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CHOLESTANE-TYPE STEROIDS FROM THE OCTOCORAL *VERRUCELLA CORONA*

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ABSTRACT

Using various chromatographic separations, three cholestane-type steroids were isolated from the methanol extract of the octocoral *Verrucella corona*. Their structures were elucidated to be (22*E*)-cholesta-5,22-dien-3*β*-ol-7-one (**1**), *trans*-liagosterol (**2**), and guggulsterol-II (**3**), by detailed analysis of the 1D and 2D-NMR data as well as comparison with those reported. Among them, compound **2** showed significant cytotoxicity against eight human cancer cell lines as HepG2, HL-60, KB, LNCaP, LU-1, MCF7, SK-Mel2, and SW480.

Keywords: *Verrucella corona*, octocoral, steroid, cytotoxic activity.

1. INTRODUCTION

Verrucella corona is an octocoral belonging to Ellisellidae family, Alcyonacea order, Octocorallia subclass, Anthozoa class, and Cnidaria phylum. *Verrucella* is a little investigated genus with some papers reported the isolation of steroid [1, 2], briarane-type diterpenoid [3], and *N*-atom-containing constituents [4]. Recently, we have reported seven new steroids from *V. corona* and their *in vitro* cytotoxic effects [5]. In this paper, we further report the isolation, structural elucidation, and cytotoxic activity of three steroids as (22*E*)-cholesta-5,22-dien-3*β*-ol-7-one (**1**), *trans*-liagosterol (**2**), and guggulsterol-II (**3**) from this species.

2. EXPERIMENTAL

2.1. General methods

Optical rotations were measured on a JASCO P-2000 polarimeter. The ^{1}H NMR (500

MHz) and ^{13}C NMR (125 MHz) spectra were recorded on a Bruker AVANCE III HD 500 spectrometer with TMS used as an internal standard. Medium pressure liquid chromatography (MPLC) was carried out on a Biotage - Isolera One system. Column chromatography (CC) was performed on silica gel (Kieselgel 60, 70–230 mesh and 230–400 mesh, Merck) and YMC*GEL resins (ODS-A, 12 nm S-150 μm, YMC Co., Ltd.). Thin layer chromatography (TLC) used pre-coated silica gel 60 F_{254} (Merck) and RP-18 F_{254S} plates (Merck), and spots were visualized by spraying with aqueous 10% H_2SO_4 and heating for 3–5 min.

2.2. Biological materials

The samples of the octocoral *Verrucella corona* (Grasshoff, 1999) (Ellisellidae) were collected at Vinh Moc, Quang Tri province, Vietnam, in May 2016, and immediately frozen after collection. The scientific name was identified by Prof. Do Cong Thung from Institute of Marine Environment and Resources, Vietnam Academy of Science and Technology (VAST). A voucher specimen (VM-QT-SH2) is deposited at the Institute of Marine Biochemistry and Institute of Marine Environment and Resources, VAST.

2.3. Extraction and isolation

The dried samples (5 kg) of the octocoral *V. corona* were extracted five times (1 h each) with MeOH in ultrasonic condition at room temperature. The resulted solutions were filtered, combined, and concentrated (at below 50 ^oC) by rotary vapors to obtain the MeOH residue (M, 300 g). The MeOH residue was suspended in water and partitioned in turn with hexane and EtOAc to give the extracts of hexane (H, 80 g), EtOAc (E, 20 g), and aqueous layer. The H and E extracts were combined and separated by normal phase MPLC with the mobile phase of hexane/acetone (gradient 100:1→1:1, v/v) to obtain seven fractions, H1–H7.

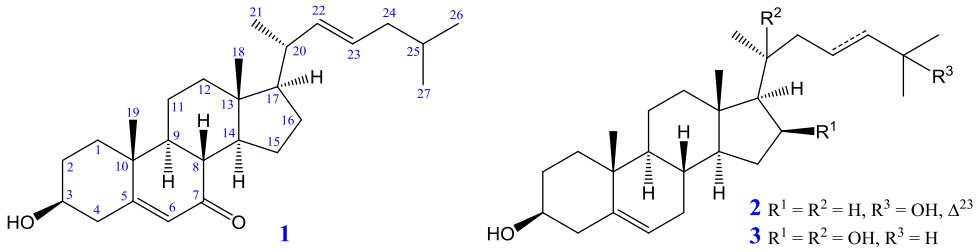

Figure 1. Chemical structures of compounds **1**–**3**.

