Monocyclic Diterpene Benzenoids from Taiwanese Marine Sponge *Strongylophora durissima*

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ABSTRACT

Cacospongin D (1) was isolated from a Taiwanese marine sponge *Strongylophora durissima*. Compound 1 is unstable and appears to be a possible biogenetic precursor of strongylophorines. Its structure was determined on the basis of extensive spectral analysis of 1 and its dimethylate (2) and methyl ester derivatives (3). Cacospongin dimethyl ester (3) showed moderate cytotoxicity against human oral epidermoid carcinoma cells.

Key words: Strongylophora durissima; Monocyclic diterpene benzenoids; Cytotoxicity.

INTRODUCTION

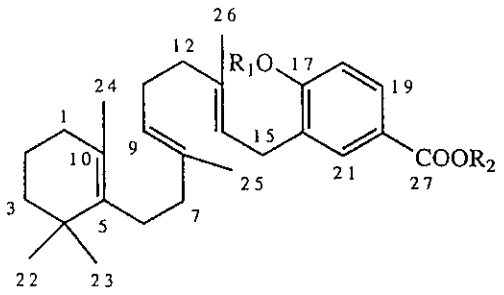
The marine sponges of the genus *Strongylophora* were reported to contain novel meroditerpenoids with interesting biological activities.[1,2] Nevertheless, very little is known about the biogenetic pathway of these particular compounds such as strongylophorines. In our pursuit of isolating biologically active secondary metabolites from marine sponges,[3-6] we have undertaken the chemical investigation of the marine sponge *Strongylophora durissima*.[7] Its crude extract showed potent ichthyotoxicity and some meroditerpenoids exhibited significant cytotoxicities against mouse leukemia cells (P-338), human mouth epidermoid carcinoma (KB), lung (A-549) and colon (HT-29) cancer cells. Recently we have reported the isolation of three new meroditerpenoids, strongylophorines 9, 11 and 12 in addition to the known strongylophorines 1, 2, 3, 7 and 8.[8,9] During this investigation a rare compound, cacospongin D,[10] which might be a precursor of meroditerpenoids, was isolated. The structure of **1** was determined on the basis of HMBC and NOESY spectral analysis and its mono- (**2**) and dimethylates (**3**). In this note, we wish to report the the isolation and structural elucidation of **1**, which has never been reported from the Taiwanese sponge *S. durissima*.

RESULTS AND DISCUSSION

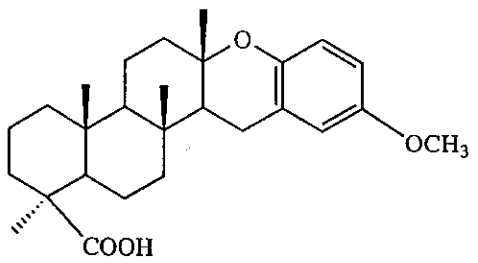
The acetone extract of the fresh sponge *S. durissima* was partitioned between EtOAc and H_2O. The EtOAc-soluble fraction was evaporated and subjected to Si gel column and preparative TLC chromatography to yield cacospongin D (**1**). The

EtOAc-soluble fraction of the acetone extract from another batch of *S. durissima* was chromatographyed on a silica gel column followed by methylation and HPLC to furnish cacospongin D methyl ester (**3**).

