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GYMNASTATINS I-K, CANCER CELL GROWTH INHIBITORS FROM A SPONGE-DERIVED *GYMNASCELLA DANKALIENSIS*

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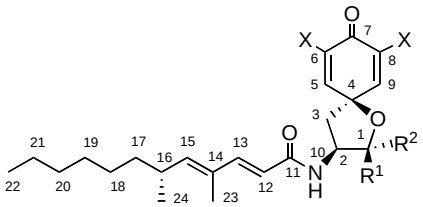
Abstract – The *Halichondria* sponge-derived fungus, *Gymnascella dankaliensis*, was cultured in a malt extract medium containing the bromine source. Three new metabolites, gymnastatins I (**5**)–K (**7**), have been isolated from the mycelial MeOH extract. The spectroscopic analyses using 1D and 2D NMR techniques have established the stereostructures of **5**–**7** in which chlorine atoms in gymnastatins A (**1**)–C (**3**) are respectively replaced by bromine atoms. All of the isolated metabolites (**5**–**7**) exhibited growth inhibition against the P388 cancer cell line. Furthermore, gymnastatins I (**5**) and J (**6**) showed appreciable growth inhibition against the human cancer cell lines.

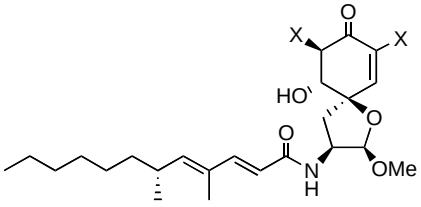
INTRODUCTION

Marine microorganisms are potentially prolific sources of highly bioactive secondary metabolites that might represent useful leads in the development of new pharmaceutical agents. As part of our ongoing search for potential new antitumor materials from marine-derived microorganisms,[1-3] we have already reported the structures and cytostatic activities of twelve polyketide-alkaloids, gymnastatins A–C (**1**–**3**),[4,5] D–G, H (**4**),[4–6] Q and R,[7] and dankastatins A and B,[7] and six unusual steroids[8–10] which were isolated from the fungus *Gymnascella dankaliensis* OUPS-N134 separated from the sponge *Halichondria japonica*. All the polyketide-alkaloids except for gymnastatin H (**4**) have chlorine atoms in the molecule, but no other halogen atoms. It was presumed that this fact was related to halogen sources in culture media because only chlorine ions were used as halogen sources for our previous experiment.[4–7] It has actually been reported that the bromo analogues of antibiotics with chlorine atoms were obtained by the

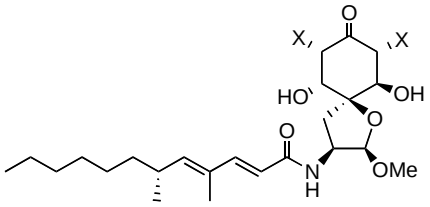
substitution of bromide ion for chloride ion in certain media used for the fermentations.[11] This consideration prompted us to examine polyketide-alkaloids from this fungal strain in culture conditions using bromine sources, and the fermentation produced polyketide-alkaloids with bromines, designated gymnastatins I–K (**5**–**7**). We report herein the isolation and structure elucidation of these compounds together with their growth inhibition against cancer cell lines.[12]



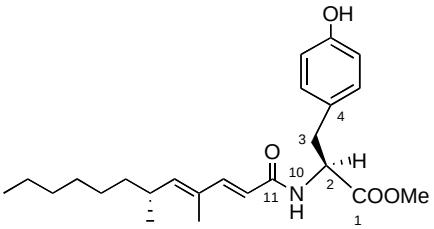
1a : R^1 = OH, R^2 = H, X = Cl
1b : R^1 = H, R^2 = OH, X = Cl
5a : R^1 = OH, R^2 = H, X = Br
5b : R^1 = H, R^2= OH, X = Br
8 : R^1 = R^2= O, X = Br



2 : X = Cl
6 : X = Br



3 : X = Cl
7 : X = Br



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RESULTS AND DISCUSSION

In the previous experiment,[4–7] the fungal strain was cultured at 27 °C for 4 weeks in the malt-glucose-peptone medium in artificial seawater consisting of 2% NaCl, 0.2% KCl, 0.4% $MgSO_4$•$7H_2O$ and 0.03% $CaCl_2$•$2H_2O$. In this experiment, the fungal strain was cultured with the same condition in a medium, in which the chlorine ions of artificial seawater were replaced by bromine ions (2% NaBr, 0.2% KBr, 0.4% $MgSO_4$•$7H_2O$ and 0.03% $CaBr_2$•$2H_2O$). The MeOH extract of the mycelia was purified by bioassay (P388 cell line)-guided fractionation employing a combination of Sephadex LH-20 and silica gel column chromatography procedures as well as reversed-phase HPLC to afford gymnastatins I (**5**), J (**6**) and K (**7**).

