A NEW CYTOTOXIC DOLABELLANE FROM THE INDONESIAN SOFT CORAL *Anthelia* sp.

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ABSTRACT

*One new dolabellane **(1)** and two known diterpenoids stolonidiol (**2**) and clavinflol B (**3**) have been isolated from the ethyl acetate extract of the Indonesian soft coral Anthelia sp. A new compound **1** exhibited a moderate cytotoxicity against NBT-T2 cells at 10 µg/mL, while known compounds **2** and **3** showed cytotoxicity at 1 and 0.5 µg/mL, respectively. Structure of the new compound **1** was elucidated by interpretation of NMR spectroscopic data (1D and 2D NMR data) and mass spectrometry (ESIMS data) as well as comparison with those of related ones. This finding should be useful for anti cancer drug development of the promising dolabellane-types compound.*

Keywords: *Anthelia sp.; soft coral; dolabellane; NBT-T2; cytotoxicity; marine*

ABSTRAK

*Senyawa baru dolabellane (**1**) dan dua senyawa diterpenoid, yaitu stolonidiol (**2**), dan clavinflol B (**3**) telah diisolasi dari fraksi etil asetat koral lunak Anthelia sp. yang berasal dari perairan Indonesia. Senyawa baru **1** menunjukkan aktivitas sitotoksik moderat terhadap sel tumor NBT-T2 pada konsentrasi 10 µg/mL, sedangkan senyawa **2** dan **3** menunjukkan sitotoksik pada konsentrasi berturut-turut 1 dan 0.5 µg/mL. Struktur senyawa baru **1** dielusidasi dengan interprestasi spektroskopi NMR (1D dan 2D) dan spektrometri massa (ESIMS) serta perbandingan data spektroskopi dengan senyawa-senyawa sejenis. Penemuan ini berguna untuk pengembangan obat anti kanker dari senyawa-senyawa tipe dolabellane yang menjanjikan.*

Kata Kunci: *Anthelia sp.; koral lunak; dolabellane; NBT-T2; sitotoksisitas; laut*

INTRODUCTION

Tropical waters particularly coral reefs are a large treasure trove of bioorganic molecules that contain unique molecular structures and diversed biological functions. The molecules and their bioactivities can develop and even create a new scientific field [1]. Marine organisms especially marine invertebrates such as sponge and soft coral are no value as food, but they are important sources of biologically active substances that have potential to be developed into new drugs and other useful products such as health care or cosmetics [2].

Soft corals are marine invertebrates that do not produce calcium carbonate skeletons. Soft corals have been proved to be a source of structurally diverse and biologically active terpenoids [3]. One of the important compounds derived from soft corals is hippuristanol that can be used to inhibit poliovirus replication [4]. Species of the genus *Anthelia* belonging to the family Xeniidae have been reported to contain at least three types of metabolites including polyhydroxylated steroid [5], xenicane-type [6], and C_{24}-acetoacetylated diterpenoid-type [7]. Generally, the activity of those metabolites was reported to show cytotoxic activity against various cell lines [5-7]. Moreover, waixenicin A, a xenicane-type compound was identified as a specific inhibitor of TRPM7 ion channels for the treatment of gut motor disorders, gastric and breast cancer [8]. Due to a little chemical information about *Anthelia* species and its cytotoxicity, we investigated the chemical constituents of an Indonesian *Anthelia* sp. that have afforded a new bioactive diterpenoid **1** together with known ones **2** and **3**, whose structures and biological activities are described herein. The compounds **1**-**3** can be developed as tools for study of anti cancer drugs from marine soft corals.

