

CHOLESTANE-TYPE STEROIDS FROM THE OCTOCORAL *VERRUCELLA CORONA*

Ninh Thi Ngoc¹, Tran Thi Hong Hanh¹, Nguyen Xuan Cuong^{1,*},
Nguyen Van Thanh¹, Do Thi Thao², Nguyen Hoai Nam¹,
Do Cong Thung³, Phan Van Kiem¹, Chau Van Minh¹

¹*Institute of Marine Biochemistry (IMBC), Vietnam Academy of Science and Technology (VAST), 18 Hoang Quoc Viet, Ha Noi*

²*Institute of Biotechnology (IBT), VAST, 18 Hoang Quoc Viet, Ha Noi*

³*Institute of Marine Environment and Resources (IMER), VAST, 246 Da Nang, Hai Phong*

*Email: cuongnx@imbc.vast.vn

Received: 23 July 2018, Accepted for publication: 14 October 2018

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 ¹H 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 F₂₅₄ (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 °C) 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.

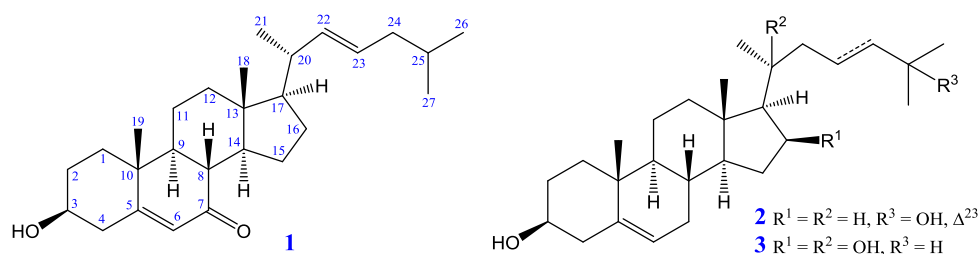


Figure 1. Chemical structures of compounds 1–3.

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.

C	1			^e δ _C	2		^f δ _C	3	
	^a δ _C	δ _C ^{b,c}	δ _H ^{b,d} mult. (<i>J</i> = Hz)		δ _C ^{b,c}	δ _H ^{b,d} mult. (<i>J</i> = Hz)		δ _C ^{b,c}	δ _H ^{b,d} mult. (<i>J</i> = 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)

^aδ_C of (22*E*)-cholesta-5,22-dien-3β-ol-7-one [6], ^brecorded in CDCl₃, ^c125 MHz, ^d500 MHz, ^eδ_C for the side chain of 6β,25-dihydroxycholesta-4,23(*E*)-dien-3-one [7], ^fδ_C of guggulsterol-II [8].

separated by silica gel CC with eluent of CH₂Cl₂/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/H₂O (3:1, v/v).

(22*E*)-Cholesta-5,22-dien-3 β -ol-7-one (**1**): White powder; $[\alpha]_D -95$ (c 0.05, MeOH); ¹H-NMR (500 MHz, CDCl₃) and ¹³C-NMR (125 MHz, CDCl₃) see Table 1.

trans-Liagosterol (**2**): White powder; $[\alpha]_D -35$ (c 0.05, MeOH); ¹H-NMR (500 MHz, CDCl₃) and ¹³C-NMR (125 MHz, CDCl₃) see Table 1.

Guggulsterol-II (**3**): White powder; $[\alpha]_D -40$ (c 0.05, MeOH); ¹H-NMR (500 MHz, CDCl₃) and ¹³C-NMR (125 MHz, CDCl₃) 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 ¹H- and ¹³C-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 reticulata* [14].

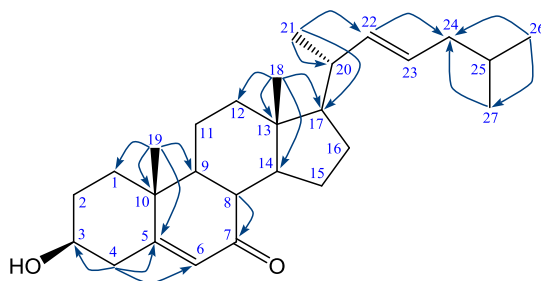


Figure 2. Key HMBC correlations of compound **1**.

The ^1H - 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 $6\beta,25$ -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- $3\beta,12\beta,16\beta,20\alpha$ -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 \pm 4.18	54.55 \pm 6.82	39.88 \pm 2.35	43.71 \pm 5.19	45.18 \pm 3.94	31.74 \pm 2.48	57.49 \pm 5.93	39.93 \pm 1.09
2	21.76 \pm 2.57	25.50 \pm 3.23	17.12 \pm 1.69	23.65 \pm 2.59	29.20 \pm 3.31	23.93 \pm 1.60	18.51 \pm 2.10	22.29 \pm 1.26
3	>100	>100	>100	>100	87.58 \pm 3.60	77.10 \pm 5.34	87.13 \pm 3.19	86.80 \pm 4.17
Ellipticine ^a	1.71 \pm 0.24	1.67 \pm 0.28	1.42 \pm 0.12	1.54 \pm 0.20	1.50 \pm 0.16	1.67 \pm 0.08	1.87 \pm 0.08	1.99 \pm 0.16

^a Positive control. Results are the means \pm 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 IC₅₀ values ranging from 17.12 \pm 1.69 to 29.20 \pm 3.31 μM , relative to the positive control, ellipticine (IC₅₀ values ranging from 1.42 \pm 0.12 to 1.99 \pm 0.16 μM). Moderate cytotoxic effect against these cancer cell lines (IC₅₀ values ranging from 39.88 \pm 2.35 to 57.49 \pm 5.93 μM) was observed for (22*E*)-cholesta-5,22-dien- 3β -ol-7-one (**1**), whereas guggulsterol-II (**3**) showed weak activity.

