

Article

Oxygenated Ylangene-Derived Sesquiterpenoids from the Soft Coral *Lemnalia philippinensis*

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Abstract: Chemical examination of a Taiwanese soft coral *Lemnalia philippinensis* led to the isolation of three oxygenated ylangene-derived sesquiterpenoids **1–3**, including two new metabolites, philippinlins A and B (**1** and **2**). The structures of these compounds were elucidated on the basis of detailed spectroscopic data. Compound **1** was shown to exhibit cytotoxicity against HepG2, MDA-MB231 and A549 cancer cell lines.

Keywords: *Lemnalia philippinensis*; ylangene; philippinlins A and B; cytotoxicity

1. Introduction

In recent years, soft corals have become one of the most prolific sources for the discovery of novel secondary metabolites [1]. Previous chemical investigations on soft corals of the genus *Lemnalia* have led to the isolation and identification of variety of ylangene-derived sesquiterpenoids [2–5]. Some of these have been found to possess various biological activities, such as cytotoxic [4] and anti-inflammatory properties [6]. Using our experience searching for new bioactive metabolites from soft corals of *Lemnalia* [7] and *Paralemnalia* [8–11], we carried out the first chemical investigation of the soft coral *Lemnalia philippinensis* with the aim of discovering interesting new metabolites. Our chemical examination of this soft coral led to the isolation of two new oxygenated ylangene-type sesquiterpenoids, philippinlins A and B (**1** and **2**), and one known compound lemnalol (**3**) [2]. The structures of **1–3** were established by detailed spectroscopic analysis, including extensive examination of 2D NMR (COSY, HSQC, HMBC and NOESY) correlations. The cytotoxicity of metabolites **1–3** towards human liver carcinoma (HepG2), human breast carcinoma (MDA-MB231) and human lung adenocarcinoma epithelial cells (A549) was evaluated, and **1** was found to be cytotoxic against the above cancer cells.

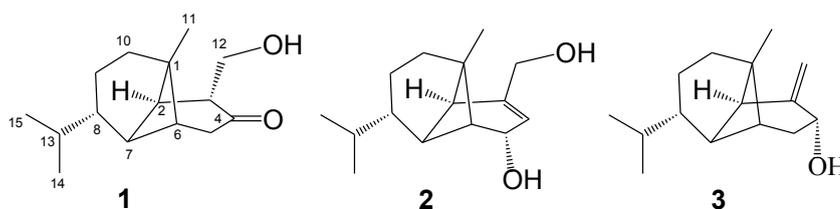
2. Results and Discussion

The EtOAc extract of the frozen specimen was fractionated by silica gel column chromatography and the eluted fractions were further separated utilizing normal phase HPLC to yield metabolites **1–3** (Figure 1). The new compounds were given the trivial names philippinlins A and B (**1** and **2**). By comparison of NMR and MS data, compound **3** was found to be identical with known lemnalol [2]. The specific optical rotation of **3** ($[\alpha]_D^{25} -8.0$) was close to that reported for lemnalol ($[\alpha]_D^{25} -9.3$) [2], which ensures that we are dealing with the same enantiomer. Compounds **1** and **2** were isolated with **3** from the same organism and should be formed by a shared biosynthetic pathway. Thus, both new compounds are suggested to possess the absolute configurations as shown in formulae **1** and **2**.

Figure 1. The soft coral *Lemnalia philippinensis* and the structures of philippinlins A (**1**), B (**2**), and lemnalol (**3**).



