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A New Cyclitol Derivative from a Sponge *Stelletta* Species

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Abstract – Guided by the brine shrimp lethality assay, a new (**4**) and three known cyclitol derivatives (**1-3**) were isolated from the marine sponge *Stelletta* sp. Norsarcotride A (**4**) showed significant cytotoxicity against a small panel of five human tumor cell lines.
Keywords – *Stelletta* sp., cyclitol derivative, cytotoxicity, marine sponge

Introduction

Marine sponges of the genus *Stelletta* are reported to contain various sterols (Miyamoto *et al.*, 2002; Yan *et al.*, 2001; Li *et al.*, 1994; Guerriero *et al.*, 1991), terpenoids (Oku *et al.*, 2000; McCormick *et al.*, 1996; Ryu *et al.*, 1996; Su *et al.*, 1994; McCabe *et al.*, 1982), alkaloids (Nozawa *et al.*, 2001; Matsunaga *et al.*, 1999; Tsukamoto *et al.*, 1999a, 1999b, 1996; Shin *et al.*, 1997; Fusetani *et al.*, 1994; Hirota *et al.*, 1990), and fatty acids (Bergquist *et al.*, 1984). In our study on the cytotoxic constituents of the marine sponge *Stelletta* sp. collected from Korean waters, four acetylenic acids, two phosphatidylcholines, two ω-hydroxy fatty acid methyl esters, and six monoglycerides have been isolated (Zhao *et al.*, 2003a; Zhao *et al.*, 2003b). In our continuing study on the cytotoxic compounds from the same sponge, a new cyclitol derivative (**4**), along with three known ones (**1-3**) (Liu *et al.*, 2002; Kim *et al.*, 1999), were isolated. The gross structures of the compounds were elucidated by the aid of NMR and MS analyses. The isolation, structure elucidation, and biological evaluation of the compounds are described herein.

Experimental

General – Optical rotations were obtained using a JASCO DIP-1000 digital polarimeter. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Inova 500 and Bruker AC200. Chemical shifts were reported with reference to the respective residual solvent peaks (δ_H 3.30 and δ_C 49.0 for CD_3OD). FAB-CID tandem MS data were obtained using a JEOL JMS SX-102A. Gel filtration chromatography was performed with Sephadex LH-20 (Pharmacia Biotech AB). HPLC was performed with YMC-Pack CN (250×10 mm I.D., 5 μm, 120 Å) column using a Shodex RI-71 detector.

Animal material – The sponge was collected by hand using SCUBA (20 m depth) in October 2001, off the coast of Ullung Island, Korea. The specimen was identified as *Stelletta* sp. by Prof. C. J. Sim, Hannam University. A voucher specimen (registry No. Spo. 37) was deposited at the Natural History Museum, Hannam University, Korea, and has been described elsewhere (Zhao *et al.*, 2003a).

Isolation – The frozen sponge (15 kg) was extracted with MeOH at room temperature. The MeOH extract displayed moderate toxicity to brine shrimp larvae (LD_{50}, 296 μg/mL). 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 aqueous MeOH (5.2 g) and *n*-hexane soluble (19.1 g) fractions. The aqueous MeOH fraction was subjected to a step gradient reversed-phase flash column chromatography (YMC Gel ODS-A, 60 Å 500/400 mesh) eluting with a solvent system of 50 to 0% H_2O/MeOH, to afford twenty-two fractions. These fractions were evaluated for activity employing the brine shrimp assay, and the fractions 8-16 were found active. The fraction 13 was further separated by a Sephadex LH-20 column chromatography eluting with MeOH, to afford eighteen fractions. The

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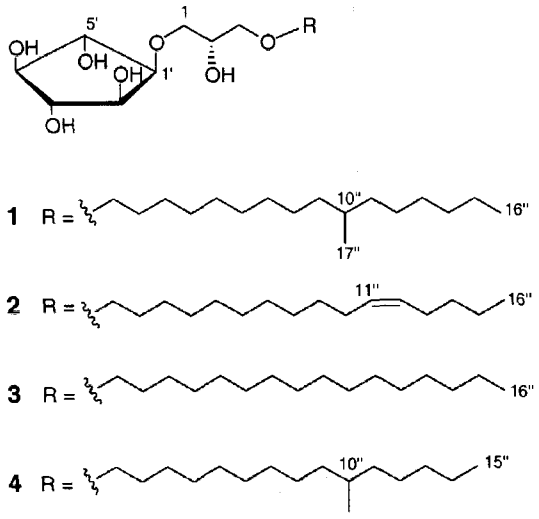
subfraction 13-11 and 13-12 were purified by reversed-phase HPLC (YMC-Pack CN, 250×10 mm I.D., 5 μm, 120 Å) eluting with 43% H_2O/MeOH to yield compounds **1** (10.0 mg), **2** (0.6 mg), **3** (3.9 mg), and **4** (7.1 mg).