Gymnastatin I (**5**) had the molecular formula $C_{23}H_{31}Br_2NO_4$ established by respective $[M - OH]^+$ and M^+ peaks of **5** and its oxidative derivative (**8**) in HREIMS, and the ratio of relative intensity of isotope peaks (M^+ : $[M + 2]^+$: $[M + 4]^+$ = *ca.* 1 : 2 : 1) of **8** in EIMS. The UV and IR spectra of **5** exhibited the presence of a hydroxyl group, a conjugated ketone and an amide. The ^1H and ^{13}C NMR spectra (Table 1) of **5** suggested that it existed in a 3 : 1 mixture of two stereoisomers (**5a** and **5b**) on a hemiacetal group (δ_H 5.54, d, 4.4 Hz, and δ_H 5.62, s; δ_C 96.5 and δ_C 103.2). The general features of the ^1H and ^{13}C NMR spectra (Table 1) of major isomer **5a** closely resembled those of major isomer **1a** of gymnastatin A[5] except that

the signals for H-5, H-9, C-4, C-5, C-6, C-8 and C-9 in **5a** revealed a chemical shift difference relative to those of **1a**, suggesting that chlorine atoms in **1a** were replaced by bromine atoms in **5a**. The planar structure of **5a** thus deduced from the 1D NMR spectral analysis was confirmed by analysis of ^{1}H-^{1}H COSY and HMBC correlations (H-1/C-4, H-3α/C-5, H-3β/C-4, H-3β/C-9, H-5/C-6, H-5/C-7, H-5/C-9, H-9/C-7, H-9/C-8, H-13/C-11, H-23/C-13) (Table 2).

Table 1. NMR spectroscopic data ($CDCl_3$) for gymnastatin I (**5**)

	5a (Major Isomer)			**5b** (Minor Isomer)		
positon	δ_C, mult.	δ_H[a]		δ_C, mult.	δ_H[a]	
1	96.5, CH	5.54 d	4.4 (2)	103.2, CH	5.62 s	
2	52.1, CH	4.79 dtd	11.3 (3β), 8.2 (3α, 10), 4.4 (1)	57.8, CH	4.64 m	
3α	37.9, CH_2	2.59 dd	12.8 (3β), 8.2 (2)	39.8, CH_2	2.80 dd	14.2 (3β), 8.1 (2)
β		2.21 dd	12.8 (3α), 11.3 (2)		2.27 dd	14.2 (3α), 1.8 (2)
4	80.8, qC			82.3, qC		
5	148.8, CH	7.39 d	2.5 (9)	152.4, CH	7.54 d	2.5 (9)
6	121.2, qC			120.8, qC		
7	172.1, qC			172.3, qC		
8	121.2, qC			119.5, qC		
9	151.4, CH	7.30 d	2.5 (5)	149.8, CH	7.28 d	2.5 (5)
10		6.11 d	8.2 (2)		6.18 br d	5.5 (2)
11	166.8, qC			167.4, qC		
12	116.7, CH	5.77 d	15.3 (13)	116.7, CH	5.83 d	15.3 (13)
13	147.7, CH	7.27 d	15.3 (12)	147.7, CH	7.29 d	15.3 (12)
14	130.7, qC			130.7, qC		
15	148.9, CH	5.68 d	9.4 (16)	148.9, CH	5.70 d	9.6 (16)
16	33.2, CH	2.51 m		33.2, CH	2.51 m	
17A	37.2, CH_2	1.27 m		37.2, CH_2	1.27 m	
B		1.35 m			1.35 m	
18	27.5, CH_2	1.22 m		27.5, CH_2	1.22 m	
19	29.4, CH_2	1.24 m		29.4, CH_2	1.24 m	
20	31.8, CH_2	1.23 m		31.8, CH_2	1.23 m	
21	22.6, CH_2	1.27 m		22.6, CH_2	1.27 m	
22	14.1, CH_3	0.87 t	6.8 (21)	14.1, CH_3	0.87 t	6.8 (21)
23	12.5, CH_3	1.77 s		12.5, CH_3	1.77 s	
24	20.5, CH_3	0.97 d	6.6 (16)	20.5, CH_3	0.97 d	6.6 (16)
OH-1		4.66 br s			4.74 br s	

