Enantiomeric Compounds with Antileishimanial Activities from a Sponge, *Plakortis* sp.

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As part of a program to discover bioactive natural products from marine organisms, two new enantiomers, *ent*-3,6-Epidioxy-4,6,8,10-tetraethyltetradeca-7,11-dienoic acid and *ent*-[3,5-Diethyl-5-(2-ethyl-hex-3-enyl)-5H-furan-2-ylidene]-acetic acid methyl ether, were isolated from a sponge *Plakortis* sp. These compounds showed strong *in vitro* antiproliferative effects on promastigotes of *Leishmania mexicania*, flagellate protozoan that causes leishmaniasis. Structures were assumed by interpretation of NMR spectroscopic data and optical rotation. Both compounds exhibited significant antileishmanial activities *in vitro* with IC_{50} of 1.0-23.0 μg/ml.

Key words: *antileishmaniasis, enantiomer, antibacterial, sponge*

Leishmania and malaria are occur widely in the tropical and subtropical regions.[1,2] Leishmaniasis is breaking out in many poorest countries, where estimated 1.5 to 2 million people are infected each year.[3,4] In the central and South Americas, the causative agent is the protozoan *Leishmania mexicana*, *L. mexicana* utilizes intermediate as host and is transmitted by flies. The most commonly used drugs for the treatment of leishmaniasis contain pentavalent antimonials and these drugs generally unacceptable side effects. The urgent need for alternative compounds has led to a program to screen natural products for potential use in the therapy of these leishmainasis.

Marine sponge has been proven to be a valuable resource for various bioactive substances and sponges of the genus *Plakortis* are well known for their ability to produce cyclic peroxides and related metabolites.[5-8]

In this paper, we describe the isolation and identification of two new enantiomeric compounds, with antileishmanial and antifungal activites from a sponge of *Plakortis* sp.

Materials and Methods

Sample. The sponge was collected from rocks at a depth of −40 m off of Jeju seashore on March 2004. The sponge formed a moderately thick encrustation of very soft, liver-like and very easily-torn texture. The sponge was an undescribed species of *Plakortis*. Vaucher specimen of these sponge has been deposited at the Natural History Museum, London, United Kingdom.

Extraction and Isolation. Sample of *Plakortis* sp. (1.3 kg, wet wt) was extracted with 6 *l* of ethanol in a blender. The extract (1.5 g) was subjected to silica flash chromatography (Merck, column 150 × 3 cm, gel size 230-400 mesh, 450 g) using a gradient elution with hexane, ethyl acetate and finally methanol (Table 1). Fractions 2-6 were further fractionated using gel permeation chromatography on Sephadex LH-20 (Pharmacia, column 4.9 × 76 cm) with methanol. Further purification was carried out using reverse-phase HPLC elution (Phenomenex Ultracarb 5 μm, ODS 30, 250 × 21.5 mm) using a linear gradient with CH_3CN-H_2O as an eluent (flow rate: 10 ml/min, UV detection: 230 nm). This process yielded *ent*-3,6-epidioxy-4,6,8,10-tetraehtyltetradeca-7,11-dienoic acid (compound 1, 13.4 mg, oil) and *ent*-[3,5-Diethyl-5-(2-ethyl-hex-3-enyl) -5H-furan-2-ylidene]-acetic acid methyl ether (compound 2, 21.1 mg, oil).

Structure analysis. IR and UV spectra were obtained using an AATI Mattson Genesis Series FTIR and Perkin-Elmer Lambda 3B UV/Vis spectrophotometer. Optical rotations were measured with an AUTOPOL IV autopolarimeter. 1D and 2D NMR spectra were recorded on a Bruker Avance DRX-400 spectrometer. Chemical shift (δ) values were expressed in ppm and are referenced to the residual solvent signals with resonances at δ_H/δ_C 7.26/77.0 ($CDCl_3$). The samples were processed on an ESI-FTMS 30es ion cyclotron HR HPLC-FT spectrometer with direct injection into an electrospray interface and positive or negative ion mode.

Table 1. Solvent compositions for extraction from the sponge (%)

Solvents	Fraction									
	1	2	3	4	5	6	7	8	9	10
Hexane	100	75	50	25	0					
EtOAc		25	50	75	100	90	75	50	25	0
MeOH						10	25	50	75	100

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Biological assay. The bioassay employed parasites of the trypanosomatide family characterized by Ramirez and Guevara[9] as *Leishmania mexicana* (NR strain). The cells were cultured in liver infusion tryptose medium (LIT), supplemented with 10% fetal calf serum (GIBCO) and transferred into a fresh medium at 5-day intervals. The bioassays were carried out in triplicate repetition. Different concentrations of the drugs dissolved in DMSO were added to 10 m*l* PBS (pH) contains 2×10^6 cells. DMSO was used as a control. The cultures were placed in a New Bauer chamber and monitored daily for 96 hrs using light dispersion at 560 nm to determine cell density. The cells were also monitored using a Nikon-diaphot microscope to determine the mobility of the parasite and the integrity of the membrane.