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Table 1. ^{1}H-NMR data for compounds **1**-**3** in $CDCl_3$

Position	Compound **1**	Compound **2**	Compound **3**
	δ_H (*J* in Hz)	δ_H (*J* in Hz)	δ_H (*J* in Hz)
1			
2a	1.24, (overlapped)	1.29, brdd, (14.7, 9.2)	1.25, m
2b	1.64, m	1.53, dd (12.0, 9.3)	1.64, m
3a	1.95, m	1.85, dd (8.5, 5.0)	1.97, m
3b	2.01, m	2.17, m	2.09, m
4			
5a	2.10, m	2.25, m	2.29, m
5b	2.41, m	2.48, brdd (16.0,8.1)	2.43, td (13.3, 4.4)
6a	2.07, m	1.65, m	1.78, m
6b	2.41, m	1.75, m	2.06, m
7	5.49, dd (11.5, 3.7)	3.17, t like (7.1)	3.96, d (11.5)
8			
9a	2.14, dd (15.0, 5.1)	2.13, dd (16.0, 2.0)	2.20, m
9b	2.83, dd (15.5, 6.1)	2.48, dd (16.0, 8.1)	
10	2.63, dd (6.1, 1.8)	3.11, dd (8.0, 1.8)	2.96, t (4.6)
11			
12	2.35, dd (9.3, 3.4)	2.28, dd (9.9, 3.8)	2.21, dd (7.4, 2.7)
13a	1.68, m	1.60, m	1.61, m
13b	1.98, m	1.94, m	1.93, m
14a	1.71, m	1.68, m	1.65, m
14b	1.74, m	1.70, m	1.76, m
15	0.85, s	0.85, s	0.85, s
16a	4.64, s	4.71, s	4.82, s
16b	4.72, s	4.80, s	5.00, s
17a	3.72, s	3.63, dd (12.4, 8.5)	3.61, dd (11.2, 7.1)
17b	4.11, s	3.78, dd (12.4, 4.8)	3.83, d (9.9)
18			
19	1.21, s	1.19, s	1.21, s
20	1.29, s	1.29, s	1.27, s

EXPERIMENTAL SECTION

Materials

Merck silica gel 60 (0.063-0.20 mm) was used for column chromatography. Analytical TLC was performed on commercial silica gel 60 F_{254} visualized with vanillin-EtOH-1% H_2SO_4. All solvents used were reagent grade. A sample of the soft coral *Anthelia* sp. was collected by hand using scuba at Krakatau Island, West Java, Indonesia and was stored in EtOH. The identification of the genus was done by Prof. Junichi Tanaka, Department of Chemistry, Biology, and Marine Science, University of the Ryukyus, Japan.

Instrumentation

Optical rotations were obtained with a JASCO P-1010 digital polarimeter. The IR spectra were taken using a DR 8020 Shimadzu spectrophotometer. The ^{1}H and ^{13}C-NMR spectra were recorded on JEOL 500 FTNMR spectrometer. The chemical shifts were expressed in δ (ppm) and coupling constant (*J*) in Hz. ESIMS data were obtained on a PE QSTAR mass spectrometer and infrared (IR) spectra were recorded on a DR 8020 Shimadzu spectrophotometer. HPLC was performed on a Hitachi L-6000 pump equipped with a Shodex RI-101 monitor and a Hitachi L-4000 UV detector using a Cosmosil 5 C_{18}AR-II or a Mightysil RP-18 column.

Procedure

The isolation procedure was performed on the basis of the previous method [9]. The soft coral specimen (wet weight 121 g) stored in EtOH was extracted four times using Me_2CO (4 x 150 mL). The combined extracts were concentrated under reduced pressure, and the residue was partitioned between EtOAc and H_2O to obtain a lipophilic extract (1.57 g). The extract showed cytotoxicity to NBT-T2 cell at 1 μg/mL. The whole extract was separated on a silica gel column by stepwise elution with hexane-EtOAc-MeOH to give 19 fractions. The eighth fraction (50.1 mg) was purified on reversed-phase HPLC to give a new compound **1** (1.0 mg). The ninth fraction (80.1 mg) was repeatedly separated by HPLC (first, RP18: MeOH-H_2O, 5:2; second, Si60, CH_2Cl_2-EtOAc, 7:3) to afford stolonidiol (**2**, 3.5 mg) and clavinflol B (**3**, 1.3 mg).

