Cytotoxic Polyketides from the Marine Sponge *Discodermia calyx*

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Received December 22, 2006

Bioassay-guided fractionation of the MeOH extract from the sponge *Discodermia calyx* collected off the coast of Jeju Island, South Korea, led to the isolation of a polyketide, icadamide C (**1**), along with previously reported theopederin K (**3**). Structure elucidation was performed by a combination of high resolution mass and 2D-NMR (principally COSY, HMBC, HSQC, and NOESY) spectroscopy. Stereochemistry of compound **1** was determined as 2*R**, 3*R**, 6*R**, 10*S**, 11*S**, 12*R**, 13*S**, 15*R** and 2′*S* by NMR data and Marfey analysis. Isolated metabolites displayed potent cytotoxic activity against a small panel of five human solid tumor cell lines with ED_{50} values of less than 0.1 μg/mL.

Key Words : Marine sponge, *Discodermia calyx*, Polyketide, Cytotoxicity, Marfey analysis

Introduction

Sponges of the order Lithistida (subclass Tetractinomorpha) are well known for producing a wide range of secondary metabolites, including macrolides and peptides. Marine sponges belonging to the genus *Discodermia* are a promising source of diverse chemical metabolites. The marine sponge *Discodermia calyx* (order Lithistida, family Theonellidae) is reported to contain the calyculins,[1-6] unique polyketides bearing nitrogen and phosphorus functions. These macrolides exhibit a variety of biological activities including antitumor and smooth muscle contractile, which are attributed to inhibition of protein phosphatase 1 and 2A.[5] Three sulfated ceramides, calyceramides, have also been isolated from the same sponge as inhibitors of neuraminidase.[7]

As a part of our continuing research aimed at the discovery of biologically active secondary metabolites from marine organisms,[8-10] we isolated two C-21 furanoterpenes and eight bisindole alkaloids from the marine sponge *Discodermia calyx*, as a first report from a sponge belonging to the subclass Tetractinomorpha.[11] In the continuation of our study on this marine sponge, we isolated two pederin polyketides (**1** and **3**) from the 90% MeOH fraction. This paper deals with the isolation, structure elucidation, and cytotoxicity evaluation of polyketides (**1** and **3**).

Results and Discussion

The MeOH crude extract of *Discodermia calyx* was subjected to activity-guided fractionation using solvent partition and reversed-phase flash column chromatography followed by repeated reversed-phase HPLC to yield compounds **1** and **3**. The structures of these metabolites were deduced using NMR (^{1}H, ^{13}C, COSY, HSQC, HMBC, and NOESY) and MS analysis, and optical rotation data.

Compound **1** was obtained as colorless oil, and its molecular formula was established as $C_{38}H_{61}N_5O_{12}$ on the basis of HRFABMS and NMR data (Table 1). The exact mass of the $[M + Na]^+$ ion (*m/z* 802.4234) matched well with the expected molecular formula $C_{38}H_{61}N_5O_{12}$ (Δ+2.0 mmu). The ^{1}H NMR spectrum revealed signals of an exo-methylene (δ_H 4.64 and 4.80), a disubstituted double bond (δ_H 5.18 and 5.75), a conjugated diene (δ_H 6.18, 6.32, 7.15, and 6.06), three methoxy groups (δ_H 3.19, 3.51, and 3.19), seven oxymethine protons (δ_H 3.90, 4.26, 3.89, 4.13, 3.48, 3.26, and 3.59), and four methyl groups (δ_H 0.83, 0.96, 0.98, and 1.18). ^{13}C NMR data (assigned by HMBC and HSQC experiments) of **1** exhibited three carbonyl carbon signals at δ_C 168.0 (C-25), 174.0 (C-8), and 178.0 (C-1′), and one ketal carbon signal at δ_C 101.0 (C-6). A signal at δ_C 158.0 was assigned as a guanidine carbon of arginine, and this analysis confirmed close relationship with the bioactive metabolites known as theopederins,[12-14] mycalamides,[15-18] and onnamides.[19-22] Analysis of the ^{1}H-^{1}H COSY spectrum revealed the presence of four spin systems comprising H_3-26 to H_3-27, H-10 to H-13, H-15 to H-24, and H-2′ to H-5′. Moreover, analysis of the HMBC spectrum confirmed connectivities of these partial structures into one molecule (Fig. 2). After careful comparison with reported NMR data, it was clear that the obtained data of **1** was quite similar with that of icadamide A (**2**), which was previously isolated from a marine sponge *Leiosella* sp. (order Dictyoceratida, family Spongiidae),[23] except one additional methoxyl group attached to C-17. Therefore, compound **1** was defined as 17-*O*-methyl derivative of icadamide A (**2**). There have been reports on the isolation of *O*-methylated derivatives along with parent compounds *i.e.* theopederins K (**3**) and L (**4**).[14] mycalamides A (**5**) and B (**6**).[16] It was mentioned that to eliminate the possibility that methoxyl group was solvent derived, an extraction of the *Mycale* sponge was again carried out with ethanol instead of methanol, which led to the isolation of the same mycalamides A (**5**) and B (**6**).[16] From above discussion, it can be assumed that icadamide C

