [h]	80.9	80.32	4.30 [h]	76.5	75.19	2.76 [h]
5″	71.1	71.61	3.68 [h]	76.3	78.18	3.85 [h]	73.3	71.80	3.13 [h]
6″	18.2	17.77	1.59 d (6.0)	62.4	62.04	4.17/4.39 [h]	18.4	17.79	1.12 d (6.0)
	Xyl			*Xyl II*					
1‴	105.3	104.67	4.85 d (7.0)	105.3	104.84	5.03 d (8.0)			
2‴	72.1	70.51	4.12 [h]	73.2	73.10	3.94 [h]			
3‴	88.2	87.09	4.26 [h]	87.7	87.99	4.15 [h]			
4‴	69.8	70.12	3.76 [h]	69.1	69.01	3.97 [h]			
5‴	66.8	67.85	4.68/5.02 [h]	66.4	66.45	3.52/4.13 [h]			
	MeGlc			*MeGlc I*					
1⁗	105.2	105.59	5.27 d (7.5)	105.3	105.64	5.20 d (7.0)			
2⁗	75.2	74.69	4.22 [h]	75.0	75.00	3.93 [h]			
3⁗	87.8	87.99	3.66 [h]	87.7	87.82	3.65 [h]			
4⁗	69.3	70.51	4.12 [h]	70.8	70.49	4.06 [h]			
5⁗	78.5	78.29	3.90 [h]	78.05	78.18	3.90 [h]			
6⁗	62.5	62.08	4.23/4.43 [h]	62.4	62.17	4.17/4.39 [h]			
3⁗-OMe	60.9	60.79	3.82 s	60.4	60.75	3.80 s			
				Glc II					
1′′′′′				102.9	102.76	4.94 d (7.0)			
2′′′′′				73.2	73.57	3.94 [h]			
3′′′′′				88.1	87.51	4.00 [h]			
4′′′′′				69.9	69.94	4.00 [h]			
5′′′′′				78.05	78.18	3.85 [h]			
6′′′′′				62.4	61.15	4.34/4.51 [h]			
				MeGlc II					
1′′′′′′				105.3	105.55	5.17 d (7.0)			
2′′′′′′				75.0	75.00	3.93 [h]			
3′′′′′′				87.7	87.90	3.65 [h]			
4′′′′′′				70.8	70.57	4.06 [h]			
5′′′′′′				78.05	78.18	3.90 [h]			
6′′′′′′				62.4	62.17	4.17/4.39 [h]			
3′′′′′′-OMe				60.6	60.72	3.81 s			

[a]δ_C of neothyonidioside [5], [b]recorded in pyridine-d_5, [c]125 MHz, [d]500 MHz, [e]δ_C of stichoposide D [8], [f]δ_C of holothurin B [7], [g]recorded in DMSO-d_6, [h]overlapped signals.

Thus, **1** was identified as neothyonidioside. However, based on 2D-NMR experiments, the reported ^{13}C-NMR data at C-21 and C-27 of neothyonidioside [5] must be reversed as shown in the table 1.

Compounds **2** and **3** were elucidated as stichoposide D [8] and holothurin B [7] by an agreement of their ^{13}C-NMR data with the reported values (tables 1 and 2) and combination with 2D-NMR data. Among isolated compounds, **1** was isolated from *S. chloronotus* for the first time.

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