Notes

Cyclitol Derivatives from the Sponge *Sarcotragus* Species

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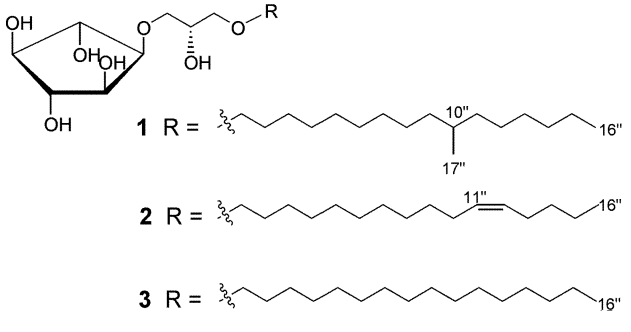
Marine sponges of the order Dictyoceratida are known to contain various furanosesterterpene derivatives that possess interesting biological properties.[1] Dozens of new furano- and pyrroloterpenoids have been isolated from *Sarcotragus* sp. (Dictyoceratida) collected from Korean waters.[2,3] In our continuing study on the cytotoxic compounds of *Sarcotragus* sp., the cyclitol derivative sarcotride A (**1**) has been isolated along with two new congeners (**2** and **3**). The gross structures of the compounds were elucidated by the aid of COSY, HMQC, and HMBC experiments. The isolation, structure elucidation, and biological activity of the compounds are described herein.

The methanol extract of the sponge displayed cytotoxicities against five human tumor cell lines (see Experimental Section) and showed toxicity to brine shrimp larvae (LD_{50}, 93 μg/mL). Guided by the brine shrimp assay, the methanol extract was successively fractionated employing reversed-phase flash column chromatography and ODS HPLC to afford compounds **1-3** as the active components.

Sarcotride A (**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* 485.3436 ($C_{25}H_{50}O_7Na$, Δ -1.8 mmu). The NMR data of **1** was identical to those reported for the cyclitol derivative from the Korean marine sponge *Petrosia* sp.[4] The location of the methyl branching in **1** was clearly recognized from the FAB-CID tandem mass spectrum of the $[M+Na]^+$ ion. The fragmentations involved parallel pathways of sequential losses of CH_2 groups differing by one carbon except for the fragmentations occurring at a branching point, where the significant losses of CH_2 groups differ by two carbons causing an obvious interruption in the main series of peaks. Thus, the location of methyl branching is clear from the 28-mass gap between the fragment ion at *m/z* 399 and the ion at *m/z* 371. The optical rotation of **1** was the same as the reported one.[4]



Sarcotride B (**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* 469.3137 ($C_{24}H_{46}O_7Na$, Δ -0.4 mmu). In the ^1H NMR spectrum of **2**, twelve crowded oxymethine and oxymethylene proton signals were observed at δ 3.43-3.91, while a total of nine oxygenated carbon signals were observed at δ 70.9-84.4 in the ^{13}C NMR spectrum (Table 1). By careful examination of these signals, a cyclopentanepentol moiety and a glycerol moiety could be unravelled. Signals characteristic of a long alkyl chain were observed at the upfield region of the NMR spectra. Confirmation of the partial structural units and determination of their connectivity were made with the aid of a HMBC spectrum, whose results are summarized in Table 1. The H-1' (δ 3.53) of cyclopentanepentol showed the HMBC correlation with one (δ_C 73.2, C-1) of the two oxymethylene carbons of the glycerol moiety. The other oxymethylene carbon (δ_C 73.1, C-3) of the glycerol unit showed the HMBC correlation to oxymethylene protons resonating at 3.45 (H-1"). The terminal methyl and the olefinic proton signals were observed as a triplet (δ 0.90, *J* =

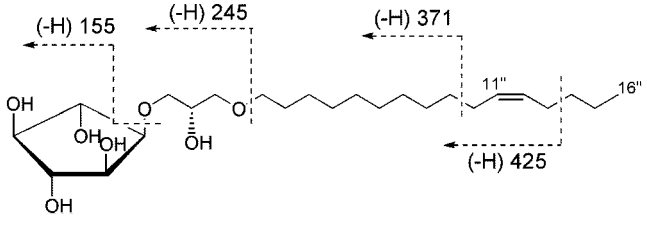


Figure 1. Key fragmentations of the $[M-Na]^+$ ion of **2** in FAB-CID MS/MS.

