SHORT REPORT

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A New Sterol-Related Metabolite from the Soft Coral *Capnella imbricata*

Zheng-Zhe Tsai [1#], Chih-Kai Hsu [1#], Po-Jen Chen [2], Yu-Li Chen [3,4], Su-Ying Chien [5], Zhi-Hong Wen [6,7], Ping-Jyun Sung [6,8,9,10,11], Kuei-Hung Lai [12,13,14*] and Hsu-Ming Chung [1*]

[1]*Department of Applied Chemistry, National Pingtung University, Pingtung 900391, Taiwan*
[2]*Clinical Immunology Laboratory, Department of Medical Research, E-Da Hospital, I-Shou University, Kaohsiung 824005, Taiwan*
[3]*Research Center for Chinese Herbal Medicine, Graduate Institute of Healthy Industry Technology, College of Human Ecology, Chang Gung University of Science and Technology, Taoyuan 33305, Taiwan*
[4]*Graduate Institute of Biomedical Sciences, Graduate Institute of Natural Products, College of Medicine, Chang Gung University, Taoyuan 33305, Taiwan*
[5]*Instrumentation Center, National Taiwan University, Taipei 106319, Taiwan*
[6]*Department of Marine Biotechnology and Resources, National Sun Yat-sen University, Kaohsiung 804201, Taiwan*
[7]*Institute of BioPharmaceutical Sciences, National Sun Yat-sen University, Kaohsiung 804201, Taiwan*
[8]*National Museum of Marine Biology and Aquarium, Pingtung, 944401, Taiwan*
[9]*Chinese Medicine Research and Development Center, China Medical University Hospital, Taichung, 404394, Taiwan*
[10]*Graduate Institute of Natural Products, Kaohsiung Medical University, Kaohsiung, 807378, Taiwan*
[11]*Fu Jen Catholic University, New Taipei City, 242062, Taiwan*
[12]*PhD Program in Clinical Drug Development of Herbal Medicine, College of Pharmacy, Taipei Medical University, Taipei 11031, Taiwan*
[13]*Graduate Institute of Pharmacognosy, College of Pharmacy, Taipei Medical University, Taipei 11031, Taiwan*
[14]*Traditional Herbal Medicine Research Center, Taipei Medical University Hospital, Taipei 11031, Taiwan*

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Abstract: Exploratory research was carried out on the ethyl acetate extract derived from the soft coral *Capnella imbricata*, sourced from the southeastern waters of Taiwan. This investigation yielded the isolation of three sterol-related compounds (**1**–**3**). Among these compounds, one previously unidentified metabolite, designated as 4β-hydroxy-24-methylene-5-cholesten-7-one (**1**), was discovered, along with two known metabolites, namely, 3β-hydroxy-24-methylene-5-cholesten-7-one (**2**) and gorgostan-5,25-dien-3β-ol (**3**). The structures of these isolated metabolites were determined through comprehensive spectroscopic analyses. Furthermore, the relative stereochemistry of metabolite **3** was established for the first time in this study using single-crystal X-ray diffraction analysis. The potential anti-inflammatory properties of metabolites **1**–**3** were evaluated by investigating their ability to suppress the expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) proteins in lipopolysaccharide (LPS)-induced RAW 264.7 macrophage cells.

Keywords: *Capnella imbricata*; sterol; X-ray; iNOS; COX-2.

*Corresponding author: E-Mail: kueihunglai@tmu.edu.tw(K.-H. Lai); shiuanmin@mail.nptu.edu.tw (H.M Chung)
# These authors contributed equally to this work and should be considered co-first authors.

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Sterols from *Capnella imbricata*