Lemnalia philippinensis



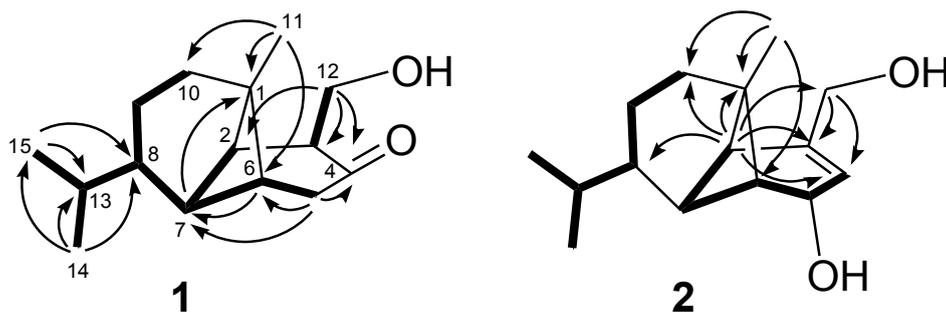
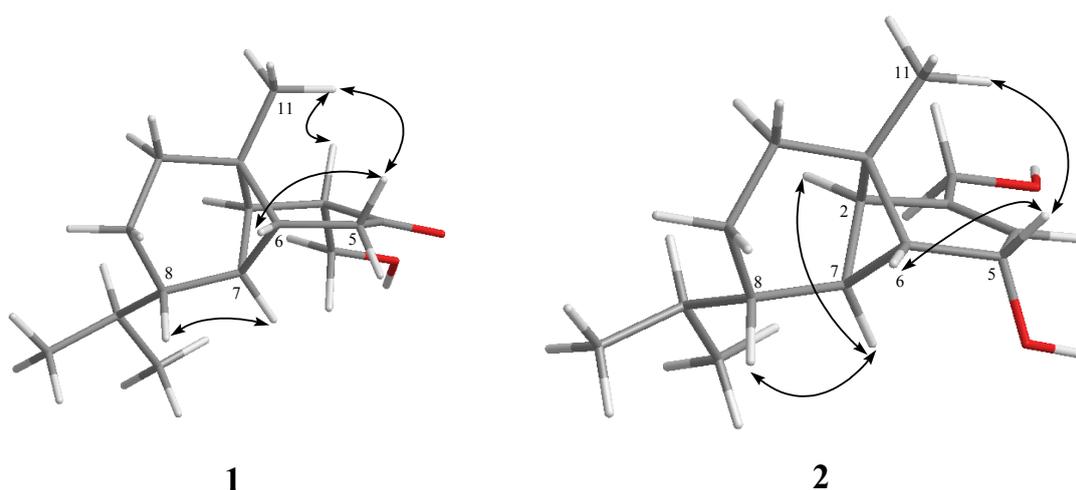
Philippinlin A (**1**) was obtained as a colorless oil. The HRESIMS of **1** exhibited a pseudomolecular ion peak at m/z 259.1672 $[M + Na]^+$, and established a molecular formula of $C_{15}H_{24}O_2$, implying four degrees of unsaturation. IR absorptions were observed at 3437 and 1714 cm^{-1} , suggesting the presence of hydroxy and carbonyl groups in **1**. In the ^{13}C NMR and DEPT spectroscopic data (Table 1), signals of three methyls, four sp^3 methylenes (including one oxymethylene), six sp^3 methines, one sp^3

quaternary carbon and one ketone were observed. From the ^1H NMR spectrum of **1**, the signals of one oxygenated methylene (δ 3.91, dd, $J = 10.5$ and 8.5 Hz; 3.55, dd, $J = 10.5$ and 5.5 Hz) and three methyls (δ 0.90, s; 0.87, d, $J = 7.0$ Hz; 0.85, d, $J = 7.0$ Hz) were observed. The planar structure and all of the ^1H and ^{13}C chemical shifts of **1** were elucidated by 2D NMR spectroscopic analysis, in particular COSY and HMBC experiments (Figure 2). To establish the proton sequences in **1**, the ^1H - ^1H COSY spectrum analysis established two proton sequences (Figure 2). The molecular framework of **1** was further established by an HMBC experiment, which showed the following key correlations (Figure 2): H₂-5 to C-4, C-6 and C-7, H-6 to C-7, H-7 to C-1, H₃-11 to C-1, C-2, C-6 and C-10, H₂-12 to C-2, C-3 and C-4, both methyls H₃-14 and H₃-15 to C-8 and C-13. From above results and the chemical shifts of CH₂-12 and C-4 (Table 1), **1** was found to possess a hydroxy group at C-12, and a ketone group at C-4. Furthermore, analysis of the ^1H and ^{13}C NMR data showed that the partial structures from C-1, C-2 and C-6 to C-15 in **1** should be identical to those of **3**. The relative configurations of all stereocenters, except C-3 and C-4 of **1**, were confirmed to be the same as those of **3** by comparison of the proton shifts and NOE correlations (Figure 3). One hydroxymethyl group at C-3 was assigned the α -configuration primarily due to the NOE correlation between H-3 and H₃-11. Thus, the structure of sesquiterpenoid **1** was established.

Table 1. ^{13}C and ^1H NMR spectral data for compounds **1** and **2**.