Fractions H4 (8 g) and H5 (7 g) were combined and separated into eleven subfractions, H4A-H4K, using RP-18 MPLC with MeOH/H_2O (gradient 2:1→100:1, v/v). Subfractions H4F (2 g) and H4G (1 g) were combined and separated into eight smaller fractions, H4F1-H4F8, using silica gel CC with CH_2Cl_2/EtOAc (10:1, v/v). Fraction H4F6 (400 mg) was separated by YMC CC with MeOH/H_2O (5:1, v/v) to give four subfractions, H4F6A–H4F6D. Compound **3** (1 mg) was isolated from subfraction H4F6B (9 mg) by silica gel CC using eluent of hexane/EtOAc (2.2:1, v/v). Subfraction H4F6D (9 mg) was continuously separated on a silica gel CC using CH_2Cl_2/acetone (30:1, v/v) as eluent to afford compound **2** (2 mg). Subfraction H4H (2.5 g) was

Table 1. The NMR spectroscopic data of compounds **1**–**3**.

		1			**2**			**3**	
C	[a]δ_C	δ_C[b,c]	δ_H[b,d] mult. (*J* = Hz)	[e]δ_C	δ_C[b,c]	δ_H[b,d] mult. (*J* = Hz)	[f]δ_C	δ_C[b,c]	δ_H[b,d] mult. (*J* = Hz)
1	36.3	36.39	1.20 m/1.95 m		37.28	1.07 m/1.85 m	23.8	37.22	1.06 m/1.84 m
2	31.2	31.23	1.61 m/1.93 m		31.69	1.50 m/1.83 m	32.3	31.66	1.49 m/1.83 m
3	70.5	70.55	3.67 m		71.83	3.52 m	72.4	71.77	3.51 m
4	41.8	41.85	2.38 m/2.50 m		42.33	2.25 m/2.28 m	43.0	42.29	2.24 m/2.28 m
5	165.0	165.08	-		140.80	-	142.2	140.97	-
6	126.1	126.14	5.69 br s		121.69	5.35 t (3.0)	122.2	121.34	5.35 t (3.0)
7	202.2	202.19	-		31.91	1.53 m/1.97 m	32.8	31.70	1.52 m/1.96 m
8	45.4	45.42	2.24 dd (11.0, 12.0)		31.94	1.45 m	32.3	30.99	1.60 m
9	50.0	50.00	1.34 m		50.15	0.94 m	51.7	50.17	0.93 m
10	38.3	38.31	-		36.53	-	37.7	36.56	-
11	21.2	21.24	1.53 m/1.57 m		21.09	1.48 m/1.52 m	21.8	20.74	1.53 m
12	38.6	38.62	1.14 m/2.00 m		39.72	1.16 m/1.99 m	40.9	40.39	1.18 m/2.14 m
13	43.0	43.04	-		42.40	-	43.9	42.85	-
14	51.2	50.09	1.50 m		56.75	1.00 m	55.9	54.79	0.85 m
15	26.3	26.36	1.23 m/2.37 m		24.33	1.10 m/1.60 m	38.4	37.47	1.28 m/2.24 m
16	28.8	28.76	1.26 m/1.74 m		28.23	1.28 m/1.86 m	74.4	74.13	4.60 dt (4.5, 7.5)
17	54.6	54.71	1.12 m		55.85	1.10 m	61.0	60.22	1.22 m
18	12.2	12.22	0.69 s		11.93	0.69 s	15.2	14.94	1.16 s
19	17.3	17.34	1.20 s		19.41	1.01 s	19.8	19.41	1.03 s
20	39.9	39.90	2.03 m	36.02	36.11	1.46 m	78.1	77.02	-
21	21.0	21.08	1.01 d (6.5)	18.61	18.69	0.91 d (6.5)	26.5	27.01	1.30 s
22	137.9	137.90	5.25 m	38.72	38.84	1.75 m/2.15 m	45.4	44.36	1.57 m/1.76 m
23	126.4	126.46	5.25 m	125.31	125.53	5.58 m	41.6	22.40	1.28 m/1.35 m
24	41.9	41.97	1.83 m	139.49	139.43	5.58 m	38.1	39.67	1.18 m
25	28.5	28.57	1.56 m	70.74	70.77	-	29.0	27.95	1.55 m
26	22.3	22.29	0.86 d (6.5)	29.90	29.94	1.31 s	22.9	22.60	0.88 d (6.5)
27	22.3	22.31	0.86 d (6.5)	29.90	29.94	1.31 s	23.1	22.71	0.88 d (6.5)

[a]δ_C of (22*E*)-cholesta-5,22-dien-3β-ol-7-one [6], [b]recorded in $CDCl_3$, [c]125 MHz, [d]500 MHz, [e]δ_C for the side chain of 6β,25-dihydroxycholesta-4,23(*E*)-dien-3-one [7], [f]δ_C of guggulsterol-II [8].

separated by silica gel CC with eluent of CH_2Cl_2/EtOAc (7:1, v/v) giving three smaller fractions, H4H1–H4H3. Fraction H4H3 (300 mg) was further separated into four subfractions, H4H3A–H4H3D using silica gel CC with hexane/EtOAc (2:1, v/v) as eluent. Compound **1** (5

mg) was isolated from subfraction H4H3C (70 mg) after subjecting it on YMC CC with eluent of MeOH/H_2O (3:1, v/v).