Table 1. 1D and 2D NMR Data for **1** in CD_3OD at 500 MHz

position	δ_C[a]	δ_H (mult., J = Hz)	COSY	HMBC
2	70.5	3.90[b]	H-26, H-3	
3	42.5	2.20 (m)	H-27, H-2	C-4, C-28
4	147.5			
5a	34.2	2.41 (d, 14.0)	H-5b	C-4, C-28, C-6
5b		2.31 (d, 14.0)	H-5a	C-4, C-28, C-6, C-3
6	101.0			
7	73.0	4.26 (s)		C-5, C-8, C-6
8	174.0			
10	75.2	5.63 (d, 9.0)	H-11	C-8
11	70.2	3.89[b]	H-10, H-12	
12	74.5	4.13 (dd, 9.5, 6.0)	H-11, H-13	C-13, C-29, C-10
13	80.6	3.48[b]	H-12	
14	41.0			
15	76.8	3.26[b]	H-16	
16	35.8	1.47 (dd, 10.5, 3.5)	H-17, H-15	
17	81.5	3.59 (m)	H-18, H-16	
18	132.5	5.18 (dd, 15.0, 8.0)	H-19, H-17	C-20
19	134.0	5.75 (dt, 15.0, 7.0)	H-18, H-20	C-17, C-20
20	36.2	2.97 (m)	H-21, H-19	C-21, C-19, C-18, C-22
21	141.8	6.18 (dt, 14.0, 7.0)	H-22, H-20	C-20
22	130.5	6.32 (dd, 14.0, 10.0)	H-23, H-21	C-23
23	140.6	7.15 (dd, 15.0, 10.0)	H-22, H-24	C-25
24	123.4	6.06 (d, 15.0)	H-23	C-22, C-25
25	168.0			
26	18.4	1.18 (d, 6.5)	H-2	C-2, C-3
27	12.6	0.98 (d, 7.0)	H-3	C-4, C-2, C-3
28a	110.0	4.80[b]		C-3, C-5
28b		4.64 (br s)		C-3, C-5
29a	87.0	5.12 (d, 7.0)	H-29b	C-12
29b		4.78[b]	H-29a	
30	23.5	0.96 (s)		C-13, C-14, C-15
31	13.5	0.83 (s)		C-13, C-14, C-15, C-30
6-OCH_3	42.0	3.19 (s)		
13-OCH_3	61.0	3.51 (s)		C-13
17-OCH_3	55.8	3.19 (s)		C-17
1'	178.0			
2'	55.0	4.34 (dd, 7.0, 5.5)	H-3'a, H-3'b	C-1'
3'a	31.2	1.88 (m)	H-2', H-3'b, H-4'	C-2', C-1', C-5', C-4'
3'b		1.72 (m)	H-3'a, H-2', H-4'	
4'	25.8	1.60 (m)	H-5', H-3'a, H-3'b	C-2', C-5', C-3'
5'	42.0	3.19[b]	H-4'	C-7'
7'	158.0			

[a]Signals were assigned by HMBC and HSQC experiments. [b]Overlapped with other signals.