Table 2. ^{13}C-NMR data for compounds **1**-**3** in $CDCl_3$*

Position	Compound **1**		Compound **2**		Compound **3**	
	δ_C	mult.	δ_C	mult.	δ_C	mult.
1	44.7	C	44.7	C	44.8	C
2	38.7	CH_2	37.9	CH_2	42.6	CH_2
3	27.6	CH_2	29.3	CH_2	25.2	CH_2
4	149.1	C	148.7	C	147.8	C
5	36.7	CH_2	31.4	CH_2	34.9	CH_2
6	26.0	CH_2	24.8	CH_2	29.3	CH_2
7	128.5	CH	57.9	CH	67.2	CH
8	137.1	C	63.6	C	75.9	C
9	27.3	CH_2	26.9	CH_2	33.8	CH_2
10	60.9	CH	56.6	CH	54.5	CH
11	75.5	C	75.9	C	76.9	C
12	49.8	CH	48.4	CH	50.3	CH
13	27.3	CH_2	27.3	CH_2	27.8	CH_2
14	38.0	CH_2	36.9	CH_2	38.9	CH_2
15	24.1	CH_3	23.5	CH_3	24.2	CH_3
16	110.6	CH_2	111.3	CH_2	113.9	CH_2
17	68.3	CH_2	65.3	CH_2	65.9	CH_2
18	74.6	C	74.6	C	75.2	C
19	29.8	CH_3	29.7	CH_3	29.7	CH_3
20	26.4	CH_3	26.1	CH_3	26.1	CH_3

*multiplicity was determined by DEPT and HMQC Spectrum

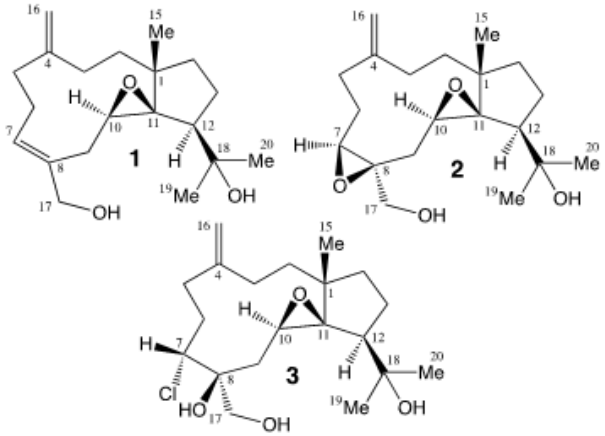

Fig 1. Chemical structure of new compound **(1)**, stolonidiol **(2)**, and clavinflolB **(3)**

*Compound **1***. Colorless oil; $[\alpha]_D^{27}$ -22°(*c* 0.01, $CHCl_3$); IR (KBr) ν_{max} 3419, 2965, 1683, 1645, 1456, 1377, 1168, 1014, 948, 892 cm^{-1}; ^{1}H and ^{13}C-NMR see Tables 1 and 2; ESIMS *m/z* 343.2052 $[M+Na]^+$(calcd for $C_{20}H_{32}O_3Na$ 343.2249).

*Compound **2***. Colorless oil; $[\alpha]_D^{27}$ -37.9°(*c* 0.29, $CHCl_3$) {$[\alpha]_D$ -31°(*c* 1.4, $CHCl_3$) [10]}; ^{1}H and ^{13}C-NMR see Tables 1 and 2.

*Compound **3***. Colorless oil; $[\alpha]_D^{27}$ +2.5°(*c* 0.29, $CHCl_3$) {$[\alpha]_D$ +8.9°(*c* 0.36, CH_2Cl_2) [11]};^{1}H and ^{13}C-NMR see Tables 1 and 2.