Cacospongin D (**1**) was obtained as a pale yellow oil and its molecular formula was determined as $C_{27}H_{38}O_3$ from a combination of low resolution EIMS (*m/z* 410, $[M]^+$) and FABMS (*m/z* 433, $[M+Na]^+$) spectra, and was also confirmed from EIMS spectrum of its dimethylate (**2**), which showed a molecular ion at *m/z* 438. The IR spectrum indicated the presence of a hydroxyl (3396 cm^{-1}), an acid carbonyl (1695 cm^{-1}) and an aromatic (1608, 1512 cm^{-1}) group respectively. The presence of a hydroxybenzoyl moiety was deduced from UV absorption bands at 254 and 287 nm, and EIMS fragments at *m/z* 151 of **1** and at *m/z* 179 of its dimethylate (**2**). The ^1H NMR spectrum (Table 1) of **1** showed the presence of three aromatic protons at δ 6.84 (H-18, d, J = 8.0 Hz), 7.88 (H-19, d, J = 8.0 Hz) and 7.88 (H-21, s), two olefinic protons at δ 5.30 (H-14, t, J = 6.9 Hz) and 5.10 (H-9, m), two methylene protons at δ 3.40 (d, J = 6.9 Hz, H-15) and five methyl singlets at δ 0.99 (× 2), 1.60, 1.64 and 1.81. The relationship of protons was obtained from the COSY spectrum, which gave cross peaks of H-1/H-2/H-3, H-9/H-11, H-14/H-15 and H-18/H-19. In addition to DEPT, the assignment of carbon signals was facilitated by HSQC and HMBC correlation spectra of **1** and **3** (Fig. 1). The HMBC of **1** revealed correlations of Me-24/C-1, C-5; Me-22/C-4, C-5; Me-25/C-8, C-9; Me-26/C-12, C-13, C-14; H-15/C-14, C-16, C-17, C-21 and H-18/C-16, C-17, C-20; H-19/C-27; H-21/C-27; H-15/C-17 and H-15/C-21, confirming the hydroxyl group at the C-17 and the acidic group at the C-20 as well as the tetraprenyl side chain. Upon methylation cacospongin D (**1**) yielded a dimethylate (**2**) which exhibited two methoxyl groups at δ 3.87 (δ 51.8) and 3.88 (δ 55.5) in the NMR spectra. The



1, $R_1 = R_2 = H$
2, $R_1 = R_2 = Me$
3, $R_1 = H$, $R_2 = Me$



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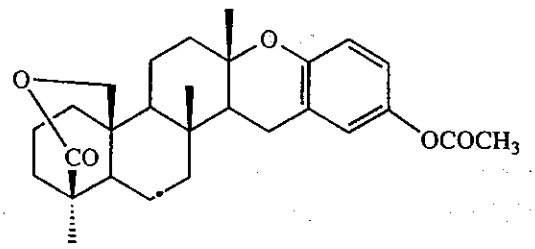

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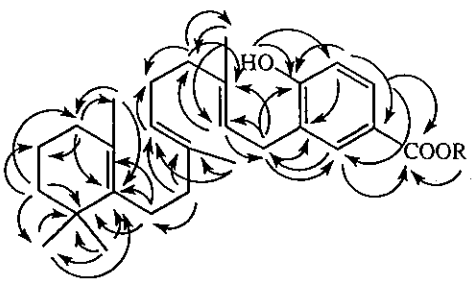

Fig. 1. HMBC correlations of cacospongin D (**1**, R = H) and its methyl ester (**3**, R = Me).

Table 1. ^{1}H NMR Spectral Data[a] (300 MHz, $CDCl_3$) for Compounds 1-3

C#	1	2	3
1	1.90 m	1.89 m	1.90 m
2	1.57 m	1.59 m	1.18 m
3	1.40 m	1.41 m	1.42 m
6	2.02 m	2.01 m	2.03 m
7	2.02 m	2.01 m	2.02 m
9	5.10 m	5.14 m	5.11 m
11	2.14 m	2.11 m	2.13 m
12	2.14 m	2.11 m	2.13 m
14	5.30 (t, 6.9)	5.32 (t, 7.8)	5.34 (t, 6.9)
15	3.40 (d, 6.9)	3.35 (d, 7.2)	3.39 (d, 7.1)
18	6.84 (d, 8.0)	6.85 (d, 8.2)	6.83 (d, 8.1)
19	7.88 (d, 8.0)	7.89 (d, 8.2)	7.80 (d, 8.1)
21	7.88 s	7.82 s	7.82 s
22	0.99 s	0.98 s	0.99 s
23	0.99 s	0.98 s	0.99 s
24	1.60 s	1.59 s	1.59 s
25	1.64 s	1.63 s	1.64 s
26	1.81 s	1.72 s	1.78 s
COOMe		3.87 s	3.88 s
OMe		3.88 s	