[a] ^{1}H chemical shift values (δ ppm) followed by multiplicity and then the coupling constants (*J*/Hz). Figures in parentheses indicate the proton coupling with that position.

The relative stereochemistry of the spiro ring in **5a** was established by a combination of a coupling constant between H-1 and H-2 ($J_{1,2}$ 4.4 Hz),[5] and NOEs from H-2 to H-1, H-9 and H-3α, and from H-3β to H-5 (Table 2). The geometry of the diene and the relative configuration of C-16 in the side chain of **5a** were determined by comparison of the NMR data of the side chain of **5a**, including ^{1}H and ^{13}C chemical

shifts, coupling constants ($J_{12,13}$ 15.3 Hz), and NOEs (H-12/H-23 and H-13/H-15) with those of gymnastatin A (**1**).[5] Based on this evidence, the relative stereostructures for gymnastatin I (**5**) as a mixture of diasteromers at C-1 were established as **5a** and **5b**, in which the chlorines in gymnastain A (**1**) were replaced by bromines. The specific rotation and CD curve of compound **5** were comparable with that of

Table 2. 2D NMR spectroscopic data ($CDCl_3$) for gymnastatins I (**5**) –K (**7**)

	gymnastatin I (**5a**)			gymnastatin J (**6**)			gymnastatin K (**7**)		
positon	^{1}H-^{1}H COSY	HMBC[a]	NOESY	^{1}H-^{1}H COSY	HMBC[a]	NOESY	^{1}H-^{1}H COSY	HMBC[a]	NOESY
1	OH-1, 2	2, 3, 4	2	2	OCH_3-1, 3	OCH_3-1, 2, 10	2	OCH_3-1, 3	OCH_3-1, 2
2	1, 3α, 3β, 10		1, 3α, 9, 10	1, 3α, 3β, 10		1, 3β, 9, 10	1, 3α, 3β, 10		1, 3α
3α	2, 3β	1, 2, 5	2, 3β, 9	2, 3β	1, 2, 4, 5, 9	2, 3β, 9	2, 3β	1, 2, 4, 5	2, 3β
β	2, 3α	2, 4, 5,9	3α, 5, 10	2, 3α	1, 2, 4, 5, 9	3α, 5	2, 3α	1, 2, 4, 9	3α, 5
4									
5	9	6, 7, 9	3β	5-OH, 6, 9	4, 6, 7, 9	OCH_3-1, 3β, 6	6	4, 6, 7	OCH_3-1, 3β, 6
6				5	5, 7	5, OH-5	5	5, 7	5, 8
7									
8							9	4, 7, 9	6
9	5	5, 7, 8	2, 3α	5	7, 8	2, 3α	8	8	OH-9
10	2	11	2, 3β, 12	2	1, 2, 11	1, 2	2	1, 2, 11	
11									
12	13	11, 14	10, 23	13	11, 13, 14	23	13	11, 13, 14	23
13	12	11, 12, 14, 15, 23	15	12	11, 12, 14, 15, 23	15	12	11, 12, 14, 15, 23	15
14									
15	16, 23	13, 16, 17, 23, 24	13, 16, 24	16, 23	13, 16, 17, 23, 24	13, 16, 24	16, 23	13, 14, 16, 17, 23, 24	13, 16, 24
16	15, 17, 24	14, 15, 17, 24	15, 23, 24	15, 17, 24	14, 15, 24	15, 23, 24	15, 17, 24	14, 15, 24	15, 23, 24
17A									
B									
18									
19									
20									
21									
22	21	20, 21		21	20, 21		21	20, 21	
23	15	13, 14, 15	12, 16	15	13, 14, 15	12, 16	15	13, 14, 15	12, 16
24	16	15, 16, 17	15, 16	16	15, 16, 17	15, 16	16	15, 16, 17	15, 16
OCH_3-1					1	1, 5		1	1, 5
OH-1									
OH-5					4, 5	6		5	
OH-9							9	4, 8, 9	

[a]HMBC correlations are from proton stated to the indicated carbon.

gymnastatin A (**1**) {lit,[5] $[\alpha]_D$ –3.8},[13] of which the absolute stereochemistry was previously determined.[5] This evidence suggests that the absolute configuration of gymnastatin I (**5**) is the same as that of gymnastatin A (**1**).