Cytotoxicity assay

The NBT-T2 cell (BRC-1370) was purchased from Riken and cultured under a standard protocol using DMEM. NBT-T2 is a cell line derived from chemically induced rat bladder carcinoma cells. The cells were seeded in 1 mL of modified Eagle's media supplemented with 10% heat-inactivated fetal bovine serum, streptomycin, amphotericin B, and glutamic acid. Cells were exposed to graded concentrations of the new and known compounds as well as their fractions at 37 °C for 72 h and observed under a microscope to evaluate the effects at 48 and 72 h.

RESULT AND DISCUSSION

A sample of the soft coral *Anthelia* sp. was thoroughly extracted with acetone. After concentration, the residue was partitioned between EtOAc and water. The EtOAc fraction showed a significant toxicity at 1 μg/mL against rat bladder tumor cells NBT-T2 was chromatographed on silica gel followed normal phase or reversed-phased HPLC to give a new dolabellane **1** together with known ones **2** and **3** (Fig. 1).

The molecular formula of compound **1**, $C_{20}H_{32}O_3$ was concluded by its ESIMS and NMR spectrum. It was indicated by five degrees of unsaturation that can be accounted for one trisubtituted epoxide [δ_H 2.63, dd, *J* = 6.1, 1.8, δ_C 60.9 (CH), 75.5 (C)], one *exo*-methylene (δ_H 4.64, s, 4.72, s, δ_C 110.6), and one trisubtituted olefin [δ_H 5.49, dd, *J* = 3.7, 11.5, δ_C 128.5 (CH), 137.1 (C)]. The two remaining unsaturation degrees were attributed to one 11-membered ring macrocyclic and one isopropyl cyclopentane system as observed for stolonidiol (**2**) and clavinflol B (**3**) [10-11]. The presence of three methyls (δ_H 0.85, s, δ_C 24.0; δ_H