Compound 1: light yellow oil; $[\alpha]^{21}_D$ -7°, (*c* 0.28, MeOH); ^{1}H NMR (500 MHz, CD_3OD) δ 3.73 (1H, dd, *J* = 9.5, 3.5 Hz, H-1_a), 3.53 (1H, dd, *J* = 9.5, 6.0 Hz, H-1_b), 3.92 (1H, m, H-2), 3.48 (1H, dd, *J* =10.0, 5.0 Hz, H-3_a), 3.45 (1H, dd, *J* = 10.0, 6.0 Hz, H-3_b), 3.53 (1H, t, *J* = 7.0 Hz, H-1'), 3.85 (1H, t, *J* = 5.5 Hz, H-2'), 3.74 (1H, t, *J* = 6.0 Hz, H-3'), 3.55 (1H, t, *J* = 6.0 Hz, H-4'), 3.82 (1H, t, *J* = 6.0 Hz, H-5'), 3.47 (2H, t, *J* = 8.0 Hz, H-1"), 1.56 (2H, quint, *J* = 7.0 Hz, H-2"), 1.26-1.36 (23H, m, H-3"-H-8", H-9_a", H-10", H-11_a", H-12"-H-15"), 1.11 (2H, m, H-9_b", H-11_b"), 0.90 (3H, t, *J* = 7.0 Hz, H-16"), 0.86 (3H, d, *J* = 7.0Hz, H-17"); ^{13}C NMR (50 MHz, CD_3OD) δ 73.2 (C-1), 70.9 (C-2), 73.1 (C-3), 84.5 (C-1'), 75.1 (C-2'), 82.0 (C-3'), 81.6 (C-4'), 80.0 (C-5'), 72.7 (C-1"), 30.6-31.1 (C-2", C-4"-C-7", and C-13"), 27.2 (C-3"), 28.1 (C-8", C-12"), 38.2 (C-9", C-11"), 33.9 (C-10"), 33.1 (C-14"), 23.7 (C-15"), 14.5 (C-16"), 20.2 (C-17"); FAB-CID MS/MS *m/z* 485 $[M + Na]^+$ (100), 469 (0.2), 455 (0.2), 411 (0.2), 427 (0.2), 413 (0.3), 399 (0.3), 371 (0.4), 357 (0.2), 343 (0.2), 329 (0.2), 315 (0.3), 301 (0.2), 245 (1.0), 229 (0.3), 215 (0.4), 171 (0.7), 155 (0.8); HRFABMS *m/z* 485.3435 (calcd for $C_{25}H_{50}O_7Na$, 485.3454).

Compound 2: light yellow oil; $[\alpha]^{21}_D$ +18°, (*c* 0.12, MeOH); ^{1}H NMR (500 MHz, CD_3OD) δ 3.73 (1H, dd, *J* = 9.5, 3.5 Hz, H-1_a), 3.53 (1H, dd, *J* = 9.5, 6.0 Hz, H-1_b), 3.92 (1H, m, H-2), 3.48 (1H, dd, *J* =10.0, 5.0 Hz, H-3_a), 3.45 (1H, dd, *J* = 10.0, 6.0 Hz, H-3_b), 3.53 (1H, t, *J* = 7.0 Hz, H-1'), 3.85 (1H, t, *J* = 5.5 Hz, H-2'), 3.74 (1H, t, *J* = 6.0 Hz, H-3'), 3.55 (1H, t, *J* = 6.0 Hz, H-4'), 3.82 (1H, t, *J* = 6.0 Hz, H-5'), 3.45 (2H, t, *J* = 8.0 Hz, H-1"), 1.56 (2H, quint, *J* = 7.0 Hz, H-2"), 1.26-1.36 (18H, m, H-3"-H-9", H-14"-H-15"), 2.02 (4H, m, H-10", H-13"), 5.34 (2H, t, *J* = 5.5 Hz, H-11", H-12"), 0.90 (3H, t, *J* = 7.0 Hz, H-16"); ^{13}C NMR (50 MHz, CD_3OD) δ 73.2 (C-1), 70.9 (C-2), 73.1 (C-3), 84.5 (C-1'), 75.1 (C-2'), 82.0 (C-3'), 81.6 (C-4'), 80.0 (C-5'), 72.7 (C-1"), 30.3-30.8 (C-2", C-4"-C-9"), 27.2 (C-3"), 28.1 (C-10"), 130.8 (C-11", C-12"), 27.9 (C-13"), 33.1 (C-14"), 23.4 (C-15"), 14.3 (C-16"); FAB-CID MS/MS *m/z* 469 $[M + Na]^+$ (100), 453 (0.3), 439 (0.3), 425 (0.4), 371 (0.4), 357 (0.2), 343 (0.2), 329 (0.2), 315 (0.3), 301 (0.2), 245 (1.0), 229 (0.3), 215 (0.4), 171 (0.7), 155 (0.8); HRFABMS *m/z* 469.3141 (calcd for $C_{24}H_{46}O_7Na$, 469.3142).