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Table 1. ^{1}H NMR (CD_3OD, 500 MHz) and ^{13}C NMR Data (CD_3OD, 50 MHz) of **2**

Position	δ_H (mult, *J*)	δ_C	HMBC (H to C)
1	3.72 (dd, 9.5, 3.5)	73.2	2, 3, 1'
	3.53 (dd, 9.5, 6.0)		
2	3.91 (m)	70.9	1, 3, 1'
3	3.47 (dd, 10.0, 5.0)	73.1	2, 3, 1"
	3.43 (dd, 10.0, 6.0)		
1'	3.53 (t, 7.0)	84.4	1, 2', 3', 5'
2'	3.82 (t, 5.5)	75.1	1, 1', 3'
3'	3.72 (t, 6.0)	81.9	2', 4'
4'	3.54 (t, 7.0)	81.6	3', 5'
5'	3.82 (t, 6.0)	80.0	1, 1', 4'
1"	3.45 (t, 8.0)	72.7	2, 3, 2", 3"
2"	1.56 (quint, 7.0)	30.3-30.8	1", 3"
3"	1.28-1.34 (m)	27.2	
4"-7"	1.28-1.34 (m)	30.3-30.8	
8"	1.28-1.34 (m)	30.3-30.8	
9"	1.28-1.34 (m)	30.3-30.8	
10"	2.02 (m)	28.1	9", 11", 14"
11"	5.34 (t,5.5)	130.8	10"
12"	5.34 (t,5.5)	130.8	13"
13"	2.02 (m)	27.9	9", 11", 12"
14"	1.28-1.34 (m)	33.1	13", 15"
15"	1.28-1.34 (m)	23.4	14", 16"
16"	0.90 (t, 7.0)	14.3	14", 15"

7.0 Hz) and a triplet (δ 5.34, J = 5.5 Hz), respectively. The chemical shifts of the allylic carbons (δ 28.1 and δ 27.9) indicated that the double bond geometry is *cis*.[5] The chemical shifts of H-2 and C-2 (δ 3.91, 70.9) indicated that the cyclitol and the alkyl chain were ether linked to the glycerol at C-1 and C-3, respectively. The double bond position in **2** was clearly recognized from the FAB-CID tandem mass spectrum of the $[M+Na]^+$ ion (Figure 1). Major fragmentations of the $[M+Na]^+$ ion were observed as odd mass ions due to the remote charge fragmentation which is characteristic of the collisional activation of alkali-metal-cationized ion. The location of the double bond was clear from the 54-mass gap between the major fragment ions of allylic cleavage at *m/z* 425 and 371.[4]

The relative stereochemistry of the five-membered cyclitol moiety of **2** was presumed to be identical to **1** based on the comparison of NMR spectral data .

Sarcotride C (**3**) was isolated as a light yellow oil. FABMS of **3** showed the $[M+H]^+$ ion at *m/z* 449 accompanied by the $[M+Na]^+$ ion at *m/z* 471. As indicated by the ^{1}H and ^{13}C NMR data, **3** was a dihydro analog of **2**. The relative stereochemistry of the five-membered cyclitol moiety and the C-2 of glycerol moiety was presumed to be identical to **1** based on the NMR data.

Compounds **1-3** showed moderate to significant cytotoxicity against a small panel of five human tumor cell lines (Table 2). Sarcotride A (**1**) inhibited DNA replication *in vitro* at the level of initiation.[4] A couple of biological activities such as antifeedant activity[6] and nerve growth factor stimulatory activity[7] were attributed to this class of compounds.

Table 2. Cytotoxicity of Compounds **1-3** against Human Solid Tumor Cells[a]

Compound	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
1[b]	>10	9.5	>10	9.8	9.4
2	11.5	5.1	7.9	7.5	10.5
3	4.8	5.3	4.6	4.3	5.3
doxorubicin	0.02	0.16	0.02	0.13	0.06

[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. [b]Data from the reference 4.