(**1**) is also a natural product rather than an artifact produced during isolation.

^1H-^1H coupling constants and the ^{13}C NMR data, which were almost superimposable on those of icadamide A (**2**), suggested the identical relative stereochemistry for **1**. The relative stereochemistry of **1** was further confirmed on the basis of NOESY data (Fig. 3) and analysis of coupling constants. The *cis* fusion of the tetrahydropyran ring and the dioxane ring was assigned from the small coupling constant ($J_{11,12}$ = 6.0 Hz) between H_{eq}-11 and H_{ax}-12 (*trans* fusion: J = ~10.0 Hz).[24-26] This was further confirmed from the NOESY correlation between H_{ax}-10 and H_{ax}-15 (*trans* fusion would demand different correlations). NOESY correlations between H_{ax}-10/H_{ax}-15. H_{ax}-10/H_{ax}-29. and H_{ax}-13/H_{ax}-29 indicated their spatial proximity. Furthermore, NOESY correlations H_3-27 to H_{ax}-5. H_{ax}-2 to H_3-6-OCH_3. and H_{ax}-12 to H_3-31 showed 1,3-diaxially oriented configurations. The *trans* geometry for double bonds was assigned from the large coupling constants (J = 14~15 Hz). Thus, the relative stereochemistry of the ring carbons of compound **1** was

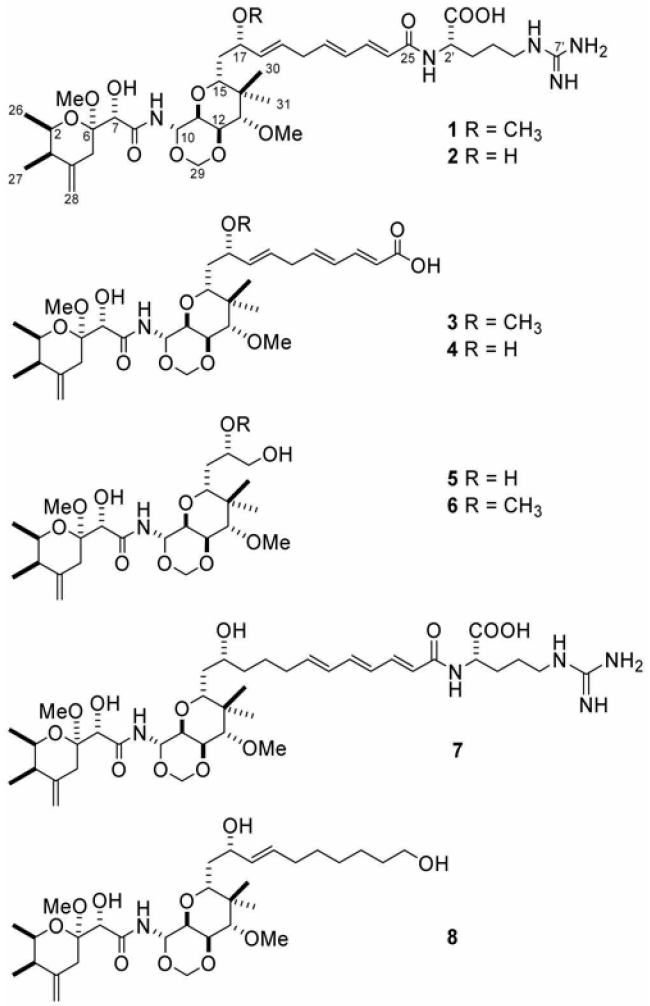

Figure 1. Structures of compounds **1**–**8**.

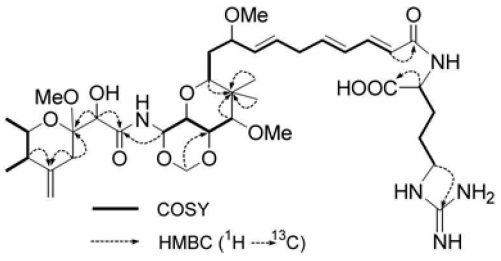

Figure 2. Key COSY and HMBC correlations for compound **1**.

