Gymnastatins and Dankastatins, Growth Inhibitory Metabolites of a *Gymnascella* Species from a *Halichondria* Sponge†

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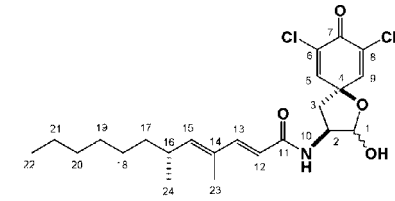
Four new metabolites, gymnastatins Q (**3**) and R (**4**) and dankastatins A (**5**) and B (**6**), have been isolated from the mycelial MeOH extract of a fungal strain of *Gymnascella dankaliensis* separated from a *Halichondria* sponge. Their stereostructures have been established on the basis of spectroscopic analysis using 1D and 2D NMR techniques. All of the isolated metabolites (**3**–**6**) exhibited growth inhibition against the P388 cancer cell line. Furthermore, gymnastatin Q (**3**) showed appreciable growth inhibition against BSY-1 (breast) and MKN7 (stomach) human cancer cell lines.

Marine natural products include a number of compounds with unique structures, in some of which the possibility of exhibiting unusual bioactivities is evident. On the basis of this consideration, we have focused our attention on potential new antitumor materials from marine-derived microorganisms and found a number of antitumor and/or cytostatic compounds.[1–3] As part of this study, we have already reported the structures and cytostatic activities of gymnastatins A (**1**), B−E,[4,5] F (**2**), G, and H,[6] gymnasterones A−D,[7,8] and dankasterones A and B,[8,9] which were isolated as cytostatic metabolites from the fungus *Gymnascella dankaliensis* OUPS-N134 separated from the sponge *Halichondria japonica*. Dankasterones A and B and the other products including gymnasterones were isolated from the fungal strains cultured in media types A and B, respectively.[8] Medium type A contained 1% malt extract, 1% soluble starch, and 0.05% peptone in artificial seawater, and the 1% soluble starch in medium type A was replaced by 1% glucose in medium type B. Further investigation of metabolites of this fungal strain using medium type A led to the isolation of four additional novel metabolites, designated gymnastatins Q (**3**) and R (**4**) and dankastatins A (**5**) and B (**6**).We report herein the isolation and structure elucidation of these compounds together with their growth inhibition against murine P388 lymphocytic leukemia and human cancer cell lines.



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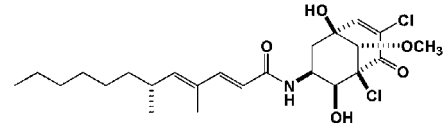

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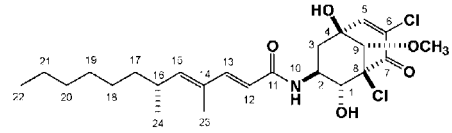

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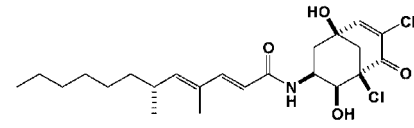

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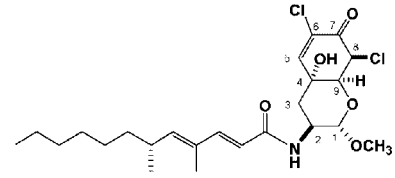

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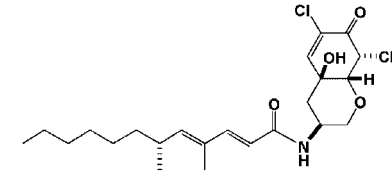

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Results and Discussion

The sponge-derived fungus *G. dankaliensis* OUPS-N134 was cultured with static conditions in a liquid medium (type A) containing 1% malt extract, 1% soluble starch, and 0.05% peptone in artificial seawater (pH 7.5) for 28 days at 27 °C. 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 Q (**3**) and R (**4**) and dankastatins A (**5**) and B (**6**).