1. Animal Material

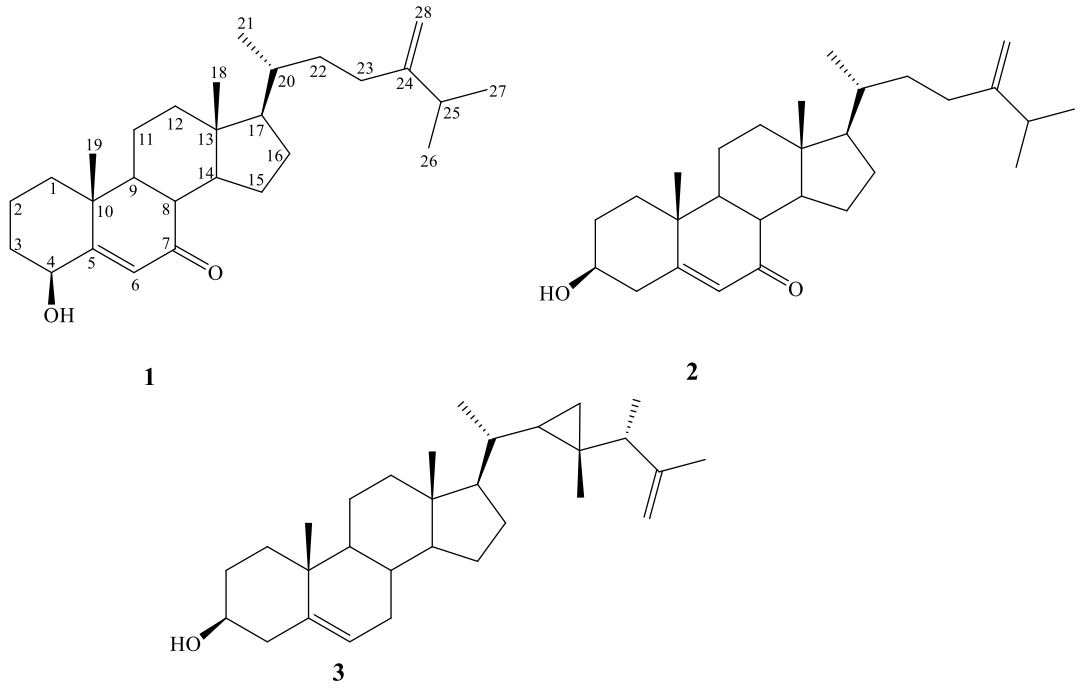
In June 2017, specimens of the soft coral *C. imbricata* were collected manually off the coast of Orchid Island (Lanyu Island), Taiwan, using self-contained underwater breathing apparatus. Following collection, these samples were carefully preserved in a freezer at -20 °C until the extraction process. To ensure proper documentation, a voucher specimen labeled with the voucher number NMMBA-TW-SI-2017-030 was deposited at the National Museum of Marine Biology and Aquarium of Taiwan.

2. Previous Studies

Over the past fifty years, soft corals classified under the genus *Capnella* have been acknowledged as abundant sources of sesquiterpenes, particularly capnellane [1–7], germacrene [8], bicyclogermacrene [8–11], xenicane diterpenes [12], and steroids [12,13]. These secondary metabolites have attracted considerable attention owing to their diverse chemical structures and potential biological activities. *Capnella imbricata* has emerged as a subject of interest due to its varied chemical composition, which yields bioactive compounds. Notably, capnellane-type [1–7] and capgermacrene-type sesquiterpenes [8–10] have been identified, exhibiting a range of biological activities including anti-inflammatory, antibacterial, and cytotoxic properties. These findings highlight the pharmaceutical potential inherent in compounds derived from *C. imbricata.*

3. Present Study

Soft coral specimens, identified as *C. imbricata* and freshly harvested from the shores of Orchid Island, underwent a preservation process involving freezing followed by freeze-drying. The resulting dried material was subsequently ground into a powder and subjected to extraction using ethyl acetate (EtOAc), resulting in the production of an extract. This extract was then subjected to further purification through column chromatography employing silica gel and high-performance liquid chromatography, ultimately leading to the isolation of three sterols: 4β-hydroxy-24-methylene-5-cholesten-7-one (**1**), 3β-hydroxy-24-methylene-5-cholesten-7-one (**2**), and gorgostan-5,25-dien-3β-ol (**3**) (see Figure 1).



1 **2** **3**

Figure 1. The structures of 4β-hydroxy-24-methylene-5-cholesten-7-one (**1**), 3β-hydroxy-24-methylene-5-cholesten-7-one (**2**), and gorgostan-5,25-dien-3β-ol (**3**).

Table 1. ^{1}H and ^{13}C NMR data for sterol **1**

Position	^{1}H (*J* in Hz)[a]	^{13}C[b],	type[c]
1a/b	1.73 m; 2.04 m	37.1,	CH_2
2	1.58 m	32.0,	CH_2
3a/b	1.26 m; 2.03 m	38.5,	CH_2
4	4.36 s	73.3,	CH
5		168.3,	C
6	5.82 s	126.3,	CH
7		202.0,	C
8	2.53 m	39.6,	CH
9	0.91 m	53.6,	CH
10		38.0,	C
11	1.50 m	22.2,	CH_2
12a/b	1.17 m; 2.05m	39.6,	CH_2
13		42.6,	C
14	1.35 m	50.8,	CH
15	1.65 m	24.1,	CH_2
16	1.89 m	28.1,	CH_2
17	1.16 m	55.9,	CH
18	0.75 s	12.0,	CH_3
19	1.38 s	19.5,	CH_3
20	1.43 m	35.8,	CH
21	0.96 d (6.6) [c]	18.7,	CH_3
22a/b	1.16 m; 1.53 m	31.0,	CH_2
23a/b	1.86 m; 2.18 m	34.6,	CH_2
24		156.8,	C
25	2.21 m	33.8,	CH
26	1.02 d (3.0)	21.9,	CH_3
27	1.03 d (3.0)	22.0,	CH_3
28a/b	4.66 s; 4.72 s	106.0,	CH_2