*(22E)-Cholesta-5,22-dien-3β-ol-7-one (**1**)*: White powder; $[\alpha]_D$ –95 (*c* 0.05, MeOH); ^{1}H-NMR (500 MHz, $CDCl_3$) and ^{13}C-NMR (125 MHz, $CDCl_3$) see Table 1.

*trans-Liagosterol (**2**)*: White powder; $[\alpha]_D$ –35 (*c* 0.05, MeOH); ^{1}H-NMR (500 MHz, $CDCl_3$) and ^{13}C-NMR (125 MHz, $CDCl_3$) see Table 1.

*Guggulsterol-II (**3**)*: White powder; $[\alpha]_D$ –40 (*c* 0.05, MeOH); ^{1}H-NMR (500 MHz, $CDCl_3$) and ^{13}C-NMR (125 MHz, $CDCl_3$) see Table 1.

2.4. Cytotoxic assays

Cytotoxicity of the isolated compounds toward five human cancer cell lines, including LNCaP, HepG2, KB, MCF-7, SK-Mel2, HL-60, LU-1, and SW480, using the sulforhodamine B method developed by Monks et al. [9]. The experimental protocols have been described in our published papers [5, 10, 11].

3. RESULTS AND DISCUSSION

Compound **1** was isolated as a white powder. Its NMR data are indicative for a cholestane-type steroid with presence of 27 carbon atoms including typical signals of one oxymethine [δ_C 70.55 (C-3)/δ_H 3.67 (1H, m, H-3)], one trisubstituted double bond [δ_C 165.08 (C-5) and 126.14 (C-6)/δ_H 5.69 (1H, br s, H-6)], one disubstituted double bond [δ_C 137.90 (CH, C-22) and 126.46 (C-23)/δ_H 5.25 (2H, m, H-22 and H-23)], one ketone [δ_C 202.19 (C-7)], two tertiary methyls [δ_C 12.22 (C-18) and 17.34 (C-19)/δ_H 0.69 (H-18) and 1.20 (H-19), each 3H, s], and three secondary methyls [δ_C 21.08 (C-21), 22.29 (C-26), and 22.31 (C-27)/δ_H 1.01 (3H, d, *J* = 6.5 Hz, H-21) and 0.86 (6H, d, *J* = 6.5 Hz, H-26 and H-27)]. Detailed analysis of HSQC correlations led to assignment of all proton signals with the corresponding carbon signals as shown in Table 1. The ^{1}H- and ^{13}C-NMR data of **1** were similar to those of (22*E*)-cholesta-5,22-dien-3*β*-ol-7-one [6]. In addition, the structure of **1** was further confirmed by HMBC experiment. Proton H-4 (δ_H 2.38 and 2.50) and H-19 (δ_H 1.20) exhibited HMBC cross-peaks with C-5 (δ_C 165.08) indicating position of the trisubstituted double bond at C-5/C-6. Detailed analysis of other HMBC correlations (Figure 2) clearly confirmed the structure of **1** as (22*E*)-cholesta-5,22-dien-3*β*-ol-7-one [6, 12]. This compound was reported from the sponges *Cliona copiosa* [12], *Stelodoryx chlorophylla* [13], red alga *Hypnea flagelliformis* [6], and gorgonian *Echinogorgia sassapo reticulate* [14].

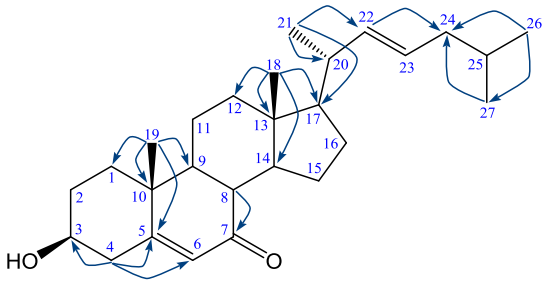

Figure 2. Key HMBC correlations of compound **1**.