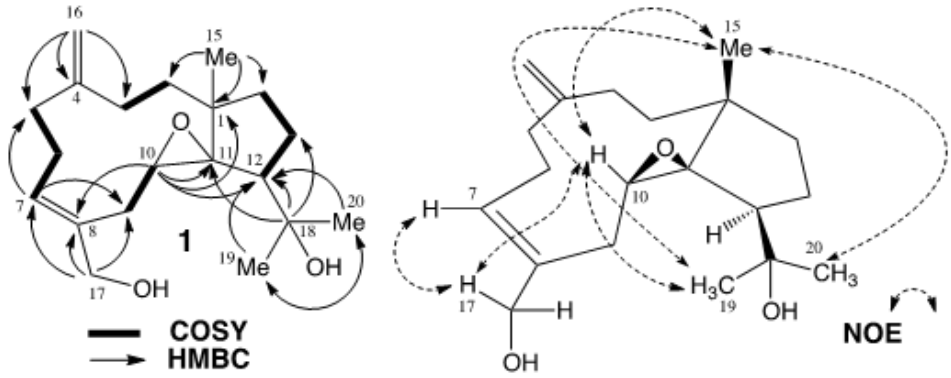

Fig 2. Key COSY, HMBC and NOE of new compound **1**

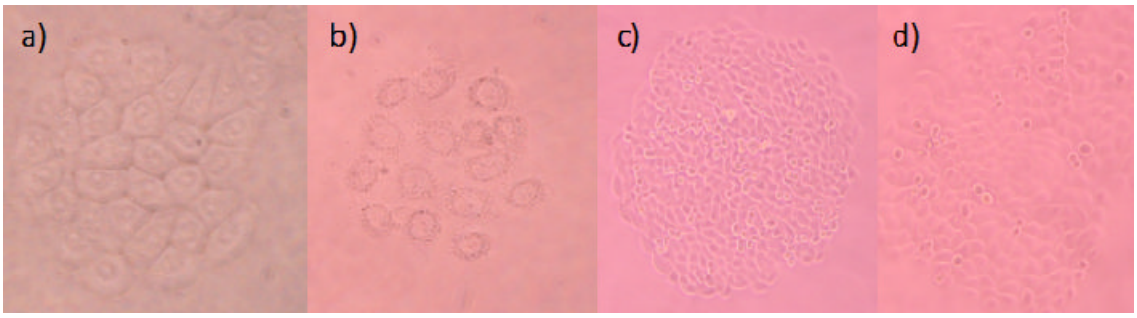

Fig 3. Cytotoxic NBT-T2 Cells assay in a) blank MeOH, 10 µg/mL; b) compound **1**, 10 µg/mL; c) compound **2**, at 1 µg/mL; d) compound **3**, at 0.5 µg/mL

1.21, s, δ_C 29.8; δ_H 1.29, s, δ_C 26.4) and one hydroxymethylene (δ_H 4.11, s, 3.72, s, δ_C 68.1) were confirmed by ^{1}H and ^{13}C-NMR spectrum. The presence of *exo*-methylene and hydroxy groups were also supported by IR absorption at 948.1645 cm^{-1} and 3419 cm^{-1}, respectively. In the ^{1}H-^{1}H COSY, it was possible to identify four structural units, which were assembled with the assistance of an HMBC experiment (Fig. 2). The Key HMBC correlations of H_3-15 to C-1, C-2, C-11, C-14; H_2-16 to C-3, C-4, C-5; H_2-17 to C-7, C-8, C-9; H-10 to C-1, C-8, C-11, C-12 permitted connections of the carbon skeleton. The olefin at C7-C8 was confirmed by HMBC correlations of H-7 and C-5. Furthermore, the isopropyl alcohol group attached at C-12 was confirmed from the simultaneous HMBC correlations of H-20 to C-12, C-19; H-19 to C-12, C-20. On the basis of the above analysis, the planar structure of **1** was established unambiguously. The $\Delta^{7,8}$ was assigned as *E* on the basis of NOE correlation between H-17and H-7.The relative stereochemistry of angular methyl (C-15) was established to be the same as that of **2** by extensive NOE analysis in which H-15 correlated with H-20, H-19, and H-10, suggesting angular methyl (H-15), isopropyl group (H-19, H-20), and H-10 to be the same side of **1** (Fig. 2). As predicted by the similarity of the ^{1}H and ^{13}C-NMR data of **1** to stolonidiol (**2**), the relative stereochemistry of **1** was assigned to be identical with that of stolonidiol, which stereochemistry was established by X-ray diffraction [10]. Therefore, the relative stereochemistry of **1** was 1*S**, 10*R**, 11*R**, and 12*S**. Two known compounds (**2-3**) were verified their structure using 1D NMR as well as their optical rotation that nearly the same (or similar) values as reported in literature [10-11].

All isolated compounds **1**-**3** were evaluated their toxicity against NBT-T2 rat bladder epithelial cells. The new compound **1** showed moderate toxicity at 10 µg/mL, while known compounds **2**-**3** showed strong toxicity at 1, 0.5 µg/mL, respectively. The compounds **1**-**3** were found as bicyclo [9.3.0]-tetradecane skeleton having the *cis*-geometry at the ring junction. Compound **1** is featured by the presence of one *E* double bond instead of one epoxide ring as in **2**. In addition, this is the first report that dolabellane diterpenoids are present in the genus *Anthellia* sp. This finding also implies that the dolabellane-type compound as in **1**-**3** may be useful for anti cancer drug development.

CONCLUSION

One new compound (**1**) along with known ones (**2-3**) has been isolated and characterized as dolabellane diterpenoid. The new compound **1** showed a moderate toxicity against NBT-T2 cell at 10 µg/mL,

while **2**-**3** showed strong toxicity at 1 and 0.5 µg/mL, respectively. The compounds show the unique structures containing bicyclo [9.3.0]-tetradecane skeleton.

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