C/H	1		2	
	δ_c (Mult.) ^a	δ_H (J in Hz) ^b	δ_c (Mult.) ^c	δ_H (J in Hz) ^d
1	41.6 (C)		48.9 (C)	
2	37.3 (CH)	2.07 dd (7.0, 2.0)	40.9 (CH)	2.24 d (6.0)
3	51.5 (CH)	2.74 ddd (8.5, 5.5, 2.0)	150.3 (C)	
4	216.7 (C)		119.7 (CH)	5.66 brs
5	43.5 (CH ₂)	α : 2.69 dd (19.0, 1.5) β : 2.52 dd (19.0, 3.5)	70.2 (CH)	4.36 brs
6	46.2 (CH)	1.70 m	54.8 (CH)	1.82 m
7	42.8 (CH)	1.74 m	42.3 (CH)	1.89 m
8	44.2 (CH)	1.65 m	44.9 (CH)	1.60 m
9	21.6 (CH ₂)	1.70 m; 1.59 m	21.9 (CH ₂)	1.67 m; 1.59 m
10	36.3 (CH ₂)	1.79 m; 1.71 m	36.8 (CH ₂)	1.73 m
11	18.2 (CH ₃)	0.90 s	19.1 (CH ₃)	0.82 s
12	63.4 (CH ₂)	3.91 dd (10.5, 8.5) 3.55 dd (10.5, 5.5)	65.4 (CH ₂)	4.06 s
13	32.4 (CH)	1.50 m	32.5 (CH)	1.58 m
14	19.8 (CH ₃)	0.85 d (7.0)	19.4 (CH ₃)	0.87 d (6.4)
15	19.5 (CH ₃)	0.87 d (7.0)	20.0 (CH ₃)	0.86 d (6.4)

Spectra recorded at ^a 125 MHz in CDCl₃; ^b 500 MHz in CDCl₃; ^c 100 MHz in CDCl₃; ^d 400 MHz in CDCl₃.

Figure 2. Selected COSY (—) and HMBC (→) correlations of **1** and **2**.**Figure 3.** Key NOESY correlations for **1** and **2**.

Philippinlin B (**2**) was obtained as a white powder and showed a $[M + Na]^+$ ion peak in the HRESIMS spectrum corresponding to the molecular formula $C_{15}H_{24}O_2$, the same as that of **1**. IR absorption was observed at 3358 cm^{-1} , again suggesting the presence of the hydroxy group in **2**. Also, unlike **1**, the absence of carbonyl absorption was shown in the IR spectrum. The planar structure and all of the ^1H and ^{13}C chemical shifts of **2** were elucidated by 2D NMR spectroscopic analysis, in particular COSY and HMBC experiments (Figure 2). Thus, **2** was found to possess one double bond at C-3/C-4, two hydroxy groups at C-5 and C-12. The relative configurations of the six chiral centers at C-1, C-2, C-5, C-6, C-7 and C-8 in **2** were elucidated by detailed NOE analysis, as shown in Figure 2. In these experiments, it was found that H-5 showed NOE interactions with H-6 and H₃-11. Thus, assuming the β -orientation of H-5, both H-6 and H₃-11 should be positioned on the β face. On the basis of the above findings and detailed examination of other NOE correlations (Figure 3), the relative structure of compound **2** was determined.

The cytotoxicity of metabolites **1–3** against the growth of HepG2, MDA-MB231 and A549 carcinoma cells was studied. Compound **1** showed cytotoxicity towards HepG2, MDA-MB231, and A549 cancer cell lines with IC_{50} values of 16.0, 16.3, and 15.8 $\mu\text{g/mL}$, respectively. While lemnalol has been shown to possess remarkable anti-inflammatory activities [6,12,13], compounds **2** and **3** did not exhibit cytotoxicity towards the above cancer cell lines.