Compound 3: light yellow oil; $[\alpha]^{21}_D$ +5°, (*c* 0.01, MeOH); ^{1}H NMR (500 MHz, CD_3OD) δ 3.72 (1H, dd, *J* = 9.5, 3.5 Hz, H-1_a), 3.53 (1H, dd, *J* = 9.5, 6.0 Hz, H-1_b), 3.91 (1H, m, H-2), 3.47 (1H, dd, *J* =10.0, 5.0 Hz, H-3_a), 3.43 (1H, dd, *J* = 10.0, 6.0 Hz, H-3_b), 3.53 (1H, t, *J* = 7.0 Hz, H-1'), 3.82 (1H, t, *J* = 5.5 Hz, H-2'), 3.72 (1H, t, *J* = 6.0 Hz, H-3'), 3.54 (1H, t, *J* = 7.0 Hz, H-4'), 3.82 (1H, t, *J* = 6.0 Hz, H-5'), 3.45 (2H, t, *J* = 8.0 Hz, H-1"), 1.56 (2H, quint, *J* = 7.0 Hz, H-2"), 1.26-1.36 (20H, m, H-3"-H-15"), 0.90 (3H, t, *J* = 7.0 Hz, H-16"); ^{13}C NMR (50 MHz, CD_3OD) δ 73.3 (C-1), 70.9 (C-2), 73.1 (C-3), 84.5 (C-1'), 75.1 (C-2'), 82.0 (C-3'), 81.6 (C-4'), 80.0 (C-5'), 72.7 (C-1"), 30.5-30.8 (C-2", C-4"-13"), 27.2 (C-3"), 33.1 (C-14"), 23.7 (C-15"), 14.4 (C-16"); FAB-CID MS/MS *m/z* 471 $[M + Na]^+$ (100), 455 (0.5), 441 (0.3), 427 (0.3), 413 (0.3), 399 (0.3), 385 (0.3), 371 (0.3), 357 (0.3), 343 (0.3), 329 (0.2), 315 (0.3), 301 (0.2), 245 (1.0), 229 (0.3), 215 (0.4), 171 (0.7), 155 (0.8); HRFABMS *m/z* 471.3294 (calcd for $C_{24}H_{48}O_7Na$, 471.3297).



Compound 4: light yellow oil; $[\alpha]^{21}_D$ -5°, (*c* 0.16, MeOH); ^{1}H and ^{13}C NMR data, see Table 1; FAB-CID MS/MS *m/z* 471 $[M + Na]^+$ (100), 455 (0.4), 441 (0.2), 427 (0.3), 413 (0.4), 399 (0.4), 371 (0.4), 357 (0.4), 343 (0.2), 329 (0.2), 315 (0.3), 301 (0.3), 245 (1.2), 229 (0.5), 215 (0.4), 171 (0.9), 155 (1.1); HRFABMS *m/z* 471.3294 (calcd for $C_{24}H_{48}O_7Na$, 471.3297).

Results and Discussion

The MeOH extract of the sponge showed toxicity to brine shrimp larvae (LD_{50}, 296 μg/mL). Guided by the brine shrimp lethality assay, the MeOH extract was successively fractionated employing reversed-phase flash column chromatography, Sephadex LH-20 gel filtration column chromatography, and ODS HPLC to afford compounds **1-4** as a group of the active components.

Compound **1** was isolated as a light yellow oil. The molecular formula of **1** was established as $C_{25}H_{50}O_7$ on the basis of HRFABMS. The $[M + Na]^+$ ion was observed at *m/z* 485.3435 ($C_{25}H_{50}O_7Na$, Δ -1.9 mmu). The NMR and FAB-

CID tandem mass data of **1** were identical to sarcotride A reported from the Korean marine sponge *Petrosia* sp. (Kim *et al.*, 1999) and *Sarcotragus* sp. (Liu *et al.*, 2002).

Compound **2** was isolated as a light yellow oil. The molecular formula of **2** was established as $C_{24}H_{46}O_7$ on the basis of HRFABMS. The $[M + Na]^+$ ion was observed at *m/z* 469.3141 ($C_{24}H_{46}O_7Na$, Δ -0.3 mmu). The NMR and FAB-CID tandem mass data of **2** were identical to sarcotride B which was reported from the Korean marine sponge *Sarcotragus* sp. (Liu *et al.*, 2002).

