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New eudesmane sesquiterpenes from the marine-derived fungus *Penicillium thomii*

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ABSTRACT

Four new eudesmane-type sesquiterpenes thomimarines A–D (**1**–**4**) were isolated from the marine-derived fungus *Penicillium thomii*. Their structures were established based on spectroscopic methods. The absolute configurations of **1**–**4** were determined by time-dependent density functional theory (TD-DFT) calculations of ECD spectra. Inhibitory effects of compounds **1**, **2** and **4** on NO production in LPS-induced RAW 264.7 murine macrophages were evaluated.

1. Introduction

A family of eudesmane-type sesquiterpenes derived from decalin framework are found particularly in higher plants and another sources such as marine organisms and terrestrial fungi. They show a wide range of biological activities, including cytotoxic (Bomfim et al., 2013; Zhao et al., 2014), antibacterial (Iken and Baker, 2003; Li et al., 2015), insecticidal (Vera et al., 2008), ichthyotoxic (Gavagnin et al., 2003) and inhibitory activity against acetylcholinesterase and nitric oxide production in LPS-induced murine macrophages (Jiang et al., 2013; Liu et al., 2014; Morikawa et al., 2002; Xu et al., 2012; Yang et al., 2015).

During our ongoing search for of structurally novel and bioactive metabolites from marine-derived fungi, we have investigated the strain *Penicillium thomii* KMM 4667 isolated from sea grass *Zostera marina* (Sea of Japan). Herein, we report the isolation, structure elucidation and biological assay results of the four new eudesmane sesquiterpenes produced by this fungus (Fig. 1).

2. Results and discussion

The molecular formula of compound **1** was established as $C_{15}H_{24}O_3Na$ by a HRESIMS peak $[M+Na]^+$ (*m/z* 275.1623) and by ^{13}C NMR analyses. A close inspection of ^1H and ^{13}C NMR data of **1** (Tables 1 and 2) by DEPT and HSQC revealed the presence of three methyl (δ_H 0.93, δ_C 17.6, δ_H 1.27, δ_C 23.6 and δ_H 1.93, δ_C 21.9) groups, five methylenes (δ_C 54.8, 22.9, 20.7, 37.0, 69.0) including one oxygen-bearing (δ_H 3.67, 3.46). two methines (δ_H 2.84, δ_C 43.3, δ_H 1.87, δ_C 37.6) along with one carbonyl carbon (δ_C 199.4), trisubstituted double bond (δ_C 163.9 (C), 126.6 (CH)) and one oxygenated quaternary carbon (δ_C 75.9). The ^{13}C NMR data observed for the ring A of **1** resemble those reported for oxyphyllanene A (Xu et al., 2012). This information and HMBC correlations from H_3-14 to C-1 (δ_C 54.8), C-5 (δ_C 43.3), C-10 (δ_C 36.9); from H_3-15 to C-3 (δ_C 126.6), C-4 (δ_C 163.9), C-5 (δ_C 43.3) and from H_2-1 to C-2 (δ_C 199.4), C-3 and C-5 indicated the presence of a cyclohex-2-enone ring system in **1** and the location of the methyl groups at the C-4 and C-10. The COSY spectrum of **1** and long-range correlations from H_3-14 to C-9 (δ_C 37.0); from H_2-6 to C-5, C-7 (δ_C 37.6), C-8 (δ_C 20.7) and from H-7 to C-5, C-9 established the structure of ring B in **1**. The HMBC correlations from H-7 to C-11 (δ_C 75.9), C-12 (δ_C 69.0) and C-13 (δ_C 23.6); from H_3-13 to C-7, C-11 and

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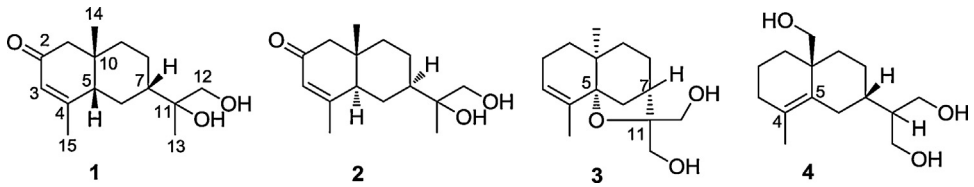

Fig. 1. Chemical structures of thomimarines A–D (**1**–**4**).