[a] δ in ppm (*J* in Hz); TMS (Tetramethylsilane) as internal standard

EIMS fragmentation of **1-3** also agreed with the structural assignment. The presence of a base peak at *m/z* 137 ($C_{10}H_{17}^+$) and the peaks at *m/z* 259 ($C_{19}H_{31}^+$), *m/z* 151 ($C_8H_7O_3^+$), 165 ($C_9H_9O_3^+$) and 179 ($C_{10}H_{11}O_3^+$) suggested that these compounds underwent allylic cleavage between C-6 and C-7, C-11 and C-12, and benzylic cleavage between C-14 and C-15 in EIMS (Fig. 2). The NOESY spectra of **2** and **3** were also consistent with the structural features of **1-3**, in which the cross peaks of H-18/OMe-17 and H-21/H-15 were observed (Fig. 3).

Cacospongin D represents a rare monocyclic diterpene-benzenoid similar to jaspaquinol.[11] Although compound **1** has been reported in the sponge *Cacospongia* sp.,[10] this is the first report of **1** in *S. durissima*. The occurrence of cacospongin D (**1**) may be of significance from a biogenetic point of view. Pharmacological screening revealed that compound **3** exhibited moderate cytotoxicity against human oral epidermoid carcinoma cells with an IC_{50} value of 6.7 μg/mL while compound **2** was inactive.

EXPERIMENTAL

General Procedures

Optical rotations were recorded on a JASCO DIP-1000 polarimeter. IR and UV spectra were measured on Hitachi T-2001 and Hitachi U-3210 spectrophotometers, respectively. EIMS and CIMS spectra were recorded on a VG Quattro 5022 Mass spectrometer. The high resolution EIMS and FABMS data were collected on a JEOL JMS-HX 110 mass spectrometer. The ^{1}H, ^{13}C NMR, DEPT, COSY,

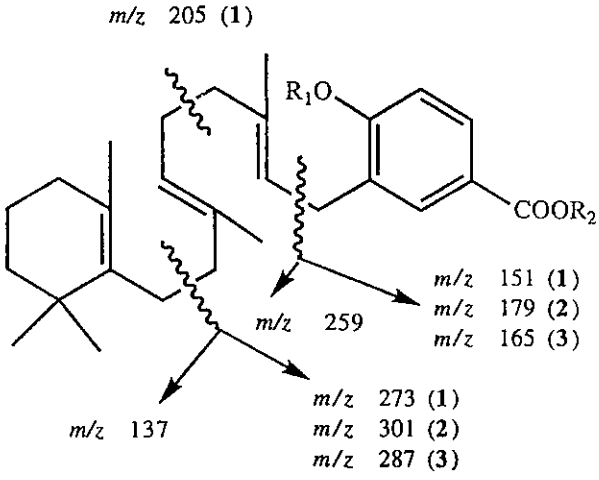

Fig. 2. EIMS fragmentation of cacospongin D (**1**, $R_1 = R_2 = H$), cacospongin D dimethylate (**2**, $R_1 = R_2 = Me$) and cacospongin D methyl ester (**3**, $R_1 = H$, $R_2 = Me$).

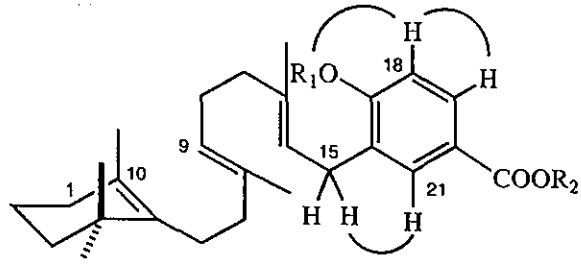

Fig. 3. Selective NOESY of Compounds **1-3**.