Gymnastatins J (**6**) was assigned the molecular formula $C_{24}H_{35}Br_2NO_5$ as deduced from a molecular ion

peak in HREIMS, and the ratio of relative intensity of isotope peaks in EIMS. The general spectral features of **6** (Table 3) closely resembled those of gymnastatin B (**2**)[5] except that the signals for H-6, H-9, C-4, C-6, C-8 and C-9 in the NMR spectra revealed a chemical-shift difference relative to those of **2**. In addition to this evidence, analysis of ^{1}H-^{1}H COSY and HMBC (Table 2) led to planar structure **6** for gymnastatin J.

The observations of a coupling constant between H-1 and H-2 ($J_{1,2}$ 3.7 Hz), and NOEs from H-2 to H-1, H-9 and H-3α, and from H-5 to H-3β and 1-OCH_3 in **6** (Table 2) showed that H-1 and H-2 are on the same side as H-9 and H-3α. Also, NOEs from H-5 to H-3β and 1-OCH_3 implied that H-5 is oriented to the 1β-methoxy group and hence arranged pseudoequatorially in the half-chair cyclohexene of **6**. Furthermore, a NOE from H-6 to 5-OH with a pseudoaxial arrangement indicated that H-6 is arranged

Table 3. NMR spectroscopic data ($CDCl_3$) for gymnastatins J (**6**) and K (**7**)

	gymnastatin J (**6**)			gymnastatin K (**7**)		
position	δ_C, mult.	δ_H[a]		δ_C, mult.	δ_H[a]	
1	96.7, CH	4.67 d	3.7 (2)	97.3, CH	4.66 d	3.9 (2)
2	46.4, CH	4.11 dddd	12.4 (3α), 8.7 (10), 4.8 (3β), 3.7 (1)	46.2, CH	4.21 m	
3α	38.3, CH_2	2.26 dd	12.4 (3α), 4.8 (2)	34.7, CH_2	2.47 dd	12.5 (3β), 5.3 (2)
β		1.95 t	12.4 (2, 3β)		1.74 t	12.5 (2, 3α)
4	70.5, qC			72.4, qC		
5	75.9, CH	4.34 t	2.3 (6,9)	74.4, CH	4.36 d	3.4 (6)
6	51.8, CH	5.56 d	2.3 (5)	52.4, CH	5.34 d	3.4 (5)
7	183.4, qC			189.0, qC		
8	123.7, qC			59.0 CH	4.99 d	10.9 (9)
9	147.4, CH	7.25 d	2.3 (5)	74.0, CH	4.21 dd	10.9 (8), 5.6 (9-OH)
10		5.79 d	8.7 (2)		5.87 br d	8.7 (2)
11	166.5, qC			166.5, qC		
12	116.6, CH	5.70 d	15.2 (13)	116.5, CH	5.70 d	15.1 (13)
13	147.6, CH	7.13 d	15.2 (12)	148.0, CH	7.20 d	15.1 (12)
14	130.7, qC			130.7, qC		
15	149.0, CH	5.69 d	9.9 (16)	149.0, CH	5.69 d	9.6 (16)
16	33.3, CH	2.51 m		33.3, CH	2.49 m	
17A	37.2, CH_2	1.28 m		37.2, CH_2	1.27 m	
B		1.35 m			1.35	
18	27.5, CH_2	1.22		27.5, CH_2	1.22 m	
19	29.4, CH_2	1.24 m		29.4, CH_2	1.24 m	
20	31.8, CH_2	1.24 m		31.8, CH_2	1.23 m	
21	22.6, CH_2	1.27 m		22.6, CH_2	1.27 m	
22	14.1, CH_3	0.87 t	6.8 (21)	14.1, CH_3	0.87 t	6.8 (21)
23	12.5, CH_3	1.76 s		12.5, CH_3	1.77 s	
24	20.5, CH_3	0.97 d	6.6 (16)	20.5, CH_3	0.98 d	6.7 (16)
OCH_3-1	55.2, CH_3	3.48 s		55.4, CH_3	3.49 s	
OH-5		4.75 br s			4.35 br s	
OH-9					5.77 br d	5.6 (9)

