

Full Paper

## Glycerolipids from a *Sarcotragus* Species Sponge

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**Abstract:** One known and two new glycerolipids have been isolated from a *Sarcotragus* sp. marine sponge. The gross structures were established based on NMR and MS analysis.

**Keywords:** Glycerolipid, marine sponge, *Sarcotragus*.

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### Introduction

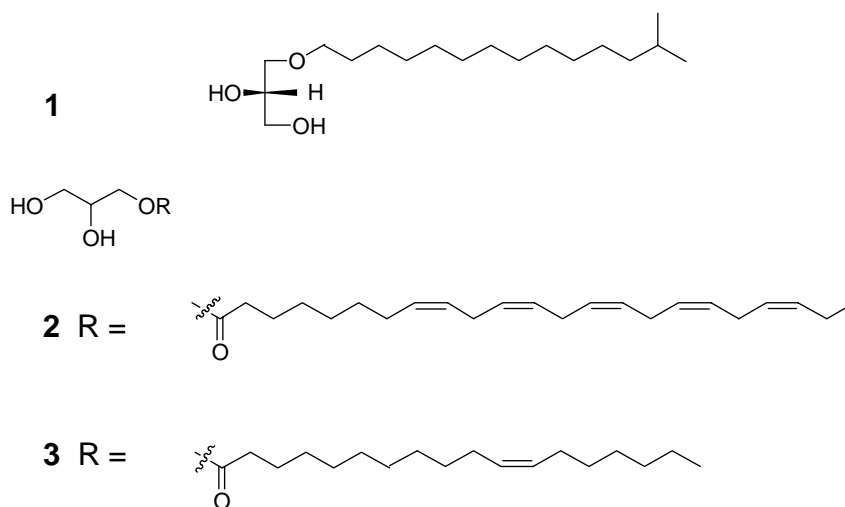
Marine sponges of the order Dictyoceratida are known to contain various linear furano-esterpenes and their derivatives that display a wide range of interesting bioactivities [1, 2]. Thirty five furano- and pyrroloterpenoids, three cyclitol derivatives and a macrolide have been isolated from *Sarcotragus* sp. (Dictyoceratida) collected from Korean waters [3-7]. During our continuing study on the cytotoxic compounds of *Sarcotragus* sp., three glycerolipids **1-3** have now been isolated. Compounds **2** and **3** have not been previously described. The isolation and elucidation of the gross structures of these compounds by COSY, HSQC, and HMBC experiments are described.

### Results and Discussion

The methanol extract of the sponge displayed cytotoxicity against five human tumor cell lines (see Extraction and Isolation section of the Experimental) and also showed toxicity towards brine shrimp larvae (LD<sub>50</sub>, 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 separate the causative components **1-3** (Figure 1) from the inactive fractions. Compound **1** was isolated as an amorphous solid. Its molecular formula was established as  $C_{18}H_{38}O_3$  based on MS and NMR spectral analyses. The FABMS of **1** showed the  $[M+H]^+$  ion at  $m/z$  303, accompanied by the  $[M+Na]^+$  ion at  $m/z$  325. Although this compound was previously identified in rabbit harderian glands by MS spectrometry [8], it has now been isolated for the first time from the sponge by HPLC and its spectroscopic data is herein described for the first time. The  $^1H$ -NMR spectrum exhibited signals for an isopropyl terminated aliphatic long chain ( $\delta$  0.87, d,  $J = 6.5$  Hz,  $\delta_C$  22.3) and signals associated with the presence of a glycerol monoether moiety. Proton signals for three pairs of oxymethylene protons and one oxymethine were observed: a two proton AB multiplet (an apparent triplet of doublets,  $J = 6.5, 1.5$  Hz) at  $\delta$  3.45, two AB doublets of doublets centered at  $\delta$  3.72 ( $J = 11.0, 4.5$  Hz) and  $\delta$  3.49 ( $J = 11.0, 6.5$  Hz), two AB doublets of doublets centered at  $\delta$  3.47 ( $J = 10.0, 5.0$  Hz) and  $\delta$  3.40 ( $J = 10.0, 6.5$  Hz) and a pseudo quintuplet at  $\delta$  3.74 for the methine proton. The  $^{13}C$ -NMR spectrum was in agreement with this structure and showed three oxymethylene carbons at  $\delta$  73.3 (C-1),  $\delta$  72.7 (C-1'), and  $\delta$  64.7 (C-3), and an oxymethine at  $\delta$  72.3 (C-2) [9]. The configuration at C-2 was established from the positive optical rotation, which is a general feature of long-chain 1-O-alkyl-*sn*-glycerols [10].