Gymnastatin Q (**3**) gave the same molecular formula ($C_{24}H_{35}Cl_2NO_5$) as gymnastatin F (**2**),[6] as deduced from HREIMS. The IR spectrum showed bands at 3384, 1713, 1652, and 1611 cm^{-1}, characteristic of a hydroxyl group, a ketone, and an amide carbonyl and a double bond. The general features of its ^1H and ^{13}C NMR spectra (Table 1) closely resembled those of **2**, and analysis of $^1H-^1H$ COSY (H-1/H-2, H-2/H-3α, H-2/H-3β, and others) and HMBC (Table S1, Supporting Information) correlations of **3** led to the same planar structure as **2**, implying that **3** is a stereoisomer of **2**. Compound **3** showed NOEs from H-9 to H-1 and H-3β (Figure 1) and coupling constants of $J_{1,2}$ 10.3 Hz, $J_{2,3\beta}$ 7.3 Hz, and $J_{2,3\alpha}$ 5.5 Hz. This evidence suggested that the cyclohexane ring (C-1−C-4−C9−C-8) in acetone-d_6 exists in a chair conformation with H-1,

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Table 1. NMR Spectroscopic Data (acetone-d_6) for Gymnastatins Q (**3**) and R (**4**)

	gymnastatin Q (**3**)				gymnastatin R (**4**)			
position	δ_C, mult.	δ_H[a]		*J*/Hz	δ_C, mult.	δ_H[a]		*J*/Hz
1	79.2, CH	4.12	dd	10.3 (2), 3.4(OH-1)	71.8, CH	3.81	dd	2.5 (2), 1.3 (9α)
2	51.0, CH	3.93	dddd	12.4 (3*β*), 10.3 (1), 7.3 (10), 5.5 (3α)	47.5, CH	4.00	ddd	12.1 (3*β*), 5.0 (3α), 2.5 (1)
3α	33.8, CH_2	2.26	dd	13.0 (3*β*), 5.5 (2)	34.6, CH_2	1.88	ddd	12.4 (3*β*), 5.0 (2), 2.0 (9α)
3*β*		2.07	dd	13.0 (3α), 12.4 (2)		2.07	dd	12.4 (3α), 12.1 (2)
4	74.9, qC				71.4, qC			
5	148.6, CH	7.03	d	2.3 (9)	154.5, CH	7.31	d	2.5 (9*β*)
6	131.3, qC				130.4, qC			
7	182.6, qC				187.1, qC			
8	83.6, qC				76.3, qC			
9α	92.8, CH				46.7, CH_2	2.51	ddd	11.7 (9*β*), 2.0 (3a), 1.3 (1)
9*β*		3.73	d	2.3 (9)		2.75	dd	11.7 (9α), 2.5 (5)
10		7.46	br d	7.3 (2)		n.d.[b]		
11	167.6, qC				165.7, qC			
12	119.6, CH	5.95	d	15.3 (13)	119.9, CH	6.07	br d	15.5 (13)
13	146.3, CH	7.16	dd	15.3 (12), 0.6(15)	145.9, CH	7.14	br d	15.5 (12)
14	132.1, qC				132.2, qC			
15	147.3, CH	5.64	ddd	9.8 (16), 1.1 (23), 0.6 (13)	146.8, CH	5.61	dd	10.3 (16), 1.3 (23)
16	33.8, CH	2.56	m		33.7, CH	2.56	m	
17A	38.0, CH_2	1.26	m		38.1, CH_2	1.26	m	
17B		1.37	m			1.37	m	
18	28.2, CH_2	1.26	m		28.2, CH_2	1.28	m	
19	30.1, CH_2	1.26	m		29.9, CH_2	1.26	m	
20	32.6, CH_2	1.28	m		32.6, CH_2	1.25	m	
21	23.3, CH_2	1.27	m		23.3, CH_2	1.27	m	
22	14.3, CH_3	0.87	t	6.8 (21)	14.3, CH_3	0.86	t	7.0 (21)
23	12.7, CH_3	1.76	d	1.1 (15)	12.7, CH_3	1.76	d	1.3 (15)
24	20.9, CH_3	0.98	d	6.6 (16)	20.9, CH_3	0.97	d	6.6 (16)
OCH_3-9	62.8, CH_3	3.62	s					
OH-1		5.26	br d	3.4 (1)		n.d.[b]		

[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. [b] Not detected.

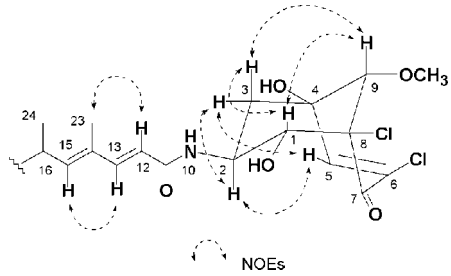


Figure 1. Observed NOEs and conformation for gymnastatin Q (**3**).

