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# Two New Lobane Diterpenes from a Bornean Soft Coral *Sinularia* sp.

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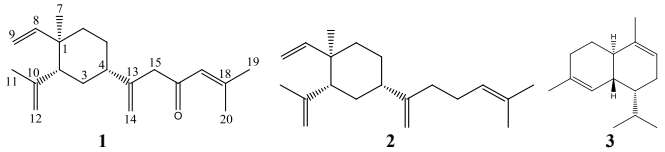
Two new lobane diterpenoids, prenyl-α-elemenone (**1**) and *ent*-prenyl-β-elemene (**2**), along with a known compound, α-murrolene (**3**) were isolated from a population of Bornean soft coral *Sinularia* sp. The structures of these compounds were elucidated on the basis of spectroscopic, including 2D NMR, and HR-MS data. These compounds were tested for their cytotoxicity and antibacterial activities against antibiotic resistant clinical strains.

**Keywords:** *Sinularia* sp., Alcyoniidae, Borneo, Soft coral, Diterpene, Lobane.

Soft corals belonging to the genus *Sinularia* (family Alcyoniidae) are rich sources of sesquiterpenes, cembranes, lobanes, steroids, steroidal glycosides, sphingosine derivatives, glycolipids and spermidine derivatives [1a-1f]. Our previous investigation of Bornean *Sinularia* led to the isolation of various cembranoids [2,3]. Our search for bioactive metabolites from Bornean *Sinularia* sp. from Mantanani Island has led to the isolation of a new lobane diterpenoid, prenyl-α-elemenone (**1**), a new stereoisomer, *ent*-prenyl-β-elemene (**2**), and a known compound, α-murrolene (**3**) (Figure 1). These compounds were tested for their cytotoxicity potentials against B16-F10 and HT-29 cells. In addition, the antibacterial activities of these compounds were tested against antibiotic resistant clinical bacterial strains such as *Staphylococcus aureus* and *Escherichia coli*. This paper reports the isolation, structure elucidation and biological potential of these compounds.

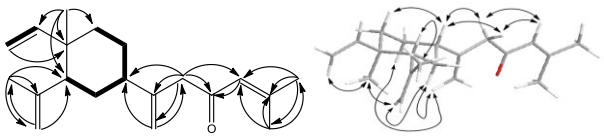


**1** **2** **3**

**Figure 1:** Structures of metabolites **1**-**3**.

**Table 1:** $^{1}$H and $^{13}$C NMR data (600 MHz and 150 MHz, $CDCl_3$) for **1** (δ in ppm, *J* in Hz).

| | Compound 1 | |
|---|---|---|
| Position | $\delta_C$ | $\delta_H$ (mult., *J* in Hz) |
| 1 | 39.8 (s) | |
| 2 | 52.6 (d) | 1.99 dd (12.7, 6.4) |
| 3 | 33.0 (t) | 1.58, 1.51 m |
| 4 | 44.2 (d) | 1.95 tt (11.9, 3.4) |
| 5 | 27.0 (t) | 1.65, 1.40 m |
| 6 | 39.8 (t) | 1.43-1.48 m |
| 7 | 16.6 (q) | 0.99 s |
| 8 | 150.2 (d) | 5.80 dd (17.2, 11.0) |
| 9 | 109.9 (t) | 4.89 d (17.2), 4.88 d (11.0) |
| 10 | 147.6 (s) | |
| 11 | 24.8 (q) | 1.70 s |
| 12 | 112.1 (t) | 4.81, 4.57 s |
| 13 | 148.4 (s) | |
| 14 | 112.1 (t) | 5.00, 4.86 s |
| 15 | 51.2 (t) | 3.15 s |
| 16 | 198.9 (s) | |
| 17 | 123.0 (d) | 6.14 s |
| 18 | 155.9 (s) | |
| 19 | 20.7 (q) | 2.15 s |
| 20 | 27.7 (q) | 1.88 s |



(**a**) (**b**)