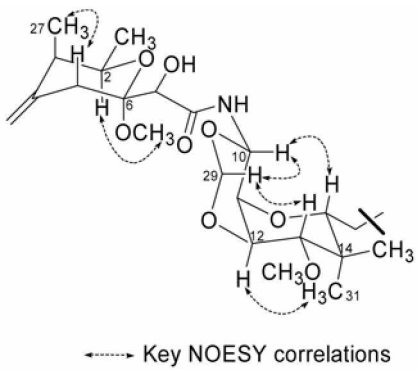

Figure 3. Relative stereochemistry of compound **1**.

determined to be 2*R**, 3*R**, 6*R**, 10*S**, 11*S**, 12*R**, 13*S**, and 15*R**. The absolute configuration of the arginine portion of **1** was defined as *S*, the same as **2** and **7**. Acid hydrolysis of **1** and derivatization with Marfey's reagent[27] followed by HPLC analysis, established L-configuration for arginine. Small amount of icadamide C (**1**) did not permit us to define absolute stereochemistry at C-7 and C-17. However, the optical rotation showed the same sign as other pederin class of compounds (**1**: $[\alpha]_D^{21}$ +35.8°, MeOH; **2**: $[\alpha]_D$ +81°, MeOH; **7**: $[\alpha]_D$ +99.1°, MeOH). Therefore, the absolute configuration of **1** was presumed to be the same with reported analogous compounds.

Compound **3** was isolated as white solid. The HRFABMS of **3** supported the molecular formula $C_{32}H_{49}NO_{11}$. The exact mass of the $[M + Na]^+$ ion (*m*/*z* 646.3197) matched well with the expected molecular formula $C_{32}H_{49}NO_{11}$ (Δ−0.5 mmu). The ^{1}H NMR spectrum of **3** showed marked similarities to that of **1**, which indicated that **3** also belong to the pederin class. Compound **3** was identified as theopedrin K by comparison of NMR, MS spectral, and optical rotation data with those of reported, which was previously isolated from a marine sponge *Discodermia* sp.[14]

Icadamide A (**2**) and B (**8**) were reported to show in vitro cytotoxicity against HCT-116 human colon carcinoma cell line with IC_{50} value of 63 nM and 0.17 nM, respectively. Among pederin type of compounds, only icadamide B (**8**) was studied for *in vivo* antitumor activity and exhibited activity against intraperitoneally and subcutaneously implanted tumors such as P-388 mouse leukemia, M109 mouse lung tumors, and asbestos-induced pulmonary squamous cell carcinoma.[23] Compound **3** was reported to demonstrate *in vitro* cytotoxicity against the cultured murine P-388 tumor cell line and the human lung adenocarcinoma A-549 cell line, with IC_{50} values of 0.1 nM and 1.5 nM, respectively.[14] The cytotoxicity of the isolated compounds against A-549, SK-OV-3, SK-MEL-2, XF-498, and HCT-15 solid tumor cell lines was studied. The results (Table 2) showed that both compounds (**1** and **3**) are potent cytotoxins against all of the cell lines tested. Moreover, it can be concluded that the presence of arginine group has negative effect on the cytotoxic profile of compound **1**.

There have been a number of reports on the isolation of pederins from numerous terrestrial insects and marine sponges. The first compound, pederin, was isolated in 1953 from the beetle *Paederus fauscipes*,[28] and thereafter scientists started reporting similar compounds from marine sponges, which are completely different source from terrestrial beetles. The taxonomy of these marine sponges is quite different as shown here: *Mycale* sp.[15,16,18] and *Stylinos* sp.[17] (subclass Ceractinomorpha, order Poescilosclerida, family Mycalidae). *Letosella* sp. (subclass Ceractinomorpha, order Dictyoceratida, family Spongiidae).[23] *Theonella* sp.[12,13,16-18] and *Discodermia* sp.[14] (subclass Tetractinomorpha, order Lithistida, family Theonellidae), and *Trachycladus laevispiralifer* (subclass Tetractinomorpha, order Axinellida, family