C-12; from H_2-12 to C-7, C-11 and C-13 indicated the presence of 1,2-dixydroxypropyl residue attached to C-7 in **1** (Fig. 2). Thus, the planar structure of **1** was established. The relative configuration of **1** was assigned on the basis of a NOESY experiment. Observed mutual NOE correlations H_3-14/H-5 (δ_H 2.84), H-7 (δ_H 1.87) indicated these protons to be on the same side of molecule and a *cis*-ring fusion of the A and B rings. To further resolve the absolute stereochemistry of **1** the ECD spectroscopy methods were used. To obtain statistically averaged ECD spectrum of *5S,7S,10S,11S* and *5S,7S,10S,11R* the most stable conformations of **1** in MeOH solvent were selected using B3LYP/6-31G(d) method according to their Gibbs free energies. It was found, that the energetically most preferable conjunction of the six-membered cycles A and B is the "*envelope-chair*" type. The *cis*-orientation of H-5 and CH_3-14 relative to C-5–C-10 bond results in the axial position for C-6. These results in that nearly all atoms of the six-membered cycle B and substituent at C-7 lie at the same side relatively to the *plane* (further the *ring A plane*), in which all atoms of ring A lie, for the exceptions of C-10. The presence of the branchy substituent in ring B, in which two OH groups can form intramolecular hydrogen bonds, results in a complicated set of conformations (rotameric forms), which contribute to spectral properties of **1**. As a result, the individual ECD spectra for ten conformations (*5S,7S,10S,11S*) and for twelve conformations (*5S,7S,10S,11R*) were calculated and statistically averaged. The comparison of both theoretical spectra with the experimental one (Fig. 3) showed, that all spectra are qualitatively similar in the region $\lambda \leq 280$ nm, where the pronounced Cotton effects occur. Therefore, we could not assign correctly the configuration of C-11 center for **1**. Finally, the configuration of **1** was established as *5S,7S,10S* and named thomimarine A.

Compound **2** was determined to have the same molecular formula as **1** on the basis of ion peak $[M+H]^+$ (m/z 253.1816) in the HRAPPIMS. The general features of the ^1H and ^{13}C NMR spectra (Tables 1 and 2) of **2** resembled those of **1** with the exception of the H-5 proton and C-5, C-7 and C-9 carbon signals. COSY and HMBC correlations established the structure of rings A and B and the presence of 1,2-dixydroxypropyl residue as side chain in **2**. NOESY cross-peaks H_3-14/H-1β (δ_H 2.27), H-6β (δ_H 1.15), H-9β (δ_H 1.62); H-5/H-1α (δ_H 2.19), H-6α (1.95), H-7 (δ_H1.68), H-9α (δ_H 1.41) and H-7/H-9α indicated a *trans*-ring fusion of the A and B rings as well as the β-orientation of H_3-14, and the α-orientation of H-5 and H-7. The absolute stereochemistry of **2** was proved as for **1**. First, the conformational analysis was performed using B3LYP/6-311+G(d,p) method and seven most stable conformations were selected. As in the cause of **1** the "*envelope-chair*" type of conjunction for two six-

Table 1
^1H NMR spectroscopic data (δ, J in Hz) for thomimarines A–D (**1**–**4**).

Pos.	1[a]	2[b]	3[a]	4[c]
1	a: 2.26, d (16.1)	a: 2.27, d (16.1)	a: 1.74, m	a: 1.82, m
	b: 2.22, d (16.1)	b: 2.19, d (16.1)	b: 1.12, dd (5.5, 12.7)	b: 0.91, m
2			a: 2.03, m	a: 1.53, m
			b: 1.97, m	b: 1.42, m
3	5.86, s	5.88, s	5.67, brs	a: 1.94, m
				b: 1.82, m
5	2.84, d (13.5)	2.35, d (11.2)		
6	a: 2.21, dt (3.7, 13.8)	a: 1.95, m	a: 2.15, ddd (1.5, 4.8, 12.5)	a: 2.49, dt (2.2, 15.7)
	b: 1.47, dt (7.2, 13.8)	b: 1.15, dd (3.6, 12.2)	b: 1.73, m	b: 1.85, m
7	1.87, m	1.68, dt (3.8, 12.1)	2.33, q (3.5)	1.70, m
8	1.73, m (2H)	a: 1.72, m	a: 1.76, m	a: 1.60, t (4.2)
		b: 1.43, m	b: 1.74, m	b: 1.47, m
9	a: 1.70, m	a: 1.62, m	a: 1.62, dd (7.2, 13.4)	a: 1.66, dt (3.7, 13.4)
	b: 1.37, m	b: 1.41, m	b: 1.30, ddd (2.0, 5.8, 13.8)	b: 0.98, td (5.3, 14.0)
10				
11				1.37,
12	a: 3.67, d (10.7)	a: 3.64, d (10.9)	a: 4.05, d (11.0)	a: 3.55,
	b: 3.46, d (10.7)	b: 3.49, d (11.0)	b: 3.95, d (11.0)	b: 3.37,
13	1.27, s	1.18, s	a: 3.69, d (10.5)	a: 3.59,
			b: 3.65, d (10.5)	b: 3.41,
14	0.93, s	0.87, s	0.93, s	a: 3.55,
				b: 3.22, dd (5.4; 10.9)
15	1.93, s	1.90, s	1.69, s	1.57, s
OH-4				
OH-9				
OH-11				
OH-12				4.14, t
OH-13				4.19, t
OH-14				4.31, t

[a] Chemical shifts were measured at 500.13 MHz in $CDCl_3$.
[b] Chemical shifts were measured at 700.13 MHz in $CDCl_3$.
[c] Chemical shifts were measured at 500.13 MHz in DMSO-d6.