[a]As in Table 1.

pseudoequatorially and consequently *trans* to pseudoequatorial H-5 (Figure 1). The stereochemistry of the side chain was determined by comparison of the ^{1}H and ^{13}C NMR data of **6** with those of **5**. The above-mentioned evidence allowed assignment of relative stereostructure **6** to gymnastatin J.

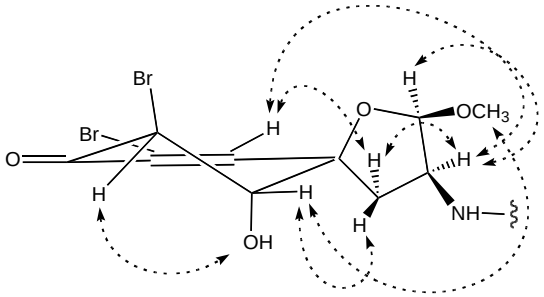

Figure 1. Selected NOE correlations for gymnastatin J (**6**)

Gymnastatin K (**7**) had the molecular formula $C_{24}H_{37}Br_2NO_6$ established by HREIMS. The general spectral features of **7** (Table 3) closely resembled those of gymnastatin C (**3**) except that the signals for H-6, H-8, C-6, C-7 and C-8 in the NMR spectra revealed a chemical-shift difference relative to those of **3**. Analysis of ^{1}H-^{1}H COSY and HMBC (Table 2) led to planar structure of **7**. Incidentally, assignments for 5-OH and 9-OH were deduced from their HMBC correlations (5-OH to C-5, and 9-OH to C-4, C-8 and C-9) (Table 2).

The observation of an NOE from H-6 to H-8 and the large coupling constant ($J_{8,9}$ 10.9 Hz) between H-8 and H-9 in **7** implied that the cyclohexane ring of **7** exists in a chair conformation with H-6 and H-8 in a coaxial arrangement and with H-8 and H-9 in a *trans*-axial arrangement. In addition, an NOE from H-5 to 1-OCH_3 and the small coupling constant ($J_{5,6}$ 3.4 Hz) between H-5 and H-6 in **7** indicated that H-5 is arranged equatorially, cis to axial H-6 and on the same side as 1-OCH_3. Further NOEs from H-2 to H-1 and H-3a implied that these protons are on the same side. The stereochemistry of the side chain was determined by comparison of the ^{1}H and ^{13}C NMR data of **7** with those of **5**. The evidence summarized above allowed assignment of relative stereostructure **7** to gymnastatin K.

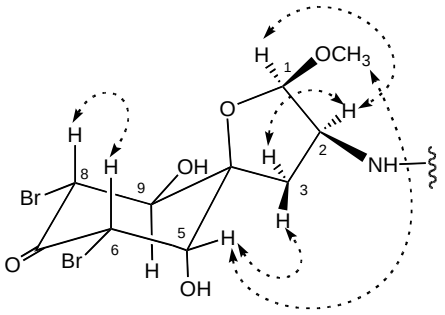

Figure 2. Selected NOE correlations for gymnastatin K (**7**)

The absolute configurations of C-16 and C-2 in gymnastatins J (**6**) and K (**7**) have not been established independently, but are assumed to be the same as for its co-metabolites, gymnastatins A (**1**), D, and E, of

Table 4. Growth inhibition of gymnastatins I (**5**) and J (**6**) against a panel of 39 human cancer cell lines