Figure 1.



Compound **2** was isolated as a colorless oil. Its molecular formula was established as  $C_{26}H_{44}O_4$  based on MS and NMR spectral analyses (Table 1). The FABMS of **2** showed a  $[M+H]^+$  ion at  $m/z$  421 accompanied by a  $[M+Na]^+$  ion at  $m/z$  443. The  $^1H$ - and  $^1H$ - $^1H$ -COSY spectra showed signals at  $\delta$  0.97 (3H, t,  $J = 7.5$  Hz) and  $\delta$  2.08 (4H, dq,  $J = 7.5, 7.5$  Hz, H-7', H-22'); the latter was coupled with the signal at  $\delta$  5.36 (10H, m). In addition it displayed signals for ten olefinic carbons, eleven methylenes and a terminal methyl group. A ten proton multiplet at  $\delta$  5.36 and an eight-proton multiplet at  $\delta$  2.83 ppm indicated the presence of five non-conjugated double bonds and four doubly allylic methylenes, respectively. The *Z* nature of the olefinic double bonds was indicated by the allylic methylenes [5]. In addition to these signals two mutually coupled sets of ABX type protons [ $\delta$  4.14 (1H, dd,  $J = 11.5, 4.5$  Hz), 4.05 (1H, dd,  $J = 11.5, 6.5$ ), 3.81(1H, m), 3.54 (2H, p,  $J = 5.0$ ,

2.5 Hz)] for a glycerol moiety were observed. Although the stereochemistry of the glycerol moiety has not been established at this time, **2** is a new monoacyl glycerol, to the best of our knowledge.

**Table 1.**  $^1\text{H}$ -NMR ( $\text{CD}_3\text{OD}$ , 500 MHz) and  $^{13}\text{C}$ -NMR Data ( $\text{CD}_3\text{OD}$ , 50 MHz) of **2**.

Position	$\delta_{\text{H}}$ (mult, $J$ )	$\delta_{\text{C}}$
1	4.14 (dd, 11.5, 4.5) 4.05(dd,11.5, 6.5)	71.2
2	3.81 (m)	71.0
3	3.54 (dd, 5.0, 2.5)	73.1
1'		175.4
2'	2.34 (t, 7.5)	34.9
3'	1.62 (quint, 8.0)	25.9
4'	1.28 - 1.39 (m)	28.0
5'	1.28 - 1.39 (m)	29.8
6'	1.28 - 1.39 (m)	29.8
7'	2.08 (m)	28.0
8'	5.36 (m)	132.8
9'	5.36 (m)	130.9
10'	2.83 (m)	26.6
11'	5.36 (m)	129.5
12'	5.36 (m)	129.4
13'	2.83 (m)	26.6
14'	5.36 (m)	129.0
15'	5.36 (m)	129.0
16'	2.83 (m)	26.6
17'	5.36 (m)	129.0
18'	5.36 (m)	129.0
19'	2.83 (m)	26.4
20'	5.36 (m)	128.9
21'	5.36 (m)	128.2
22'	2.08 (m)	21.5
23'	0.97 (t, 7.5)	14.6