Table 2. Cytotoxicity Data of Compounds 1 and **3**[a]

compound	A-549	SK-OV-3	SK-MEL-2	XF-498	HCT-15
1	0.019	0.025	0.01	0.017	0.014
3	0.0003	0.0005	0.0001	0.0001	0.0001
doxorubicin	0.029	0.11	0.016	0.015	0.10

[a]Data expressed in ED_{50} values (μg/mL). A-549, human lung cancer; SK-OV-3, human ovarian cancer; SK-MEL-2, human skin cancer; XF-498, human CNS cancer; HCT-15, human colon cancer.

Spirastrellidae).[22] Reports on isolation of pederin class of compounds from taxonomically distant organisms indicate the possible microbial origin of the compounds.

Experimental Section

General procedures. Optical rotations were measured with a Jasco P-1020 polarimeter using a 1 dm path length cell. The ^{1}H and 2D NMR spectra were recorded at 500 MHz using Varian INOVA 500 spectrometer. FABMS data were obtained on a JEOL JMS SX-102A spectrometer. HRFABMS data were obtained on a JEOL JMS SX-101A spectrometer. 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). HPLC was performed on a Gilson 370 pump with a Shodex C18M 10E (preparative, 250 × 10 mm i.d., 5 μm, 100 Å) and YMC-Pack CN (preparative, 250 × 10 mm i.d., 5 μm, 120 Å) columns using a Shodex RI-101 detector.

Animal material. The sponge was collected in November 2004, off the coast of Jeju Island, South Korea, using scuba. The samples were frozen immediately after collection and stored at −20 °C until extraction. This organism was identified as *Discodermia calyx* by Prof. Chung Ja Sim of Hannam University. A voucher specimen (registry no. Spo. 49) was deposited in the Natural History Museum, Hannam University, Korea.

Extraction and Isolation. The freeze dried sponge *Discodermia calyx* (4.5 kg) was cut into small pieces and extensively extracted with MeOH at room temperature. The MeOH extract was partitioned between CH_2Cl_2 and H_2O. The CH_2Cl_2 layer (8.0 gm) was further partitioned between aqueous MeOH and *n*-hexane. The aqueous MeOH fraction (4.2 gm) was subjected to a reversed-phase flash column chromatography (YMC Gel ODS-A, 60 Å 500/400 mesh), using gradient elution from 50 to 100% MeOH/H_2O to yield nineteen fractions (DC1-DC19). These fractions were evaluated for activity by the brine shrimp lethality assay,[29] and fractions DC4 and DC5 were selected for further separation on the basis of toxicity to brine shrimp larvae (LD_{50} 700 μg/mL and 132 μg/mL, respectively). Fraction DC5 was further subjected to a reversed-phase HPLC on a Shodex C18M 10E (preparative, 250 × 10 mm i.d., 5 μm, 100 Å) column, eluting with 65% aqueous MeOH (1 mL/min), to afford four subfractions. Compound **1** (1.0 mg) was obtained by purifying subfraction DC5-3 by reversed-phase HPLC (Shodex C18M 10E, preparative, 250 × 10 mm i.d., 5 μm, 100 Å), eluting with 40% aqueous CH_3CN (2 mL/min, t_R 15 min.). Similarly, fraction DC4 was subjected to a reversed-phase HPLC on a Shodex C18M 10E (preparative, 250 × 10 mm i.d., 5 μm, 100 Å) column, eluting with 57% aqueous MeOH (2 mL/min), to afford six subfractions. Subfraction DC4-3 was subjected to reversed-phase HPLC (Shodex C18M 10E, preparative, 250 × 10 mm i.d., 5 μm, 100 Å), eluting with 45% aqueous CH_3CN (0.5 mL/min), to yield five subfractions. Compound **3** (1.2 mg) was obtained by purification of subfraction DC4-3-1 by reversed-phase HPLC (YMC-Pack CN, preparative, 250 × 10 mm i.d., 5 μm, 120 Å), eluting with 20% aqueous CH_3CN (1 mL/min, t_R 40 min.).

