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Trisoxazole Macrolide from a Marine Sponge *Sarcotragus* Species

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Abstract – Bioassay-directed fractionation of the lipophilic extract of a marine sponge *Sarcotragus* sp. led to the isolation of a known trisoxazole containing macrolide, mycalolide B (**1**). Its structure was identified by NMR and MS analyses. This is the first report on the isolation of macrolide from a sponge of the genus *Sarcotragus* (Order: Dictyoceratida).
Keywords – *Sarcotragus*, macrolide, mycalolide B, sponge, spectroscopy

Introduction

Marine sponges of the order Dictyoceratida have proved to be a rich source of linear furanoterpenes (Faulkner D. J., 2001). In our previous studies on the cytotoxic compounds of the sponge *Sarcotragus* sp., twenty-three cytotoxic furanoterpenes and three cyclitols were isolated (Liu *et al.*, 2001; Liu *et al.*, 2002a; Liu *et al.*, 2002b; Liu *et al.*, 2003). During our search for further cytotoxic constituents of the same sponge, a known macrolide, mycalolide B (**1**) was isolated. This macrolide was previously isolated from a sponge of the genus *Mycale* along with two other macrolides, mycalolide A and C (Fusetani *et al.*, 1989). These macrolides were chemically unique incorporating three contiguous oxazole rings and a side chain terminating in *N*-methylformamide. Prior to this investigation, several cytotoxic and antifungal macrolides encompassing two or three oxazoles have been isolated from marine organisms such as nudibranchs and their egg masses (Roesener *et al.*, 1986; Matsunaga *et al.*, 1986; Matsunaga *et al.*, 1989), stony corals (Rashid *et al.*, 1995) and sponges of the genera *Halichondria* (Kernan *et al.*, 1987; Kernan *et al.*, 1988; Kobayashi *et al.*, 1997), *Jaspis* (Kobayashi *et al.*, 1993), and *Mycale* (Phuwapraisirian *et al.*, 2002; Matsunaga *et al.*, 1998a; Matsunaga *et al.*, 1998b). Sponges of the genus *Sarcotragus* (Order Dictyoceratida) have not previously been known to contain macrolides. Thus, it appears that the distribution of oxazole containing macrolides can be expanded to this genus. The gross structure of this compound was elucidated by the aid of COSY, HSQC, HMBC, and Mass spectroscopy and comparison with reported data. Herein we describe the isolation and structure identification of a known compound, mycalolide B from the lipophilic extract of a marine sponge *Sarcotragus* sp.

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Experimental

General Procedures – ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC 200, DMX 600, and Varian Inova 500 instruments. Chemical shifts were reported with reference to the respective solvent peaks and residual solvent peaks (δ_H 3.30 and δ_C 49.0 for CD_3OD. FABMS data were obtained on a JEOL JMS-700 double focusing (B/E configuration) instrument. HPLC was performed with a YMC ODS-H80 (semipreparative, 250×10 mm i.d., 4 μm, 80Å; preparative, 250×20 mm i.d., 4 μm, 80Å) column using a Shodex RI-71 detector. Normal-phase HPLC was performed with a YMC Silica (semipreparative, 250×10 mm i.d., 5 μm, 100Å) column using a JASCO UV-975 Intelligent UV/VIS detector.

Animal Material – The sponge was collected in July 1998 (15-25 m depth), off the coast of Jeju Island, Korea. The specimen was identified as *Sarcotragus* sp. by Prof. Chung Ja Sim, Hannam University. A voucher specimen (J98J-5) of this horny sponge (registry No. Por.33) was deposited in the Natural History Museum, Hannam University, Daejon, Korea, and has been described elsewhere (Liu *et al.*, 2001).

Extraction and Isolation – The frozen sponge (7 kg) was extracted with MeOH at room temp. The MeOH

extract of the sponge displayed moderate cytotoxicity against five human tumor cell lines (ED_{50} values for A549, SK-OV-3, SK-MEL-2, XF498, and HCT15 were 19.0, 20.3, 11.8, 15.5, and 12.6 μg/mL, respectively). The MeOH extract was partitioned between water and CH_2Cl_2. The CH_2Cl_2 layer was further partitioned between aqueous MeOH and *n*-hexane to yield 54 g and 13 g fractions, respectively. The aqueous MeOH fraction was subjected to a reversed-phase flash column chromatography (YMC Gel ODS-A, 60 Å 500/400 mesh), eluting with a solvent system of 25 to 0% H_2O/MeOH, to afford twenty fractions (Fg1-Fg20). These fractions were evaluated for activity in the brine shrimp lethality assay. Fraction Fg4 was found inactive to brine shrimp larvae, but it exhibited interesting ^1H NMR signals. Therefore, fraction Fg4 was further separated by a reversed-phase flash column chromatography (YMC Gel ODS-A, 60 Å, 500/400 mesh), eluting with 25 to 0% H_2O/MeOH, to afford fourteen fractions. Compound **1** (0.9 mg) was obtained by purification of sub-fraction Fg4-8-3 using ODS HPLC.

Results and Discussion

The MeOH extract of the sponge displayed cytotoxicity against a set of five human tumor cell lines (see Experimental) and showed toxicity to brine shrimp larvae (LD_{50}, 93 μg/mL). Bioassay-directed fractionation of the extract provided an inactive but chemically interesting fraction, which contained mycalolide B (**1**).