		Gymnastatin I (**5**)	Gymnastatin J (**6**)
Origin of cancer	Cell line	Log GI_{50} (M)[a]	Log GI_{50} (M)[a]
Breast	HBC-4	-5.79	-5.72
	BSY-1	-5.98	-5.85
	HBC-5	-6.28	-5.72
	MCF-7	-5.54	-5.51
	MDA-MB-231	-5.70	-5.70
Brain	U251	-5.68	-5.69
	SF-268	-5.60	-5.64
	SF-295	-5.62	-5.66
	SF-539	-5.71	-6.23
	SNB-75	-5.51	-5.46
	SNB-78	-5.71	-5.61
Colon	HCC2998	-5.76	-5.63
	KM-12	-5.60	-5.50
	HT-29	-5.68	-5.61
	HCT-15	-5.70	-5.61
	HCT-116	-5.87	-6.06
Lung	NCI-H23	-5.65	-5.72
	NCI-H226	-5.60	-5.60
	NCI-H522	-6.57	-6.49
	NCI-H460	-5.51	-5.68
	A549	-5.37	-5.38
	DMS273	-5.87	-5.75
	DMS114	-5.85	-5.70
Melanoma	LOX-IMVI	-5.84	-5.82
Ovary	OVCAR-3	-6.24	-6.22
	OVCAR-4	-5.73	-5.58
	OVCAR-5	-5.83	-5.71
	OVCAR-8	-5.71	-6.40
	SK-OV-3	-5.48	-4.76
Renal	RXF-631L	-5.70	-5.74
	ACHN	-5.92	-5.72
Stomach	St-4	-5.63	-5.51
	MKN1	-6.23	-5.85
	MKN7	-5.76	-5.70
	MKN28	-5.74	-5.61
	MKN45	-5.87	-5.70
	MKN74	-5.86	-5.66
Prostate	DU-145	-5.63	-5.60
	PC-3	-5.72	-5.73
MG-MID[b]		-5.77	-5.71
Delta[c]		0.80	0.77
Range[d]		1.20	1.72

[a] Log concentration of compounds for inhibition of cell growth at 50% compared to control.
[b] Mean value of log GI50 over all cell lines tested.
[c] The difference in log GI50 value of the most sensitive cell and MG-MID value.
[d] The difference in log GI50 value of the most sensitive cell and the least sensitive cell.

which the absolute stereochemistries have already been determined by X-ray crystal structure analyses, modified Moscher's method, and some chemical transformations.[5]

The cancer cell growth inhibitory properties of the isolated metabolites were examined using the murine P388 lymphocytic leukemia cell line and a disease-oriented panel of 39 human cancer cell lines (HCC panel) in the Japanese Foundation for Cancer Research.[14] All of gymnastatins I–K (**5**–**7**) exhibited potent growth inhibition against the P388 cell line (ED_{50} 0.021, 0.021, and 0.21 μg/mL, respectively). In the screening test by HCC panel, the mean log GI_{50} (MG-MID) of gymnastatins I (**5**) and J (**6**) was –5.77 and –5.71 (Table 4), respectively, implying that both the compounds exhibited appreciable growth inhibition. Furthermore, the delta and range values were respectively 0.8 and 1.2 for compound **5**, and 0.77 and 1.72 for compound **6**, disclosing that both the compounds showed differential activities (effective value: delta ≥ 0.5 as well as range ≥ 1.0). As shown in Table 4, gymnastatins I (**5**) and J (**6**) were respectively more effective against HBC-5 (breast), NCI-H522 (lung), OVCAR-3 (ovarian) and MKN1(stomach) cell lines, and against SF-539 (brain), HCT-116 (colon), NCI-H522 (lung), OVCAR-3 (ovarian) and OVCAR-8 (ovarian) cell lines. The above evidence suggested that a conjugated ketone system was important for enhancement of cancer cell growth inhibition in gymnastatins I (**5**) and J (**6**), and P388 cell growth inhibition of gymnastatin K (**7**) resulted from a conjugated ketone which might be derived from itself in the test system.

EXPERIMENTAL

General procedures

Optical rotations were obtained on a JASCO ORD/UV-5 spectropolarimeter. UV spectra were recorded on a Shimadzu spectrophotometer and IR spectra on a Perkin Elmer FT-IR 1720X spectrometer. CD spectra were recorded on a JASCO J-500A spectrometer. 1D and 2D NMR spectra were recorded at 27 ˚C on a Varian UNITY INOVA-500 spectrometer, operating at 500 and 125.7 MHz for ^{1}H and ^{13}C, respectively, with TMS as an internal reference. EIMS was determined using a Hitachi M-4000H mass spectrometer. Liquid chromatography over silica gel (mesh 230-400) was performed at medium pressure. HPLC was run on a Waters ALC-200 instrument equipped with a differential refractometer (R401) and Shim-pack PREP-ODS (250 mm x 20 mm i.d.). Analytical TLC was performed on precoated Merck aluminum sheets (DC-Alufolien Kieselgel 60 F254, 0.2 mm) with the solvent CH_2Cl_2-MeOH (19:1), and compounds were observed under a UV lamp and sprayed with 10% H_2SO_4 followed by heating.