Marfey analysis of arginine: Compound **1** (0.2 mg) was dissolved in 6 N HCl (1 mL) and hydrolyzed at 110 °C for 12 h. The acid hydrolyzate was dried under N_2, and to it was added 0.1% FDAA (Marfey's reagent, 1-fluro-2,4-dinitrophenyl-5-L-alanine amide) solution in acetone (100 μL) and 1 M $NaHCO_3$ (20 μL) followed by heating at 60 °C for 1 h. After cooling to room temperature, the reaction mixture was neutralized with 2 M HCl (10 μL) and diluted with 100 μL of MeOH. This solution was analyzed by reversed-phase preparative HPLC (Shodex C18M 10 E, 250 × 10 mm i.d., 5 μm, 100 Å) column with isocratic elution (5% aqueous MeOH; 1 mL/min) to furnish a peak of L-Arg-FDAA complex (t_R 15 min.; D-Arg-FDAA complex: t_R 20 min.).

Icadamide C (**1**), colorless oil: $[\alpha]_D^{21}$ +35.8° (*c* 0.1, MeOH); ^{1}H and ^{13}C NMR data (CD_3OD) see Table 1; LRFABMS *m/z* 802.5 $[M + Na]^+$; HRFABMS *m/z* 802.4234 $[M + Na]^-$ (calc. for $C_{38}H_{61}N_5O_{12}Na$, 802.42140).

Theopederin K (**3**), white solid: $[\alpha]_D^{21}$ +59.9° (*c* 0.1, MeOH); ^{1}H NMR (CD_3OD, 500 MHz) δ 7.05 (1H, dd, *J* = 15.0, 15.0 Hz, H-23), 6.26 (1H, dd, *J* = 15.0, 15.0 Hz, H-22), 6.07 (1H, dt, *J* = 15.0, 7.0 Hz, H-21), 5.85 (1H, d, *J* = 15.0 Hz, H-24), 5.75 (1H, dt, *J* = 16.0, 6.5 Hz, H-19), 5.64 (1H, d, *J* = 9.0 Hz, H-10), 5.17 (1H, dd, *J* = 16.0, 9.5 Hz, H-18), 5.12 (1H, d, *J* = 7.0 Hz, H-29), 4.80 (1H, overlapped, H-28a), 4.78 (1H, overlapped, H-29), 4.64 (1H, br s, H-28b), 4.25 (1H, s, H-7), 4.13 (1H, dd, *J* = 9.0, 5.5 Hz, H-12), 3.91 (1H, m, H-11), 3.89 (1H, overlapped, H-2), 3.58 (1H, m, H-17), 3.52 (3H, s, 13-OCH_3), 3.41 (1H, overlapped, H-13), 3.28 (1H, overlapped, H-15), 3.24 (3H, s, 6-OCH_3), 3.16 (3H, s, 17-OCH_3), 2.94 (2H, m, H-20), 2.41 (1H, d, *J* = 14.0 Hz, H-5a), 2.31 (1H, d, *J* = 14.0 Hz, H-5b), 2.19 (1H, m, H-3), 1.48 (2H, dd, *J* = 11.5, 5.5 Hz, H-16), 1.18 (3H, d, *J* = 7.0 Hz, H-26), 0.97 (3H, d, *J* = 7.0 Hz, H-27), 0.95 (3H, s, H-30), 0.83 (3H, s, H-31); LRFABMS *m/z* 646.4 $[M + Na]^-$; HRFABMS *m/z* 646.3197 $[M + Na]^-$ (calc. for $C_{32}H_{49}NO_{11}Na$, 646.32020).

Acknowledgments. Our thanks are due to Prof. Chung Ja Sim of Hannam University for the identification of the sponge. This study was financially supported by a grant from Marine Bio 21, Ministry of Maritime Affairs and Fisheries, Korea. P. B. S. is grateful to Korea Science and Engineering Foundation (KOSEF) for doctoral fellowship.

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