Compound **1** was obtained as colorless oil, and its molecular formula was established as $C_{52}H_{74}N_4O_{17}$ by HRFABMS and ^{13}C NMR data (Table 1). The exact mass of $[M + Na]^+$ ion (*m/z* 1049.4924) matched well with the expected molecular formula $C_{52}H_{74}N_4O_{17}$ (△− 2.2 mmu). The ^1H NMR spectrum immediately revealed three singlets attributable to trisoxazole moiety (δ 8.07, 8.56,

Table 1. NMR Data of Compound **1** in CD_3OD

Position	δ $^1H^c$	δ $^{13}C^d$	Position	δ $^1H^c$	δ $^{13}C^d$
1		174.0	25a	1.60 (m)	31.8
2a	2.70 (dd, 14.5, 3.0)	43.5	25b	1.51 (m)	
2b	2.63 (dd, 14.5, 10.0)		26	3.10 (m)	82.0
3	4.42 (m)	68.0	26-OMe	3.33 (s)	58.2
4a	2.65 (m)	43.7	27	1.85 (m)	35.3
4b	2.49 (m)		27-Me	0.86 (d, 6.5)	16.2
5	7.39 (dt, 16.0, 9.5)	144.5	28a	1.64 (m)	28.2
6	6.15 (d, 16.0)	133.2	28b	0.97 (m)	
7		214.0	29a	1.50 (m)	31.2
8	4.23 (dd, 8.0, 6.5)	48.5	29b	1.58 (m)	
8-Me	0.89 (d, 6.5)	13.0	30	5.09 (m)	74.7
9	4.36 (d, 8.0)	79.4	31	1.92 (m)	39.0
9-OMe	3.16 (s)	57.2	31-Me	1.00 (d, 7.0)	10.0
10		140.7	32	4.74 (dd, 10.0, 2.5)	78.4
11	8.04 (s)	139.1	32-OAc		172.6
12		157.0		2.05 (s)	21.0
13		131.3	33	2.68 (m)	38.0
14	8.58 (s)	140.4	33-Me	1.00 (s)	19.8
15		158.2	34	5.10 (m)	112.0
16		130.9		[5.29 (m)]	[114.0]^e
17	8.50 (s)	140.7	35	6.75 (d, 14.0)	130.2
18		164.5			[126.5]
19	6.47 (d, 16.0)	118.2	35-NMe	2.99 (s)	27.6
20	7.13 (ddd, 16.0, 8.5, 6.0)	141.6			[33.5]
21a	2.76 (m)	34.0	35-NCHO	8.31 (s)	163.0
21b	2.51 (m)			[8.07 (s)]^e	[164.0]
22	3.48 (m)	82.0	36		170.2
22-OMe	3.36 (s)	57.8	37	3.93 (m)	81.5
23	1.90 (m)	40.8	37-OMe	3.43 (s)	59.0
23-Me	0.97 (d, 7.0)	10.0	38a	3.62 (m)	74.0
24	5.24 (m)	74.4	38b	3.65 (m)	
			38-OMe	3.33 (s)	59.5

[a-b]Assignments with the same superscript in the same column may be interchanged.
[c]Multiplicities and coupling constants are shown in parentheses.
[d]Assignments are based on HSQC and HMBC experiments.
[e]Chemical shifts for the minor conformer are shown in square brackets.

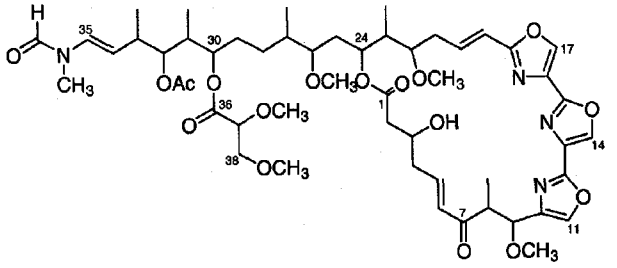

Fig. 1. Structure of compound **1**.

and 8.61) and the 2:1 singlet pair of the formamide signals (δ 8.35, 8.10), which are characteristic of the mycalolides, kabiramides, ulapualides, halichondramides, and jaspisamides class of compounds. The magnitude of chemical shift differences of the doublets was proportional to the distance from the *N*-methyl formamide unit, suggesting that each pair of doublet signals was due to restricted rotation around the C-N bond of the *N*-methyl formamide group. Comparison of the ^{1}H and ^{13}C NMR data (Table 1) of compound **1** with those reported for above mentioned compounds showed that it is identical to mycalolide B (Fusetani *et al.*, 1989). Compound **1** was identified as mycalolide B by further analysis of the COSY, HMBC, and HSQC data. Along with mycalolide B, we isolated two more trisoxazole containing macrolides, but because of small amount we could not elucidate their structures. This is the first isolation of macrolides from a sponge belonging to the genus *Sarcotragus*, although this genus has been shown to contain furanoterpenes and cyclitol derivatives. It is interesting from a chemotaxonomic point of view that structurally related macrolides have been found among sponges of the genera *Halichondria*, *Jaspis*, *Mycale*, and *Sarcotragus*, which belong to different orders.

Mycalolide B has antifungal and cytotoxic effects (Fusetani *et al.*, 1989). It was shown that mycalolide B binds to G-actin with a 1:1 molecular ratio, depolymerizes actin filaments at rates that exceed the maximal rate of depolymerization achieved by cytochalasin D, and inhibits polymerization of G-actin (Saito *et al.*, 1994). Mycalolide B was suspected to bind to various intracellular proteins, probably through the Michael addition of a sulfhydryl group to C-5 of mycalolide B (Wada *et al.*, 1998).

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