Results and Discussion

3,6-Epidioxy-4,6,8,10-tetraethyltetradeca-7,11-dienoic acid (compound 1) has previously been isolated by others from a sponge,[4] *Plakortis* aff. *angulospiculatus*. Its molecular formula and $[\alpha]_D$ of compound 1 was $C_{22}H_{39}O_4$ by HRFABMS *m/z* 367.2859 (calcd. 367.2848, [M+H]) and +164 (*c* 2.4, $CHCl_3$).

[3,5-Diethyl-5-(2-ethyl-hex-3-enyl)-5H-furan-2-ylidene]-acetic acid methyl ether (compound 2) has also been purified from a sponge, *P. halichondrioides* and obtained as a major compound (3.0% dry weight). Its molecular formula and $[\alpha]_D$ was $C_{19}H_{30}O_3$ as revealed by HRMS *m/z* 306. 2201 (calcd. 306.2195) and +175 (*c* 1.40, CCl_4). The IR spectrum

Table 2. Comparison of ^{13}C NMR assignments for *ent*-compound 1 and compound 1 in $CDCl_3$

No	*ent*-compound 1	compound 1
1	177.1(s)	178.2(s)
2	31.1(t)	31.4(t)
3	78.3(d)	78.4(d)
4	35.1(d)	35.3(d)
5	35.5(t)	35.7(t)
6	84.0(s)	84.3(s)
7	126.8(d)	127.1(d)
8	142.0(s)	141.9(s)
9	42.3(t)	42.4(t)
10	42.3(d)	42.6(d)
11	132.9(d)	133.0(d)
12	131.4(d)	131.7(d)
13	25.4(t)	25.6(t)
14	13.9(q)	13.9(q)
15	24.7(t)	24.9(t)
16	10.7(q)	10.9(q)
17	32.6(t)	32.8 (t)
18	7.4(q)	7.6(q)
19	22.5(t)	22.8(t)
20	11.8(q)	12.1(q)
21	27.6(t)	27.9(t)
22	11.3(q)	11.6(q)
OMe	-	

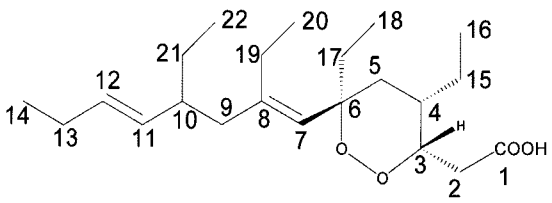

Fig. 1. Structure of *ent*-compound 1.

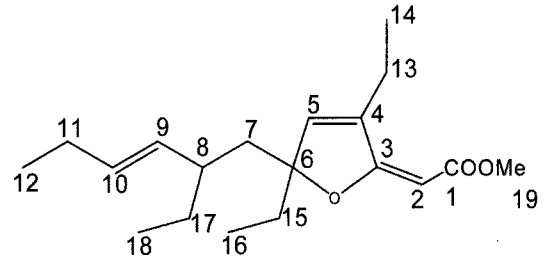

Fig. 2. Structure of *ent*-compound 2.

contained a complex group of bands at 1710, 1690, 1640, 1280, 1170 and 980 cm^{-1}).[10]

ent-3,6-Epidioxy-4,6,8,10-tetraehtyltetradeca-7,11-dienoic acid (*ent*-compound 1) was obtained as a colorless oil and its molecular formula was determined to be $C_{22}H_{39}O_4$, using positive ion HR-ESIMS [M+H] 367.2770 (calcd. 367.2961). The IR and UV spectra indicated its physical and spectral characteristics were identical to those of the already known 3,6-epidioxy-4,6,8,10-tetraethyltetradeca-7,11-dienoic acid, except for their optical rotation values (Table 2). *ent*-Compound 1 was characterized by an $[\alpha]_D^{25}$ of −165 (*c* 2.4, $CHCl_3$), whereas compound 1 rotates +164 with the same solvent. Also, *ent*-compound 2 was almost the same to

Table 3. Comparison of ^{13}C NMR assignments for *ent*-compound 2 and compound 2 in $CDCl_3$