HSQC, HMBC and NOESY spectra were recorded on Bruker Avance FT-300 and Varian FT-500 spectrometers. The chemical shifts are given in δ (ppm) and coupling constants in Hz.

Animal Materials

The sponge *Strongylophora durissima* was collected at Ken-Ting and Lan-Yu, respectively, at a depth of 10-15 m in September, 1998. The sponge was identified by G. H. Lee, Institute of Oceanography, Academia Sinica. A voucher specimen (SP-76) was deposited in the Institute of Marine Resources, National Sun Yat-sen University, Kaohsiung.

Extraction and Isolation

The fresh sponge (2.65 Kg) was extracted and fractionated as described previously to give 7 fractions: A (1.42 g), B (0.82 g), C (0.68 g), D (0.72 g), E (0.66 g), F (0.82 g) and G (0.73 g).[8] Fraction G on further chromatography on a silica gel column (10 g) with $CHCl_3$/MeOH (10:1) gave cacospongin D (**1**, 5.3 mg).

In another batch of fresh sponge (500 g) was crushed in a blender and was extracted with acetone (500 mL × 2). The combined filtrate was concentrated under vacuum to give a suspension, which was adjusted to 500 mL in volume with addition of H_2O (500 mL). The suspension was extracted exhaustively with EtOAc (500 mL). The EtOAc layer (9.5 g) was chromatographed on a silica gel column and eluted with *n*-hexane/EtOAc of increasing polarity to give seven fractions, A (curcuphenol, 40 mg), B (remeirin, 496 mg), C (strongylophrine-1, **4**, 1.97 g), D (melyne-C, 713 mg), E (900 mg), F (409 mg) and G (3.3 g). Fraction E was methylated with diazomethane (Et_2O/acetone, 5 °C, overnight) to give a residue, which was chromatographed on a silica gel column and eluted with *n*-hexane/CH_2Cl_2 of increasing polarity. Further purification by HPLC (Si gel, *n*-hexane/CH_2Cl_2/MeOH, 12:12:1) afforded cacospongin D methyl ester (**3**, 40 mg). Strongylophorin-11 (**5**, 8 mg) and strongylophorine-3 (247 mg) were obtained from fraction F. Fraction G yielded strongylophorine-2 (650 mg), strongylophorine-7 (10 mg) and strongylophorine-8 (40 mg) by Si gel column chromatography (*n*-hexane/CH_2Cl_2/MeOH, 20:20:1).

Cacospongin D (1)

Isolated as a pale yellow oil, $[\alpha]_D^{26}$ -57.3 (*c* 0.1, MeOH); UV (MeOH) λ_{max} 254 (3.50) and 287 (2.98); IR (neat) ν_{max} 3396, 2923, 2857, 1695, 1652, 1608, 1512, 1457, 1419, 1396, 1274, 1214 and 1112 cm^{-1}; ^1H- and ^{13}C-NMR ($CDCl_3$) spectral data are listed in Tables 1 and 2, respectively. EIMS (70 eV) *m/z* 410 ($[M]^+$, 15), 395 (1.5), 286 (4), 273 (8), 259 (13), 231 (6), 205 (6), 204 (10), 189 (12), 177 (7), 151 (28), 137 (100), 123 (35), 107 (25), 95 (80), 81 (69), 69 (37), 55 (33); FABMS *m/z* 433 ($[M+Na]^+$), 455 ($[M+2Na-H]^+$).

Cacospongin D dimethylate (2)

Methylation (excess CH_2N_2 in Et_2O/acetone, 5 °C, 3 days) of cacospongin D (**1**, 1.5 mg) and usual work-up gave cacospongin D dimethylate (**2**, 1.4 mg); ^1H- and ^{13}C-NMR ($CDCl_3$) spectral data are listed in Tables 1 and 2. EIMS (70 eV) *m/z* 438 ($[M]^+$, 12), 423 (1), 407 (1), 314 (4), 301 (9), 287 (5), 271 (4), 259 (25), 245 (8), 233 (12), 219 (11), 201 (23), 179 (76), 173 (26), 159 (23), 149 (27), 137 (100), 123 (89), 109 (21), 95 (57), 81 (32), 69 (19), 55 (18); FABMS *m/z* 439 ($[M+H]^+$), 461 ($[M+Na]^+$).