Biological material

The fungal strain (OUPS-N134) was isolated from the sponge *Halichondria japonica*, collected in the Osaka Bay of Japan, and identified as *Gymnascella dankaliensis* as previously reported.[5–7,9]

Culture conditions

The fungal strain was grown in a liquid media (30 L) containing 1% malt extract, 1% glucose, and 0.05% peptone in artificial seawater (1% NaBr, 0.2% KBr, 0.4% $MgSO_4 \cdot 7H_2O$, 0.03% $CaBr_2 \cdot 2H_2O$) adjusted to pH 7.5 for four weeks at 27 °C.

Extraction and isolation

The culture was filtered under suction and the mycelium collected was extracted three times with MeOH. The combined extracts were evaporated in vacuo to give the crude extract (3.8 g). The CH_2Cl_2–MeOH (1:1)-soluble portion of the crude extract was passed through Sephadex LH-20 using CH_2Cl_2–MeOH (1:1) as eluent. The second fraction (F1; 2.8 g), in which the activity was concentrated, was chromatographed on a silica gel column with an *n*-hexane–CH_2Cl_2–MeOH gradient as eluent. The MeOH–CH_2Cl_2 (1 : 99) eluate [F2 (250.4 mg)] was further separated by silica gel column chromatography with a CH_2Cl_2–MeOH gradient as eluent. The MeOH–CH_2Cl_2 (1 : 99) eluate [F 3 (132.2 mg)] was purified by HPLC (ODS) using acetone–H_2O (4 : 1) as eluent to afford 2 fractions [F 4 (21.2mg) and F 5 (50.3 mg)]. Fractions F 4 and F 5 were purified by HPLC using acetone–H_2O (7 : 3) and (3 : 1) as eluents, respectively, to afford gymnastatin K (**7**) (9.2 mg), and gymnastatins J (**6**) (10.3 mg) and I (**5**) (27.1 mg), respectively.

Gymnastatin I (5): colorless powder; mp 80.5–81.0 °C; $[\alpha]^{26}_D$ –8.5 (*c* 1.78, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 265 (4.67) nm; CD (EtOH) λ_{max} (Δε) [*c* 5.22 × 10^{-5} M] 315 (0), 275 (+2.38), 267 (+2.02), 254 (0), 236 (−2.12), 214 (0); IR (KBr) ν_{max} 3359, 3287 (OH, NH), 1685 (C=C−C=O), 1650 (CONH), 1606 (C=C) cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; EIMS *m/z* 526 $[M - OH]^+$ (1.3), 207 $[C_{14}H_{23}O]^+$ (82.8), 179 $[C_{13}H_{23}]^+$ (94.9), 95 $[C_5H_5NO]^+$ (100); HREIMS *m/z* 526.0577 $[M - OH]^+$ (calcd for $C_{23}H_{30}Br_2NO_3$, 526.0591).

Gymnastatin J (6): colorless needles; mp 88–89 °C (MeOH); $[\alpha]^{20}_D$ –129.5 (*c* 0.72, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 265 (4.56) nm; CD (EtOH) λ_{max} (Δε) [*c* 5.03 × 10^{-5} M] 302 (0), 266 (−13.19), 249 (0), 238 (+4.16), 221 (0), 207 (−3.07); IR (KBr) ν_{max} 3381, 3282 (OH, NH), 1715 (C=C−C=O), 1650 (CONH), 1607 (C=C) cm^{-1}; ^1H and ^{13}C NMR data, see Table 3; EIMS *m/z* 559 $[M + 2 - H_2O]^+$ (1.9), 224 $[C_{14}H_{26}NO]^+$ (40.0), 207 $[C_{14}H_{23}O]^+$ (100), 179 $[C_{13}H_{23}]^+$ (85.9), 95 $[C_5H_5NO]^+$ (76.5); HREIMS *m/z* 575.0875 $[M]^+$ (calcd for $C_{24}H_{35}Br_2NO_5$, 575.0880).