No	*ent*-compound 2	compound 2
1	172.4(s)	166.9(s)
2	83.6(d)	83.7(d)
3	167.2(s)	171.7(s)
4	140.6(s)	140.0(s)
5	139.5(d)	139.8(d)
6	98.1(s)	98.0(s)
7	43.8(t)	41.7(t)
8	40.0(d)	27.1(d)
9	132.4(d)	134.4(d)
10	134.3(d)	129.0(d)
11	29.7(t)	23.0(t)
12	11.7(q)	14.1(q)
13	25.9(t)	18.6(t)
14	12.1(q)	12.1(q)
15	32.6(t)	31.7(t)
16	14.2(q)	8.1(q)
17	18.8(t)	34.3(t)
18	8.3(q)	10.6(q)
OMe	50.7(q)	50.5(q)

Table 4. Biological activity IC_{50} data for *ent*-compound 1 and *ent*-compound 2

Compds	Leishmania (μg/m*l*)	Microbes (μg/m*l*)		
		C. albicans	*C. neoformans*	*A. funigatus*
ent-com-1	1.0	0.6	0.5	50
ent-com-2	23.0	-a	-	-

[a]-: Not active

spectral data (^1H, ^{13}C NMR, MS, IR and UV) of compound 2, except for their optical rotation values (Table 3). *ent*-Compound 2 provided an $[\alpha]_D^{25}$ of -179°, whereas compound 2 rotates +175° with the both samples measured in CCl_4. These data supported our conclusion that *ent*-compounds 1 and 2 are the enantiomers of the known 3,6-epidioxy-4,6,8,10-tetraethyltetradeca-7,11-dienoic acid and the [3,5-Diethyl-5-(2-ethyl-hex-3-enyl)-5H-furan-2-ylidene]-acetic acid methyl ether.

The relative stereochemistry for C-6 of compound 1 has not yet been determined in the results that was reported by Compagnone et al (1998).[9] On the basis of NOE correlation, the configuration of C-7 was determined as β position, while the ethyl group at C-6 was in the α position: H-7 (δ 5.15) to H-5 (δ 1.71) and H-4β (δ 2.09), H-2 (δ 3.06) to H-5α (δ 1.25) and H-17 (δ 0.85).

Enantiomers are generally called optical isomers because their solutions rotate the plane of polarized light passing through them. If one enantiomer rotates light in the clockwise direction, a solution of the other enantiomer will rotate it in the opposite direction. Differences in biological activities between pairs of enantiomers have frequently been observed.[11,12] With nuclear magnetic resonance, elucidation of D and L enantiomer without addition of a chiral reagent or solvent is difficult.[13]

***ent*-3,6-epidioxy-4,6,8,10-tetraehtyltetradeca-7,11-dienoic acid (*ent*-compound 1)**: Clear, colorless oil; $[\alpha]_D^{25} = -165$ (*c* 0.18, $CHCl_3$); IR V_{max} 3442, 2963, 2933, 2876, 1713, 1461, 1289, 976/cm; HRESIMS *m/z* 367.2770 ([M+H], calcd. for $C_{22}H_{39}O_4$, 367.2961); For ^{13}C NMR ($CDCl_3$) data see Table 3.

***ent*-α,β-unsaturated ester (*ent*-compound 2)**: Light yellowish oil, $[\alpha]_D^{25} = -179$ (*c* 1.40, CCl_4); IR V_{max} 1714, 1688, 1626, 1161, 973, 85/cm HRESIMS *m/z* 307.2235 ([M+H], calcd. for $C_{19}H_{31}O_3$, 307.2273); For ^{13}C NMR ($CDCl_3$) data see Table 4.

ent-Compound 1 was examined for its effects on the proliferation of *L. mexicana* promastigotes (Table 4). Compound 1 was very potent against the protozoan (IC_{50} 1.00 μg/m*l*) and *ent*-compound 1 also showed good activity at the same concentration. These data compare well with the antileishmanical activity to the sponge metabolite ilimaquinone (IC_{50} 5.6 μg/m*l*) but seem less effective than ketoconozole (IC_{50} 0.06 μg/m*l*). In case of compound 2, their effect was potent giving IC_{50} of 2.71 μg/m*l*, whereas *ent*-compound 2 shows at shows at IC_{50} value of 23.0 μg/m*l*. *ent*-Compounds 1 and 2 were also assayed for activities against *Candica albicans*, *Cryptococcus neoformans* and *Aspergillus funigatus*. *ent*-Compound 1 exhibited significant antifungal acitivites *in vitro* with IC_{50} values of 0.5-50 μg/m*l*, but *ent*-compound 2 has no activities for those fungi.

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