[a]Spectroscopic data were recorded at 600 MHz in $CDCl_3$ at 25 °C.
[b]Spectroscopic data was recorded at 150 MHz in $CDCl_3$ at 25 °C.
[c]Attached protons were deduced by HSQC experiments.

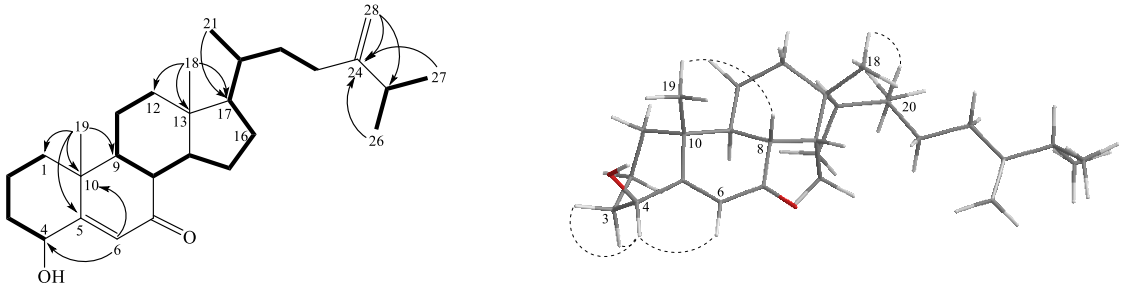


Figure 2. Key COSY (▬), HMBC (⌒), and NOESY (┈) correlations of sterol **1.**

The ^{1}H–^{1}H correlation spectroscopy (COSY) experiment conducted on sterol **1** facilitated the identification of two spin systems: H_2-1/H_2-2/H_2-3/H-4; H-8/H-9/H_2-11/H_2-12; H-8/H-14/H_2-15/H_2-16/H-17/H-20/H_2-22/H_2-23; H-20/H_3-21 and H_3-26/H-25/H_3-27 (refer to Figure 2). These spin systems were elucidated with the aid of a heteronuclear multiple-bond coherence (HMBC) experiment. Analysis via HMBC revealed correlations between protons and carbons, including H-6/C-4, C-10; H_3-18/C-12, C-13, C-14, C-17; H_3-19/C-1, C-5, C-9, C-10; H_3-21/C-17, C-20, C-22; H_3-26/C-24, C-25, C-27 and H_3-27/C-24, C-25, C-26 (see Figure 2), facilitating the establishment of the primary carbon skeleton of sterol **1**.

The relative configuration of sterol **1** was determined through observations in a nuclear Overhauser effect spectroscopy (NOESY) experiment (Figure 2). The NOESY correlations between H_3-18/H-20 and H_3-19/H-8 confirmed that these protons were on the same face, leading to their assignment as β-protons. The β-orientation of OH-4 at C-4 was established because H_3-19 showed no correlation with H-4, and a NOESY correlation was observed between H-4/H_2-3 and H-6. By comparing the ^{1}H NMR data between sterol **1** and the established sterol, 4β-Hydroxy-5-cholesten-7-one [14], we have successfully elucidated the relative configuration of sterol **1**. The stereochemistry analysis indicates that OH-6 is positioned in the β-orientation in sterol **1** (Figure 3).

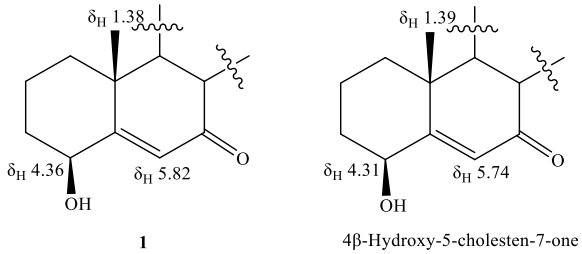


Figure 3. Comparison of the ^{1}H NMR chemical shifts in sterol **1**, with those of the known 4β-Hydroxy-5-cholesten-7-one.