Compounds analogous to **1-3** appear to be widely distributed in various sponges. They were reported from Caribbean sponges *Pseudoceratina crassa*,[6] *Verongula gigantea*, *Aplysina fistularis fulva*, *Aplysina cauliformis*, *Neofibularia nolitangere*.[8] Okinawan sponges *Luffariella* sp., *Biemna* sp., *Nestopongia* sp.,[7] and a Korean sponge *Petrosia* sp.[4] They might be ubiquitous metabolites of sponges and play some significant role in the life of the organisms.

Experimental Section

General Experimental Procedures. 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). FABMS data were obtained on a JEOL JMS-700 double focusing (B/E configuration) instrument. HPLC was performed with an YMC ODS-H80 (semipreparative, 250 × 10 mm i.d., 4 μm, 80 Å; preparative, 250 × 20 mm i.d., 4 μm, 80 Å) column using a Shodex RI-71 detector.

Animal Material. The sponge was collected in July 1998 (15-25 m depth), off Cheju Island, Korea. This specimen was identified as *Sarcotragus* sp. by Prof. Chung Ja Sim, Hannam University. A voucher specimen (J98J-5) of the sponge (registry No. Por. 33) was deposited in the Natural History Museum, Hannam University, Daejon, Korea, and has been described elsewhere.[2]

Isolation. The frozen sponge (7 kg) was extracted with MeOH at room temp. The MeOH extract of the sponge displayed cytotoxicities against five human tumor cell lines (A549, SK-OV-3, SK-MEL-2, XF498, HCT15), the ED_{50} values (μg/mL) were 19.0, 20.3, 11.8, 15.5, and 12.6, respectively. The MeOH extract was partitioned between water and CH_2Cl_2. The CH_2Cl_2 soluble was further partitioned between 90% methanol and *n*-hexane to yield 90% methanol (54 g) and *n*-hexane soluble (13 g) fractions. The 90 % methanol 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 → 0% H_2O/MeOH, to obtain twenty fractions (Fg1-Fg20). These fractions were evaluated for activity in the brine shrimp assay, and fractions Fg6-Fg9 were found active. Compound **1** (5.3 mg), **2** (4.7

mg), and **3** (1.2 mg) were obtained by purification of fraction Fg 6-8, Fg 6-5, and Fg 10-6, respectively, by ODS HPLC.

Sarcotride A (1): light yellow oil; $[\alpha]_D^{21}$ -6°, (*c* 0.15, MeOH); FABMS *m/z* 485 $[M+Na]^+$ (100), 295 (2), 245 (3), 322 (15), 136 (5); HRFABMS *m/z* 485.3436 (calcd for $C_{25}H_{50}O_5Na$, 485.3454).

Sarcotride B (2): light yellow oil; $[\alpha]_D^{21}$ +24°, (*c* 0.14, MeOH) ^1H and ^{13}C NMR data, see Table 1; FABMS *m/z* 469 $[M+H]^+$ (79), 455 (8), 371 (3), 322 (15), 245 (6), 171 (69), 164 (23), 155 (7), 132 (71), 95 (1); HRFABMS *m/z* 469.3137 (calcd for $C_{24}H_{16}O$-Na, 469.3141).

Sarcotride C (3): light yellow oil; $[\alpha]_D^{21}$ +10°, (*c* 0.04, MeOH); ^1H NMR (500 MHz, CD_3OD) δ 3.72 (1H, dd, *J* =9.5, 3.5 Hz, H-1a), 3.53 (1H, dd, *J* = 9.5, 6.0 Hz, H-1b), 3.91 (1H, m, H-2), 3.47 (1H, dd, *J* =10.0, 5.0 Hz, H-3a), 3.43 (1H, dd, *J* = 10.0, 6.0 Hz, H-3b), 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"); FABMS *m/z* 471 $[M+Na]^+$ (100), 449 $[M+H]^+$ (20), 307 (5), 135 (12).

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