**Figure 2:** (a) $^{1}$H-$^{1}$H COSY and key HMBC and (b) Selected NOE correlations of **1**.

Compound **1** was obtained as colorless oil, pseudomolecular ion $[M + H]^+$ at *m/z* 287.2361, corresponded to $C_{20}H_{30}O$, accounting for six degrees of unsaturation. The 1D NMR data of **1** (Table 1) displayed the presence of a monosubstituted alkene at $\delta_C$ 150.2 (C) and 109.9 ($CH_2$); $\delta_H$ 5.80 (1H, dd, *J* = 17.2, 11.0 Hz), 4.89 (1H, d, *J* = 17.2 Hz) and 4.88 (1H, d, *J* = 11.0 Hz), two 1,1-disubstituted olefins at $\delta_C$ 148.4 (C), 147.6 (C) and two overlapped signals at 112.1 ($CH_2$); $\delta_H$ 5.00 (1H, s), 4.86 (1H, s), 4.81 (1H, s) and 4.57 (1H, s), one trisubstituted double bond at $\delta_C$ 155.9 (C) and 123.0 (CH); $\delta_H$ 6.14 (1H, s) and a ketone moiety at 198.9 (C). These signals explained five degrees of unsaturation, implying one ring was present in the structure of **1**. The $^{1}$H-$^{1}$H COSY experiment (Figure 2a) only revealed two separate consecutive spin systems: H-2/$H_2$-3/H-4/$H_2$-5/$H_2$-6 and H-8/$H_2$-9. The key HMBC correlations (Figure 2a) facilitated connection of these partial structures and establishment of a lobane-type skeleton of **1**: $H_3$-7 to C-1, C-2, C-6 and C-8; $H_3$-11 to C-2, C-10 and C-12; $H_2$-14 to C-4, C-13 and C-15; $H_3$-19 to C-17, C-18 and C-20; and $H_3$-20 to C-17, C-18 and C-19. Spectroscopic data of **1** were closely comparable with those of **2** except that the carbonyl group at C-16 in **1** was replaced by methylene in **2**. In this context, $^{1}$H-$^{1}$H-COSY connectivity was observed from C-15 to C-17 in **2**, but not in **1**, indicating the presence of a quaternary carbon between C-15 and C-17 in **1**. Moreover, HMBC cross peaks of $H_2$-15 and H-17 to C-16, deduced that a carbonyl moiety was attached between C-15 and C-17. Based on these findings, the planar structure of **1** was determined and shown in Figure 2a. The relative configurations of three successive chiral centers at C-1, C-2 and C-4 in **1** were determined based on NOE data (Figure 2b) and comparison with other known analogues. The NOE correlations of $H_2$-3α with $H_2$-12 and $H_2$-14; $H_2$-5α with $H_2$-14; and $H_3$-7 with $H_2$-12 indicated the α-orientation of $H_2$-3α, $H_2$-5α and $H_3$-7. However, the correlations of H-2 with $H_3$-11; and H-4 with $H_2$-6β implied that H-2 and H-4 had a β-orientation. Taking into account the known absolute configuration of lobane diterpenes previously isolated from soft coral, it can be proposed

that they have the same absolute configuration [1f,4-6]. Thus, the relative structure of **1** was reported as (1*R*,2*R*,4*S*)-loba-8,10,13(14),17(18)-tetraen-16-one.

Compound **2** was an enantiomer of prenyl-β-elemene, which was isolated from an engineered *Streptomyces* host [7]. Its HR-ESIMS showed a molecular ion peak at *m/z* 311.2147 $[M + K]^+$ (calcd for $C_{20}H_{32}K$, 311.2136), which gave a molecular formula of $C_{20}H_{32}$. The $^1H$ and $^{13}C$ NMR spectra of **2** were similar to those of prenyl-β-elemene, with the exception of its antipodal rotation of $[\alpha]_D^{25}$: -22.5 (*c* 0.40, $CHCl_3$) in comparison with that of prenyl-β-elemene $[\alpha]_D^{24}$: +15.1 (*c* 0.01, $CHCl_3$), suggesting **2** to be the enantiomer of prenyl-β-elemene [7]. Analyses of NOESY data revealed that the relative configurations of three successive chiral centers at C-1, C-2 and C-4 of **2** were similar to those of **1**; NOE correlations of H-2 with H-8 and $H_3$-11; $H_2$-3α with $H_2$-12 and $H_2$-14; $H_2$-5α with $H_3$-7, $H_2$-12 and $H_2$-14; $H_2$-6β with H-8. Based on these findings, $H_2$-5α and $H_3$-7 were deduced to have an α-orientation. Hence, H-2, H-4, $H_2$-5β had a β-orientation [1f,4-6]. Thus, the structure **2** was reported as *ent*-prenyl-β-elemene. The known compound **3** was isolated and identified as α-murrolene (**3**) based on comparison of its spectroscopic data with those reported in the literature [8].

