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Manoalide-related Sesterterpene from the Marine Sponge *Luffariella variabilis*

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A new manoalide-related sesterterpene, (4*E*,6*E*)-dehydro-25-*O*-methylmanoalide (**1**), was isolated from the organic extracts of the Bornean marine sponge *Luffariella variabilis*, together with the known compound (4*E*,6*E*)-dehydromanoalide (**2**). The structure of compound **1** was elucidated by interpretation of its spectroscopic data.

Keywords: *Luffariella variabilis*, Cytotoxic compound, Manoalide-related sesterterpene, Marine sponge.

The marine sponge *Luffariella variabilis* contains an array of secondary metabolites including manoalide-type compounds [1a]. Manoalide, the major of the manoalide-type compounds, possesses potent anti-inflammatory activities inhibiting phospholipase A2 (PLA2) [1b]. In the course of our search for cytotoxic compounds from marine invertebrates, we isolated a new manoalide-type sesquiterterpene, (4*E*,6*E*)-dehydro-25-*O*-methylmanoalide (**1**), together with (4*E*,6*E*)-dehydromanoalide (**2**) from *L. variabilis* [1c] collected at Sepangar Island, Malaysia. In this paper, we describe the isolation and structure determination of compound **1**.

The EtOAc layer of the MeOH extract of the sponge was dried and subjected to solvent partitioning, as described in the Experimental section. The 90% MeOH extract (15 g) was separated by ODS flash chromatography and silica gel column chromatography, followed by reversed-phase HPLC to afford (4*E*,6*E*)-dehydro-25-*O*-methylmanoalide (**1**) and (4*E*,6*E*)-dehydromanoalide (**2**). The known compound, (4*E*,6*E*)-dehydromanoalide (**2**), was identified by comparison of its NMR and EIMS data with published information [1d, 1e, 1f].

Compound **1** was obtained as pale yellow glass needles. It had a molecular formula of $C_{26}H_{36}O_4$, which was suggested by its HRFABMS [*m/z* 435.2511 $(M+Na)^+$, Δ +0.5 mmu]. The ^{1}H- and ^{13}C-NMR spectra of **1** (Table 1) were almost identical with those of (4*E*,6*E*)-dehydromanoalide (**2**). However, they showed the characteristic signals of an additional methoxy group [δ_H 3.57 (3H, s) and δ_C 55.3]. The location of this in **1** was established by the HMBC correlation of methoxy methyl protons to the hemiacetal carbon signal at δ 102.5 (C-25), which resonated at a lower field than the C-25 of **2** bearing a hydroxyl group (δ_C 97.9). Thus, **1** was identified as (4*E*,6*E*)-dehydro-25-*O*-methylmanoalide (Figure 1). The absolute configuration at C-25 remains undetermined.

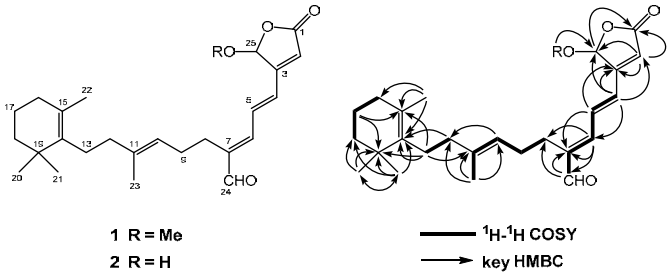

Figure 1: Chemical structures of compounds **1** and **2**, and ^{1}H-^{1}H COSY and key HMBC correlations of **1**.

The conditions of the process of extraction, partition and separation applied could not justify the conversion of **2** into **1**. Thus, we suggest that (4*E*,6*E*)-dehydro-25-*O*-methylmanoalide (**1**) and (4*E*,6*E*)-dehydromanoalide (**2**) be considered as true secondary metabolites produced by a biosynthetic pathway, rather than artifacts arising from the isolation procedure.

Anti-tumor effects of compounds **1** and **2** against ATL cells were investigated by WST-8 assay. Both compounds showed moderate cytotoxic effects against the HTLV-I-related leukemia cell line, S1T, with IC_{50} values of 1.7 and 1.9 μM, respectively. This indicates that these manoalide-related compounds show inhibitory effects on cell proliferation, warranting further investigation with the aim of developing novel anti-ATL drugs.

Table 1: NMR spectral data of **1** and **2** in CD_3OD at 300 K.