Compound **3** was isolated as a light yellow oil. The molecular formula of **3** was established as $C_{24}H_{48}O_7$ on the basis of HRFABMS. The $[M + Na]^+$ ion was observed at *m/z* 471.3294 ($C_{24}H_{48}O_7Na$, Δ -0.3 mmu). The ^1H and ^{13}C NMR data revealed that **3** was a dihydro analogue of **2**. Thus, compound **3** was identified as sarcotride C (Liu *et al.*, 2002).

Norsarcotride A (**4**) was isolated as a light yellow oil. The molecular formula of **4** was established as $C_{24}H_{48}NO_7$ on the basis of MS and NMR spectral analyses. The FABMS of **4** showed the $[M + H]^+$ peak at *m/z* 449 accompanied by the $[M + Na]^+$ peak at *m/z* 471. The exact mass of the $[M + Na]^+$ ion (*m/z* 471.3294) matched well with the expected molecular formula $C_{24}H_{48}O_7Na$ (Δ -0.3 mmu). The ^1H and ^{13}C NMR data featured the same pattern as those of **1** (Table 1). The methyl branching position in **4** was clearly recognized from the 28-mass gap between the major fragment ions at *m/z* 399 and 371 (Kim *et al.*, 1999) in the FAB-CID tandem mass spectrum of the $[M + Na]^+$ ion (Fig. 1). The relative stereochemistry of the five-membered cyclitol moiety of **4** was presumed to be identical to **1** by comparison of NMR spectral data.

Table 1. ^1H and ^{13}C NMR Data of **4**[a]

position	δ_H	δ_C
1	3.72 (dd, 9.5, 6.5)	73.2
	3.53 (dd, 9.5, 6.0)	
2	3.91 (m)	70.9
3	3.47 (dd, 10.0, 5.0)	73.1
	3.43 (dd, 10.0, 6.0)	
1'	3.53 (t, 7.0)	84.4
2'	3.82 (t, 5.5)	75.1
3'	3.72 (t, 6.0)	81.9
4'	3.54 (t, 7.0)	81.6
5'	3.82 (t, 6.0)	80.1
1"	3.45 (t, 8.0)	72.7
2"	1.56 (quint, 7.0)	30.6-31.1
3"	1.28-1.34 (m)	27.2
4"-7"	1.28-1.34 (m)	30.6-31.1
8"	1.28-1.34 (m)	28.16[c]
9"	1.28-1.34 (m)	38.22[b]
	1.11 (m)	
10"	1.28-1.34 (m)	33.9
11"	1.28-1.34 (m)	
	1.11 (m)	38.21[b]
12"	1.28-1.34 (m)	28.14[c]
13"	1.28-1.34 (m)	33.1
14"	1.28-1.34 (m)	23.7
15"	0.88 (t, 7.0)	14.5
16"	0.86 (d, 7.0)	20.2

[a]Spectra were recorded in CD_3OD at 500 and 125 MHz for ^1H and ^{13}C, respectively. [b,c]Assignments with the same superscript in the same column may be interchanged.

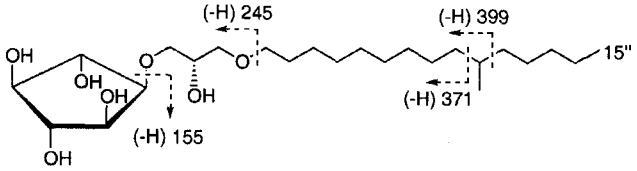


Fig. 1. Key fragmentations of the $[M + Na]^+$ ion of **4** in FAB-CID MS/MS.

Table 2. Cytotoxicity of Compound **4** against Human Solid Tumor Cells[a]

Compound	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
4	4.3	5.1	5.3	4.4	3.9
doxorubicin	0.03	0.13	0.06	0.19	0.29

[a]Data expressed in ED_{50} values (μg/mL). A549, human lung cancer; SK-OV-3, human ovarian cancer; SK-MEL-2, human skin cancer; XF498, human CNS cancer; HCT 15, human colon cancer.

It is being recognized that the cyclitol derivatives are widely distributed in sponges and they appear to be characteristic metabolites of the phylum Porifera. (Costantino *et al.*, 2002, 1994, 1993; Ishibashi *et al.*, 1993; Kobayashi *et al.*, 1993). Sarcotrides A-C (**1-3**) were reported to show moderate to significant cytotoxicity against a small panel of five human tumor cell lines (Liu *et al.*, 2002). Norsarcotride A (**4**) exhibited similar range of cytotoxicity against the same panel of five human tumor cell lines (Table 2).

Acknowledgments

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