The ^{1}H- and ^{13}C-NMR data of **2** are also indicative for a cholestane-type steroid having one oxymethine [δ_C 71.83 (C-3)/δ_H 3.52 (1H, m, H-3)], one quaternary oxygenated carbon [δ_C 70.77 (C-25)], one trisubstituted double bond [δ_C 140.80 (C-5) and 121.69 (C-6)/δ_H 5.35 (1H, t, *J* = 3.0 Hz, H-6)], one disubstituted double bond [δ_C 125.53 (C-23) and 139.43 (C-24)/δ_H 5.58 (2H, m, H-23 and H-24)], four tertiary methyls [δ_C 11.93 (C-18), 19.41 (C-19), 29.94 (C-26 and C-27)/δ_H 0.69 (3H, s, H-18), 1.01 (3H, s, H-19), and 1.31 (6H, s, H-26 and H-27)], and one secondary methyl [δ_C 18.69 (C-21)/δ_H 0.91 (3H, d, *J* = 6.5 Hz, H-21)]. Comparison of the ^{13}C-NMR data of **2** with those of cholesterol [15] and 6*β*,25-dihydroxycholesta-4,23(*E*)-dien-3-one [7], as well as detailed analysis of HSQC and HMBC experiments led to identification of **2** as *trans*-liagosterol [16]. Compound **2** was found from the red alga *Rhodymenia palmata* [16], sponge *Haliclona oculata* [17], and brown alga *Sargassum thunbergii* [18].

Comparison of the ^{13}C-NMR data of **3** with the literature values (Table 1) suggested this compound as guggulsterol-II [8]. However, there are big differences of the data at C-1 and C-23 of **3** with those reported. Further comparison the ^{13}C-NMR chemical shifts at these two carbons of **3** with that at C-1 (δ_C 37.28) of **2** and C-23 (δ_C 21.7) of cholesta-5-en-3*β*,12*β*,16*β*,20*α*-tetraol [19] as well as detailed analysis of 2D-NMR experiments led to confirmation of the ^{13}C-NMR data for C-1 and C-23 of **3** as shown in Table 1. This compound was previously obtained from resin of *Commiphora mukul* [8, 20].

Table 2. Cytotoxic activity of **1**-**3** against eight human cancer cell lines.

Compounds	**IC_{50} values** (μM)							
	LNCaP	HepG2	KB	MCF-7	SK-Mel2	HL-60	LU-1	SW480
1	51.58 ± 4.18	54.55 ± 6.82	39.88 ± 2.35	43.71 ± 5.19	45.18 ± 3.94	31.74 ± 2.48	57.49 ± 5.93	39.93 ± 1.09
2	21.76 ± 2.57	25.50 ± 3.23	17.12 ± 1.69	23.65 ± 2.59	29.20 ± 3.31	23.93 ± 1.60	18.51 ± 2.10	22.29 ± 1.26
3	>100	>100	>100	>100	87.58 ± 3.60	77.10 ± 5.34	87.13 ± 3.19	86.80 ± 4.17
Ellipticine[a]	1.71 ± 0.24	1.67 ± 0.28	1.42 ± 0.12	1.54 ± 0.20	1.50 ± 0.16	1.67 ± 0.08	1.87 ± 0.08	1.99 ± 0.16

[a] Positive control. Results are the means ± standard deviation (S.D.) of triplicate experiments.

Compounds **1**–**3** were evaluated for their cytotoxicity against eight human cancer cell lines, including LNCaP, HepG2, KB, MCF-7, SK-Mel2, HL-60, LU-1, and SW480. As the results (Table 2), *trans*-liagosterol (**2**) exhibited significant cytotoxicity against all eight cancer cell lines with IC_{50} values ranging from 17.12 ± 1.69 to 29.20 ± 3.31 μM, relative to the positive control, ellipticine (IC_{50} values ranging from 1.42 ± 0.12 to 1.99 ± 0.16 μM). Moderate cytotoxic effect against these cancer cell lines (IC_{50} values ranging from 39.88 ± 2.35 to 57.49 ± 5.93 μM) was observed for (22*E*)-cholesta-5,22-dien-3*β*-ol-7-one (**1**), whereas guggulsterol-II (**3**) showed weak activity.

4. CONCLUSION

Three cholestane-type steroids, (22*E*)-cholesta-5,22-dien-3*β*-ol-7-one (**1**), *trans*-liagosterol (**2**), and guggulsterol-II (**3**), were isolated and structurally elucidated from methanol extract of the Vietnamese octocoral *Verrucella corona*. Among them, compound **2** showed significant

cytotoxicity against eight human cancer cell lines as HepG2, HL-60, KB, LNCaP, LU-1, MCF7, SK-Mel2, and SW480. Whereas, compound **1** showed moderate cytotoxic effect and **3** exhibited weak activity on these cancer cell lines.

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