Compound **3** was isolated as a colorless oil. The molecular formula was established as  $\text{C}_{21}\text{H}_{40}\text{O}_4$ , based on MS and NMR spectral analyses. The FABMS of **3** showed a  $[\text{M}+\text{H}]^+$  ion at  $m/z$  357 accompanied by a  $[\text{M}+\text{Na}]^+$  ion at  $m/z$  379. According to  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR data, compound **3** displayed a typical fatty acid spectrum, with an intense peak at  $\delta$  1.26-1.36 ppm, due to the methylenes in the fatty acyl chain, a triplet at 0.87 ppm, revealing terminal methyl groups, another triplet at 2.36 ppm due to the methylene groups  $\alpha$  to the carbonyl, and olefinic protons which were observed at  $\delta$  5.36. In addition peaks for a glycerol moiety were observed [ $\delta$  4.22 (1H, dd,  $J$  = 11.5, 3.5 Hz), 4.16 (1H, dd,  $J$  = 11.5, 6.5 Hz), 3.94 (1H, m), 3.70 (1H, dd,  $J$  = 11.0, 4.0 Hz), 3.61 (1H, dd,  $J$  = 11.0, 6.0 Hz)]. The double bond position in **3** was clearly recognized from the FAB-CID tandem mass spectrum of the  $[\text{M}+\text{Na}]^+$  ion, where a 54-mass gap between the major fragment ions of allylic cleavage at  $m/z$  307 and 253 was observed. The *Z* nature of the olefinic double bonds was indicated by the allylic methylenes [5]. Glyceryl ether has strongly mutagenic properties which has potentially important implications for the etiology of colon cancer [11], while acyl glycerols are common primary metabolites, they may act as the second messengers, not as a primary cue of metamorphosis [12].

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## Experimental

### General

Optical rotations were obtained using a JASCO DIP-1000 digital polarimeter.  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectra were recorded on a Bruker AC200 and Varian Inova 500 instruments. Chemical shifts are reported with reference to the respective residual solvent peaks ( $\delta_{\text{H}}$  3.30 and  $\delta_{\text{C}}$  49.0 for  $\text{CD}_3\text{OD}$ ). FABMS data were obtained on a JEOL JMS-700 double focusing (B/E configuration) instrument. HPLC was performed with an YMC ODS-H80 column (semipreparative, 250 x 10 mm, 4  $\mu\text{m}$ , 80 Å; preparative, 250 x 20 mm, 4  $\mu\text{m}$ , 80 Å) using a Shodex RI-71 detector.

### Animal Material

The sponge was collected in July 1998 (15-25 m depth), off the coast of Cheju Island, Korea. This specimen was identified as *Sarcotragus* sp. by Prof. Sim, Hannam University. A voucher specimen (J98J-5) of the sponge (registry No. Por. 33) was deposited at the Natural History Museum, Hannam University, Daejeon, Korea, and has been described elsewhere [3].

### Extraction and Isolation

The frozen sponge (7 kg) was extracted with MeOH at room temp. The MeOH extract of the sponge displayed cytotoxicity against five human tumor cell lines (A549, SK-OV-3, SK-MEL-2, XF498, HCT15) with  $\text{ED}_{50}$  values of 19.0, 20.3, 11.8, 15.5 and 12.6, respectively. The MeOH extract was partitioned between water and  $\text{CH}_2\text{Cl}_2$  and the  $\text{CH}_2\text{Cl}_2$  soluble fraction was further partitioned between 90% methanol and *n*-hexane to yield alcohol (54 g) and alkane soluble (13 g) fractions. The 90 % methanol fraction was subjected to reversed-phase flash column chromatography (YMC Gel ODS-A, 60 Å 500/400 mesh), eluting with a solvent system of 25  $\rightarrow$  0%  $\text{H}_2\text{O}/\text{MeOH}$ , to give 20 fractions (F 1 – F 20). These fractions were evaluated for activity in the brine shrimp assay, and fractions F 6 – F 9 were found to be active. Further fractionation by ODS HPLC, eluting with 88% methanol afforded compounds **1** (1.9 mg), **2** (0.9 mg), and **3** (0.5 mg) from fractions F 10-7, F 10-4, and F 11-5, respectively.