Cacospongin D methyl ester (3)

Amorphous solid, $[\alpha]_D^{25}$ -3.8° (*c* 0.8, CH_2Cl_2); UV (MeOH) λ_{max} 259 (3.91); IR ($CHCl_3$) ν_{max} 3450, 2929, 1716, 1695, 1433, 1280, 1118 and 771 cm^{-1}; ^1H- and ^{13}C-NMR ($CDCl_3$) spectral data are listed in Tables 1 and 2, respectively. EIMS (70 eV) *m/z*

Table 2. ^{13}C NMR Spectral Data[a] (75 MHz, $CDCl_3$) for Compounds 1-3

C#.	1	2	3
1	32.8 t	32.7 t	32.8 s
2	19.6 t	19.6 t	19.9 t
3	39.9 t	39.8 t	39.9 t
4	35.0 s	35.0 s	35.0 s
5	137.1 s	137.2 s	137.2 s
6	27.8 t	27.9 t	27.9 t
7	40.2 t	40.3 t	40.3 t
8	136.8 s	136.7 s	136.7 s
9	122.9 d	123.6 d	123.1 d
10	126.9 s	126.9 s	127.0 s
11	26.3 t	26.7 t	26.5 t
12	39.7 t	39.8 t	39.8 t
13	139.8 s	139.8 s	139.1 s
14	120.8 d	121.6 d	121.1 d
15	29.8 t	29.7 t	29.6 t
16	126.7 s	130.1 s	127.0 s
17	159.5 s	161.1 s	159.0 s
18	115.9 d	109.5 d	115.6 d
19	130.5 d	129.4 d	129.8 d
20	121.5 s	122.1 s	122.3 s
21	132.6 d	130.8 d	131.9 d
22	28.8 q	28.6 q	28.7 q
23	28.6 q	28.3 q	28.7 q
24	19.8 q	19.8 q	19.6 q
25	16.1 q	16.0 q	16.1 q
26	16.4 s	16.2 s	16.4 q
27	170.7 s	167.2 s	167.4 s
17-OMe		55.5 q	
27-OMe		51.8 q	51.9 q

[a] Mutiplicities were obtained from DEPT

424 ([M]$^+$, 13), 409 (1), 393 (1), 287 (4), 286 (6), 273 (4), 259 (8), 245 (9), 231 (5), 220 (4), 219 (4), 205 (9), 203 (9), 187 (11), 177 (8), 165 (27), 159 (12), 137 (100), 123 (26), 109 (28), 107 (23), 95 (81), 81 (53), 69 (34), 55 (19); FABMS *m/z* 425 ([M+H]$^+$), 447 ([M+Na]$^+$); negative HRFABMS *m/z* 423.2905 ([M-H]$^-$, calcd for $C_{28}H_{39}O_3$, 423.2904); HREIMS *m/z* 424.2979 ([M]$^+$, calcd for $C_{28}H_{40}O_3$, 424.2978).

Cytotoxicity Assay

A bioassay against KB (human oral epidermoid carcinoma) tumor cells was based on reported procedures.[12] The cytotoxicity assay depends on the binding of methylene blue to fixed monolayers of cells at pH 8.5, washing the monolayer, and releasing the dye by lowering the pH value. The 96-well plate was dipped into a 0.01 M borate-buffer solution four times in order to remove the dye. Then, 100 μL/well ethanol-0.1M HCl (1/1) was added as a dye eluting solvent, and the absorbance was measured on a microtiter plate reader (Dynatech, MR 7000) at a wavelength of 650 nm. The IC_{50} value was defined by a comparison with the untreated cells as the concentration of test sample resulting in 50% reduction of absorbance. Doxorubicine was used as a standard compound, which exhibited an IC_{50} value of 0.4 μg/mL under the above conditions.

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