	compound 1		compound 2	
Position	δ_H (mult., *J* in Hz)	δ_C (mult.)	δ_H (mult., *J* in Hz)	δ_C (mult.)
1		169.6 (s)		171.0 (s)
2	6.18 (s)	120.6 (d)	6.13 (s)	119.6 (d)
3		157.7 (s)		160.2 (s)
4	6.81 (d, 15.6)	127.9 (d)	6.83 (d, 15.6)	128.3 (d)
5	7.25 (dd, 11.6, 15.6)	133.5 (d)	7.36 (dd, 11.5, 15.6)	134.1 (d)
6	6.91 (d, 11.6)	145.4 (d)	6.92 (d, 11.5)	145.9 (d)
7		146.7 (s)		146.6 (s)
8	2.49 (t, 7.5)	25.0 (t)	2.47 (t, 7.6)	24.8 (t)
9	2.15 (m)	27.5 (t)	2.14 (m)	27.3 (t)
10	5.12 (t, 7.1)	121.8 (d)	5.12 (t, 7.1)	121.9 (d)
11		138.0 (s)		138.0 (s)
12	2.00 (m)	40.2 (t)	1.99 (m)	40.2 (t)
	1.99 (m)			
13	2.00 (m)	27.9 (t)	1.98 (m)	27.8 (t)
14		137.0 (s)		137.0 (s)
15		127.1 (s)		127.2 (s)
16	1.90 (t, 6.1)	32.9 (t)	1.88 (t, 6.0)	32.9 (t)
17	1.58 (m)	19.7 (t)	1.56 (m)	19.5 (t)
18	1.40 (m)	39.8 (t)	1.40 (m)	39.9 (t)
19		35.0 (s)		35.0 (s)
20	0.98 (s)	28.8 (q)	0.96 (s)	28.6 (q)
21	0.98 (s)	28.8 (q)	0.96 (s)	28.6 (q)
22	1.57 (s)	19.9 (q)	1.56 (s)	19.8 (q)
23	1.61 (s)	16.3 (q)	1.60 (s)	16.1 (q)
24	9.54 (s)	194.0 (d)	9.51 (s)	194.5 (d)
25	6.04 (s)	102.5 (d)	6.35 (s)	97.9 (d)
OMe	3.57 (s)	55.3 (q)		

Experimental

General procedures: Optical rotation was measured at 25ºC on a JASCO DIP-370S polarimeter. NMR spectra were recorded with JEOL ECX400 and ECX600 spectrometers, and UV and IR spectra on a UV-210 and a JASCO FT/IR 5300. FAB mass spectra were obtained using a JEOL JMS-700 Mstation. Column chromatography was performed with silica gel 60 (Merck, 70—230 μm). Silica gel 60F plates (Merck, 0.25 mm thick) were used for TLC. HPLC was performed using a Waters 501 HPLC pump with a Shodex UV-41 detector. A C_{18} column (4.6 mmϕ x 250 mm) was used for HPLC [2a].

Biological materials: The marine sponge was collected at a depth of 15 m at Sepanggar Island, Sabah (6º03'N, 116º04'E), Malaysia, on August 3, 2011. The voucher specimen was deposited in the herbarium of the Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah (BORNEENSIS) and in Netherlands Centre for Biodiversity Naturalis, the Netherlands (RMNH POR 8672).

Extraction and isolation: The sample (2.64 kg, wet weight) was chopped into small pieces and extracted with MeOH (5 L) at room temperature for 1 ~ 2 weeks. Extracts were concentrated under reduced pressure at 40–45ºC and the residue (132 g) was partitioned between AcOEt (3 L) and H_2O (1 L). The AcOEt extract (28 g) was further partitioned between MeOH / H_2O (9:1) and *n*-hexane. The aq MeOH layer (15 g) was subjected to ODS flash chromatography to give 14 fractions. The active fractions (4.2 g) eluted with MeOH were further separated by silica gel flash chromatography (*n*-hexane: AcOEt = 9 : 1 – 1 : 1, gradient) to give 15 fractions. The active fraction was purified by reversed-phase HPLC (Cosmosil $5C_{18}$-AR-II, 10 x 250 mm) with 85% MeOH to furnish compounds **1** (7.3 mg) and **2** (92 mg).

Cell lines and cultures: The adult T-cell leukemia cell line S1T was maintained in RPMI-1640 supplemented with 10% fetal bovine serum, 100 U/mL penicillin, 100 μg/mL streptomycin, and 2 mM L-glutamate. Generally, cell cultures were split every 2 to 3 days, and used for *in vitro* assays during the log phase of growth [2b]

Cytotoxicity: The cells were cultured at a density of 1 x 10^4 cells per well in at least triplicates in the absence or presence of a test sample in ten-fold dilutions for 72 h in flat bottom 96-well plates at 37ºC in a humidified water-jacketed CO_2 incubator. The inhibition of cell proliferation was determined using a 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium monosodium salt (WST-8) assay kit (Dojindo, Kumamoto, Japan) [2c, 2d]. The viable cells convert the WST-8 tetrazolium salt into a water-soluble formazan. The concentration at which cell proliferation is inhibited by 50% compared with untreated control cells is expressed as the IC_{50}.

(4*E*, 6*E*)-Dehydro-25-*O*-methylmanoalide (**1**)
Pale yellow waxy solid.
$[\alpha]^{25}_D$: +2.0 (*c* 0.05, MeOH).
UV λ_{max} (MeOH): 317 nm (logε = 4.13).
IR (film): 1764, 1679 cm^{-1}.
^1H and ^{13}C NMR (CD_3OD): Table 1.
HRFABMS *m/z*: 435.2516 $[M + Na]^+$ (calcd for $C_{26}H_{36}O_4Na$ 435.2511, +0.5 mmu).

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