Cytotoxicity assays of compounds **1**-**3** against B16-F10 and HT-29 cells displayed no activity. Antibacterial activity was tested against antibiotic resistant clinical bacterial strains such as *Staphylococcus aureus* and *E. coli*. Prenyl-α-elemenone (**1**) exhibited inhibition against *S. aureus*; its MBC and MIC values were calculated to be 50 μg $mL^{-1}$ and 20 μg $mL^{-1}$, respectively. The MBC/MIC ratio of compound **1** was 2.5, which indicated bactericidal activity. In conclusion, prenyl-α-elemenone (**1**) exhibited potent antibacterial activity against antibiotic resistant bacteria. Compound **2** was reported from an engineered *Streptomyces*, but this is the first report of its presence in soft coral.

## Experimental

***General*:** NMR, JEOL ECA 600 FT-NMR; LC-IT-TOF-MS (Shimadzu); Optical rotation, AUTOPOL IV automatic polarimeter (Rudolph Research Analytic); IR, Fourier Transform Infrared Spectrophotometer (Thermo Nicolet); Preparative TLC, silica gel glass plates (Merck, Kieselgel 60 $F_{254}$).

***Biological material:*** A *Sinularia* sp. was collected from Mantanani Island, Sabah (6°42.313"N, 116°19.335"E), Malaysia in September 2014. A voucher specimen (BORMI0009) was deposited in BORNEENSIS Collection at the Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah.

***Extraction and isolation:*** Fresh soft coral (2.0 kg wet wt) was extracted in MeOH (25°C). The MeOH extract was concentrated *in vacuo* and partitioned in EtOAc/$H_2O$. The EtOAc fraction was further partitioned with *n*-hexane/90% MeOH. The *n*-hexane fraction (1.0 g) was subjected to column chromatography eluting with a gradient of *n*-hexane and EtOAc in an increasing polarity. Fraction 1 (278.2 mg) obtained from *n*-hexane-EtOAc (9:1) gave compound **1** (6.3 mg), **2** (71.1 mg) and **3** (61.7 mg).

**(1*R*,2*R*,4*S*)-Loba-8,10,13(14),17(18)-tetraen-16-one** (**1**)
Colorless oil.
$[\alpha]_D^{25}$: +4.7 (*c* 0.64, $CHCl_3$)
IR: 1741, 1686, 1636, 1619, 1440, 1375 and 891 $cm^{-1}$.
$^1H$ and $^{13}C$ NMR: Table 1. HR-ESIMS: *m/z* 287.2361, $[M + H]^+$, (calcd for $C_{20}H_{31}O$, 287.2369).

***ent*-Prenyl-β-elemene** (**2**)
$[\alpha]_D^{25}$: -22.5 (*c* 0.40, $CHCl_3$)
HR-ESIMS: *m/z* 311.2134 $[M + K]^+$ (calcd for $C_{20}H_{32}K$, 311.2136).

***Antibacterial activity*:** Antibacterial activities were conducted on *S. aureus* (ATCC 6538) and *E. coli* (ATCC 35210) based on the microdilution method [9]. The bacterial suspensions were adjusted with sterile saline to a concentration of 1.0 x $10^5$ CFU/mL and stored at 4°C. Experiments were made in triplicate. Pure compounds were added (1 and 10 mg/mL) to 100 mL Tryptic Soy Broth (TSB) with a bacteria inoculum (1.0 x $10^4$ CFU per well), reaching the desired concentration in a microtiter plate to measure the MICs and MBCs. The mixtures in microplates were incubated for 24 h at 37°C.

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