Gymnastatin K (7): colorless powder; mp 116–118 °C; $[\alpha]^{20}_D$ –93.9 (*c* 0.91, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 265 (4.52) nm; CD (EtOH) λ_{max} (Δε) [*c* 6.00 × 10^{-5} M] 320 (0), 293 (+0.43), 285 (0), 255 (−3.76),

218 (−2.49), 206 (−2.15); IR (KBr) ν_{max} 3434, 3316 (OH, NH), 1761 (C=O), 1651 (CONH), 1606 (C=C) cm^{-1}; ^1H and ^{13}C NMR data, see Table 3; EIMS *m/z* 595 $[M + 2]^+$ (0.1), 593 $[M]^+$ (0.1), 559 $[M + 2 – 2H_2O]^+$ (0.1), 224 $[C_{14}H_{26}NO]^+$ (30.8), 207 $[C_{14}H_{23}O]^+$ (66.7), 179 $[C_{13}H_{23}]^+$ (69.8), 95 $[C_5H_5NO]^+$ (100); HREIMS *m/z* 593.0967 $[M]^+$ (calcd for $C_{24}H_{37}Br_2NO_6$, 593.0986).

Oxidation of gymnastatin I (5) by a pyridine–CrO_3 complex

A solution of gymnastatin I (**5**) (16 mg) in pyridine (0.3 mL) was added to a pyridine-CrO_3 complex prepared from pyridine (0.3 mL) and CrO_3 (50 mg), and the reaction mixture was left at room temperature overnight. The mixture was diluted with water and extracted with CH_2Cl_2. The extract was evaporated under reduced pressure, and the residue was subjected to a silica gel column chromatography [CH_2Cl_2–MeOH (99 : 1)] followed by HPLC using acetone–H_2O (3 : 1) as eluent to afford lactone **8** (2.8 mg) as a colorless powder. Compound **8**: mp 73.8–74.5 °C, $[\alpha]^{20}_D$ –117.0 (*c* 0.28, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 265 (4.52) nm; CD (EtOH) λ_{max} ($\Delta\varepsilon$) [*c* 5.07 × 10^{-5} M] 303 (0), 266 (−0.95), 245 (0); IR (KBr) ν_{max} 3365, 3288 (OH, NH), 1796 (γ-lacton), 1691 (C=C-CO), 1652 (CONH), 1612 (C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.87 (3H, t, *J* = 6.9 Hz, H-22), 0.98 (3H, d, *J* = 6.6 Hz, H-24), 1.21 (2H, m, H-18 or H-19), 1.23 (2H, m, H-21 or H-20), 1.24 (2H, m, H-19 or H-18), 1.26 (1H, m, H-17A), 1.27 (2H, m, H-20 or H-21), 1.35 (1H, m, H-17B), 1.77 (3H, s, H-23), 2.52 (1H, m, H-16), 2.59 (1H, dd, *J* = 13.6, 10.1 Hz, H-3A), 2.89 (1H, dd, *J* = 13.6, 10.1 Hz, H-3B), 4.61 (1H, td, *J* = 10.1, 6.1 Hz, H-2), 5.71 (1H, d, *J* = 10.0 Hz, H-15), 5.77 (1H, d, *J* = 15.1 Hz, H-12), 6.15 (1H, d, *J* = 6.1 Hz, H-10), 7.31 (1H, d, *J* = 15.1 Hz, H-13), 7.33 (1H, d, *J* = 2.8 Hz, H-9), 7.53 (1H, d, *J* = 2.8 Hz, H-5); ^{13}C NMR ($CDCl_3$) δ 12.44 (C-23), 14.08 (C-22), 20.47 (C-24), 22.61 (C-20 or C-21), 27.45 (C-18 or C-19), 29.37 (C-19 or C-18), 31.80 (C-21 or C-20), 33.31 (C-16), 37.18 (C-17), 37.41 (C-3), 49.35 (C-2), 79 16 (C-4), 115.28 (C-12), 123.45 (C-6), 123.69 (C-8), 130.71 (C-14), 145.04 (C-9), 146.78 (C-5), 148.94 (C-13), 149.71 (C-15), 166.89 (C-11), 171.19 (C-7), 172.20 (C-1); EIMS *m/z* 545 $[M + 4]^+$ (0.1), 543 $[M + 2]^+$ (0.2), 541 $[M]^+$ (0.1).

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