3. Experimental Section

3.1. General Experimental Procedures

Optical rotation values were measured with a Jasco-P1010 digital polarimeter. Infrared spectra were obtained on a Varian Digilab FTS 1000 FT-IR spectrophotometer. NMR spectra were recorded on a Varian Mercury Plus 400 FT-NMR at 400 MHz for ^1H and 100 MHz for ^{13}C in CDCl_3 or $\text{C}_5\text{D}_5\text{N}$ at 25 °C. ESIMS and HRESIMS data were recorded on a Bruker APEX II mass spectrometer. Column chromatography was performed on silica gel (230–400 mesh, Merck, Darmstadt, Germany). TLC was carried out on precoated Kieselgel 60 F₂₅₄ (0.25 mm, Merck, Darmstadt, Germany) and spots were visualized by spraying with 10% H_2SO_4 solution followed by heating. Normal phase HPLC (NP-HPLC) was performed using a system comprised of a Hitachi L-7110 pump, a Hitachi L-7455 photodiode array detector and a Rheodyne 7725 injection port. A normal phase column (Supelco Ascentis[®] Si Cat #:581515-U, 25 cm × 21.2 mm, 5 μm) was used for NP-HPLC. Reverse phase HPLC (RP-HPLC) was performed using a system comprised of a Hitachi L-7100 pump, a Hitachi L-2455 photodiode array detector and a Rheodyne 7725 injection port. A reverse phase column (Varian Polaris C18-A, 250 mm × 10 mm, 5 μm) was used for RP-HPLC.

3.2. Animal Material

L. philippinensis, taxonomically identified by Prof. Chang-Feng Dai of National Taiwan University, was collected by hand using scuba off the coast of Lanyu, Taiwan, in August 2008, at a depth of 10–15 m, and stored in a freezer until extraction. A voucher sample was deposited at the Department of Marine Biotechnology and Resources, National Sun Yat-sen University.

3.3. Extraction and Isolation

Sliced tissues of the soft coral *L. philippinensis* (0.8 kg, wet wt) were exhaustively extracted with EtOAc. The combined EtOAc extract was concentrated under reduced pressure. The EtOAc extract was evaporated to yield a residue (10.7 g), which was subjected to open column chromatography on silica gel stepwisely eluting with *n*-hexane–EtOAc mixture and EtOAc–MeOH mixture, to give 25 fractions. Fraction 7, eluted with *n*-hexane–EtOAc (20:1), was further separated by Sephadex LH-20 column chromatography with acetone as eluent to yield 5 subfractions (7A–E). Subfraction 7D was separated by silica gel open column chromatography with gradient elution (*n*-hexane– CH_2Cl_2 , 4:1) to afford **3** (156 mg) and subfraction 7E was also separated by normal phase HPLC using *n*-hexane– CH_2Cl_2 (4:1) to afford **1** (3.9 mg). The combined fractions 18 and 19, eluted with *n*-hexane–EtOAc (1:1–1:2), were separated by silica gel open column chromatography with gradient elution (*n*-hexane–EtOAc, 7:2) to yield 8 subfractions (18A–H). Subfraction 18G was separated by normal phase HPLC using *n*-hexane–EtOAc (2:1) to afford **2** (1.1 mg).

Philippinlin A (**1**): colorless oil; $[\alpha]_D^{25} = -97$ (*c* 0.3, CHCl_3); IR (neat) ν_{max} 3437, 2941, 1714, 1459 and 1376 cm^{-1} ; ^1H and ^{13}C NMR data, see Table 1; ESIMS m/z 259 $[\text{M} + \text{Na}]^+$; HRESIMS m/z 259.1672 (calcd. for $\text{C}_{15}\text{H}_{24}\text{O}_2\text{Na}$, 259.1674).

Philippinlin B (**2**): white powder; mp 92–93 °C; $[\alpha]_D^{25} = +39$ (c 0.1, CHCl₃); IR (neat) ν_{\max} 3358, 2932, 2862 and 1464 cm⁻¹; ¹H and ¹³C NMR data, see Table 1; ESIMS m/z 259 [M + Na]⁺; HRESIMS m/z 259.1672 (calcd. for C₁₅H₂₄O₂Na, 259.1674).

3.4. Cytotoxicity Testing

Cell lines were purchased from the American Type Culture Collection (ATCC). Cytotoxicity assays of compounds **1–3** were performed using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric method [14,15]. Doxorubicin, employed as positive control, exhibited cytotoxic activity toward HepG2, MDA-MB231 and A549 cancer cell lines with IC₅₀'s 0.4, 1.4, and 1.6 µg/mL, respectively.

4. Conclusions

Two new oxygenated ylangene-derived compounds, philippinins A (**1**) and B (**2**) and a known compound lemnalol (**3**), were discovered from the soft coral *L. philippinensis*. Compound **1** was found to exhibit cytotoxicity towards HepG2, MDA-MB231 and A549 cancer cell lines.

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Conflicts of Interest

The authors declare no conflict of interest.

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