In our current study, we not only discovered a new sterol within the soft coral C. imbricata but also recognized two previously documented sterols, **2**–**3**, observed for the first time in this species. By comparing their spectroscopic characteristics with published data, the known sterols **2**–**3** were identified as 3β-hydroxy-24-methylene-5-cholesten-7-one (**2**) [15] and gorgostan-5,25-dien-3β-ol (**3**) [16].

The structural elucidation of sterol **3** was achieved using single-crystal X-ray diffraction, employing two Bruker D8 Venture diffractometers equipped with dual sources. Sterol **3** was obtained in crystalline form from n-hexane/acetone (11:1) as colorless prisms, adopting a monoclinic crystal system within the $P2_I$ space group. The crystal dimensions were measured as 0.517 x 0.175 x 0.066 mm^3, with lattice parameters a = 9.5322(4) Å, b = 7.4733(3) Å, c = 36.7188(15) Å, and a volume of 2615.65(19) Å^3. The refinement process involved collecting all 22,514 reflections, with the structural model solved using direct methods and refined via a full-matrix least-squares procedure. The refined model yielded final R1 = 0.1095 and wR2 = 0.2894 values for 10,421 observed reflections [I > 2σ(I)], employing 602 variable parameters. The absolute configuration of Sterol 3 was reliably determined, with Flack's parameter calculated as -0.1(10). A depiction of the structure is provided in the Oak Ridge Thermal Ellipsoid Plot (ORTEP) diagram (see Figure 4). Access to full crystallographic data is available in the CIF file CCDC 2313253, containing supplementary information and can be obtained at no cost from the CCDC website via www.ccdc.cam.ac.uk/structures/.

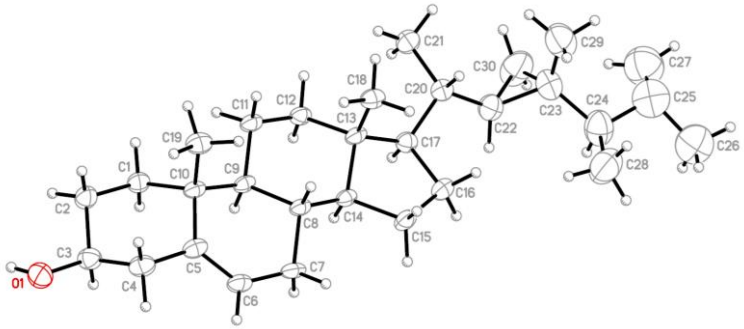


Figure 4. ORTEP revealing the structure of gorgostan-5,25-dien-3β-ol (**3**)

As part of an in vitro assessment of anti-inflammatory activity, Western blot analysis was employed to evaluate the expression of pro-inflammatory iNOS and COX-2 proteins in LPS-stimulated RAW264.7 macrophage cells (refer to Table 2). Sterols **1**–**3** demonstrated a moderate

reduction in iNOS levels (ranging from 64.409% to 77.200%) at a concentration of 10 μM, compared to control cells stimulated solely with LPS, without eliciting any cytotoxic effects (data not shown). Moreover, Sterol **3**, when administered at 10 μM, displayed a weak inhibitory effect (90.365%) on COX-2 protein expression.

Table 2. Effects of sterols **1**–**3** (at 10 μM) on LPS-induced iNOS and COX-2 protein expressions in macrophages

Compounds	iNOS	COX-2	*β*-actin
vehicle	1.588 ± 1.097	0.788 ± 0.586	113.619 ± 13.449
LPS	100.00 ± 10.472	100.00 ± 8.441	105.505 ± 5.533
1	64.409 ± 5.534	100.234 ± 5.171	108.451 ± 10.141
2	77.200 ± 9.145	101.334 ± 5.930	95.592 ± 9.602
3	73.820 ± 6.169	90.365 ± 9.600	116.316 ± 12.860
Dex[a]	63.300 ± 1.794	25.677 ± 2.626	99.454 ± 13.284

[a]Dexamethasone (DEX, 10 μM) was used as a positive control.

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

ORCID

Zheng-Zhe Tsai: 0009-0005-9704-6631
Chih-Kai Hsu: 0009-0003-4853-1695
Po-Jen Chen: 0000-0002-0135-8798
Yu-Li Chen: 0000-0003-2392-1702
Su-Ying Chien: 0009-0008-0931-3149
Zhi-Hong Wen: 0000-0003-4411-044X
Ping-Jyun Sung: 0000-0002-6644-9236
Kuei-Hung Lai: 0000-0001-7220-6356
Hsu-Ming Chung: 0000-0003-1472-9268

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