4. CONCLUSION

Three cholestane-type steroids, (22*E*)-cholesta-5,22-dien- 3β -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.

Acknowledgements. This study was supported by a grant from Vietnam Academy of Science and Technology (code: VAST.TĐ.DLB.02/16-18). The authors are grateful to MSc Dang Vu Luong, Institute of Chemistry, VAST for measurement of the NMR spectra.

REFERENCES

1. Cao F., Shao C. L., Wang Y., Xu K. X., Qi X., Wang C. Y. - Polyhydroxylated sterols from the South China Sea gorgonian *Verrucella umbraculum*, *Helvetica Chimica Acta* **97** (2014) 900-908.
2. Sun L. L., Shao C. L., Mei W. L., Huang H., Wang C. Y., Dai H. F. - Chemical constituents of gorgonian *Verrucella umbraculum* from the South China Sea, *Biochem Syst Ecol* **38** (2010) 1085-1087.
3. Huang R., Wang B., Liu Y. - Briarane-type diterpenoids from the gorgonian coral *Verrucella umbraculum*, *Chemistry of Natural Compounds* **48** (2012) 516-517.
4. Huang R., Peng Y., Zhou X., Fu M., Tian S., Liu Y. - A new pyrimidinedione derivative from the gorgonian coral *Verrucella umbraculum*, *Natural Product Research* **27** (2013) 319-322.
5. Nam N. H., Ngoc N. T., Hanh T. T. H., Cuong N. X., Thanh N. V., Thao D. T., Thung D. C., Kiem P. V., Minh C. V. - Cytotoxic steroids from the Vietnamese gorgonian *Verrucella corona*, *Steroids* **138** (2018) 57-63.
6. Nasir M., Saeidnia S., Mashinchian-Moradi A., Gohari A. R. - Sterols from the red algae, *Gracilaria salicornia* and *Hypnea flagelliformis* from Persian Gulf, *Pharmacognosy Magazine* **7** (2011) 97-100.
7. Sheu J. H., Huang S. Y., Wang G. H., Duh C. Y. - Study on cytotoxic oxygenated desmosterols isolated from the red alga *Galaxaura marginata*, *J Nat Prod* **60** (1997) 900-903.
8. Sultana N., Atta ur R., Jahan S. - Studies on the constituents of *Commiphora mukul*, *Zeitschrift für Naturforschung B* **60** (2005) 1202.
9. Monks A., Scudiero D., Skehan P., Shoemaker R., Paull K., Vistica D., Hose C., Langley J., Cronise P., Vaigro W. A., Gray G. M., Campbell H., Mayo J., Boyd M. - Feasibility of a high-flux anticancer drug screen using a diverse panel of cultured human tumor cell lines, *Journal of the National Cancer Institute* **83** (1991) 757-766.
10. Nam N. H., Kiem P. V., Ban N. K., Thao N. P., Nhiem N. X., Cuong N. X., Tistaert C., Dejaegher B., Heyden Y. V., Quetin-Leclercq J., Thao D. T., Minh C. V. - Chemical constituents of *Mallotus macrostachyus* growing in Vietnam and cytotoxic activity of some cycloartane derivatives, *Phytochem. Lett.* **4** (2011) 348-352.
11. Thao D. T., Phuong D. T., Hanh T. T. H., Thao N. P., Cuong N. X., Nam N. H., Minh C. V. - Two new neoclerodane diterpenoids from *Scutellaria barbata* D. Don growing in Vietnam, *J. Asian Nat. Prod. Res.* **16** (2014) 364-369.

12. Notaro G., Piccialli V., Sica D. - New steroidal hydroxyketones and closely related diols from the marine sponge *Cliona copiosa*, *Journal of Natural Products* **55** (1992) 1588-1594.
13. De Riccardis F., Minale L., Iorizzi M., Debitus C., Lévi C. - Marine sterols. Side-chain-oxygenated sterols, possibly of abiotic origin, from the new Caledonian sponge *Stelodoryx chlorophylla*, *Journal of Natural Products* **56** (1993) 282-287.
14. Xue L., Li P. L., Liang Z., Tang X. L., Li G. Q. - Sesquiterpenoids and steroids from gorgonian *Echinogorgia sassapo reticulate*, *Biochemical Systematics and Ecology* **57** (2014) 48-51.
15. Wilson W. K., Sumpter R. M., Warren J. J., Rogers P. S., Ruan B., Schroeffer G. J. - Analysis of unsaturated C₂₇ sterols by nuclear magnetic resonance spectroscopy, *J. Lipid Res.* **37** (1996) 1529-1555.
16. Morisaki M., Kidooka S., Ikekawa N. - Studies on sterols. XXXIX. Sterol profiles of red algae (2), *Chem Pharm Bull* **24** (12) (1976) 3214-3216.
17. Findlay J. A., Patil A. D. - Novel sterols from the finger sponge *Haliclona oculata*, *Canadian Journal of Chemistry* **63** (1985) 2406-2410.
18. He W. F., Yao L. G., Liu H. L., Guo Y. W. - Thunberol, a new sterol from the Chinese brown alga *Sargassum thunbergii*, *J Asian Nat Prod Res* **16** (2014) 685-689.
19. Hua W. G., Xiao J. L., Shi H. X. - ¹H and ¹³C NMR assignments for two new steroids from the coral *Chromonephthea* sp, *Magnetic Resonance in Chemistry* **47** (2009) 359-361.
20. Patil V. D., Nayak U. R., Dev S. - Chemistry of Ayurvedic crude drugs-I: Guggulu (resin from *Commiphora mukul*)-1: Steroidal constituents, *Tetrahedron* **28** (1972) 2341-2352.