**Compound 1:** A pale amorphous powder;  $[\alpha]_{\text{D}}^{21} + 6^\circ$ , (c 0.06, MeOH);  $^1\text{H}$ -NMR (500 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  3.72 (1H, dd,  $J = 11.0, 4.5$  Hz, H-1a), 3.49 (1H, dd,  $J = 11.0, 6.5$  Hz, H-1b), 3.74 (1H, m, H-2), 3.47 (1H, dd,  $J = 10.0, 5.0$  Hz, H-3a), 3.40 (1H, dd,  $J = 10.0, 6.5$  Hz, H-3b), 3.45 (1H, td,  $J = 6.5, 1.5$  Hz, H-1'), 1.56 (2H, quint,  $J = 6.5$  Hz, H-2'), 1.26-1.34 (20H, m, H-3'-H-12'), 1.52 (2H,

m, H-13'), 0.87 (6H, d,  $J = 6.5\text{Hz}$ , H-14', H-15');  $^{13}\text{C-NMR}$  (50 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  73.3 (C-1), 72.3 (C-2), 64.7 (C-3), 72.7 (C-1'), 31.0 (C-2'), 27.2 (C-3'), 29.2-30.8 (C-4'-C-11'), 40.3 (C-12'), 28.5 (C-13'), 22.3 (C-14', C-15'); FABMS  $m/z$  325  $[\text{M} + \text{Na}]^+$  (100), 303 (30), 137 (18).

**Compound 2:** A colorless oil;  $[\alpha]_D^{21} + 10.2^\circ$ , (c 0.15, MeOH);  $^1\text{H-}$  and  $^{13}\text{C-NMR}$  data, see Table 1; FABMS  $m/z$  443  $[\text{M} + \text{Na}]^+$  (5), 421 (1), 307 (30), 154 (100).

**Compound 3:** A colorless oil;  $[\alpha]_D^{21} - 2.9^\circ$ , (c 0.01, MeOH);  $^1\text{H-NMR}$  (500 MHz,  $\text{CDCl}_3$ )  $\delta$  4.22 (1H, dd,  $J = 11.5, 3.5\text{ Hz}$ , H-1a), 4.16 (1H, dd,  $J = 11.5, 6.5\text{ Hz}$ , H-1b), 3.94 (1H, m, H-2), 3.70 (1H, dd,  $J = 11.0, 4.0\text{ Hz}$ , H-3a), 3.61 (1H, dd,  $J = 11.0, 6.0\text{ Hz}$ , H-3b), 2.36 (2H, t,  $J = 8.0\text{ Hz}$ , H-2'), 1.64 (2H, quint,  $J = 7.0\text{ Hz}$ , H-3'), 1.26-1.36 (22H, m, H-4'-H-9', H-14'-H-17'), 2.01 (4H, m, H-10', 13'), 5.36 (2H, m, H-11', 12'), 0.87 (3H, t,  $J = 7.5\text{Hz}$ , H-18');  $^{13}\text{C-NMR}$  (50 MHz,  $\text{CDCl}_3$ )  $\delta$  65.1 (C-1), 71.8 (C-2), 63.0 (C-3), 174.3 (C-1'), 34.1 (C-2'), 27.2 (C-3'), 29.2-30.8 (C-4'-9', C-14', C-15'), 130.1 (C-11', -12'), 28.0 (C-10', C-13'), 33.2 (C-16'), 23.3 (C-17'), 14.3 (C-18'); FABMS  $m/z$  379  $[\text{M} + \text{Na}]^+$  (100), 357  $[\text{M} + \text{H}]^+$  (15), 339 (28), 321 (6), 176 (49).

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*Sample availability:* Compounds **1-3** are available from authors.

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