H-3*β*, and H-9 in coaxial arrangements, and the three protons are arranged *trans* to H-2 in an axial arrangement. In addition, the observation of NOEs from H-5 to H-2 and H-3α implied that the cyclohexenone ring (C-4−C-9) exists in a pseudo-half-boat conformation. The geometry of the diene and the relative configuration of C-16 in the side chain of **3** were determined by comparison of the NMR data of the side chain of **3**, including ^{1}H and ^{13}C chemical shifts, coupling constants (H-12/H-13), and NOEs (H-12/H-23 and H-13/H-15) with those of gymnastatins A (**1**)[5] and F (**2**).[6] The above-mentioned evidence clarified that compound **3** is the stereoisomer of **2** at C-1. The absolute configurations of C-16 and C-2 in **3** have not been established independently, but are assumed to be the same as for its co-metabolites, gymnastatins A (**1**), D, and E, of which the absolute stereochemistries have already been determined by X-ray crystal structure analyses, modified Mosher's method, and some chemical transformation.[5] This was supported by a consideration of biosynthesis of gymnastatin Q (**3**). It is most likely that gymnastatin Q (**3**) is synthesized biogenetically via gymnastatins A (**1**) (Scheme S1, Supporting Information), which is composed of L-tyrosine and 4,6 *R*-dimethyldodeca-2*E*,4*E*-dienoic acid (**7**), as reported previously for gymnastatin F (**2**).[6] On the basis of the above-mentioned considerations, the absolute stereostructure for gymnastatin Q was assumed to be **3**.

Gymnastatin R (**4**) was assigned the molecular formula $C_{23}H_{33}Cl_2NO_4$ deduced from HREIMS. The general features of the ^{1}H and ^{13}C NMR spectra (Table 1) of **4** closely resembled those of **3** except that the signals for methoxymethine in **3** were replaced by those of methylene [δ_H 2.51 (H-9α), 2.75 (H-9*β*); δ_C 46.7 (C-9)] in **4**. The planar structure of **4** deduced from this evidence was confirmed by ^{1}H−^{1}H COSY (H-1/H-2, H-2/H-3α, H-2/H-3*β*, and others) and HMBC (Table S1, Supporting Information) correlations. The observation of NOE (H-9*β*/H-3*β*), coupling constants between vicinal protons ($J_{1,2}$ 2.5, $J_{2,3\alpha}$ 5.0, and $J_{2,3\beta}$ 12.1 Hz), and *W*-type long-range couplings ($J_{1,9\alpha}$ 1.3 and $J_{3\alpha,9\alpha}$ 2.0 Hz) implied that the cyclohexane ring (C-1−C-4−C-9−C-8) in acetone-d_6 exists in a chair conformation with H-2 and H-1 in respective axial and equatorial arrangements, which are both oriented *trans* to H-3*β* and H-9*β* in coaxial arrangements. In addition, NOEs from H-5 to H-2 and H-3α suggested that the cyclohexenone ring (C-4−C-9) exists in a pseudo-half-boat conformation. The stereochemistry of the side chain was determined by comparison of the ^{1}H and ^{13}C NMR data of **4** with those of **3**. The above-mentioned evidence allowed assignment of relative stereostructure **4** to gymnastatin R. As described above for gymnastatin Q (**3**), the absolute chemistry of **4** has not been established independently, but is assumed to be the same as for its co-metabolites, gymnastatins A (**1**), D, and E. This was supported by a consideration of the biosynthetic pathway of **4** via gymnastatin A (**1**) (Scheme S1, Supporting Information) as described above for **3**.

Dankastatin A (**5**) had the molecular formula $C_{24}H_{35}Cl_2NO_5$ established by HREIMS. The IR spectrum showed bands at 3382, 1724, 1656, and 1610 cm^{-1}, characteristic of a hydroxyl group, a ketone, an amide carbonyl, and a double bond. Inspection of the ^{1}H and ^{13}C NMR spectra of **5** (Table 2) based on DEPT and HSQC (^{1}H−^{13}C COSY) experiments revealed the presence of the following functional groups: four methyl groups (C-22, C-23, C-24, and OMe) including one primary, one secondary, one vinylic, and one methoxy, six methylenes (C-3 and C-17 to C-21), four sp^3-methines (C-2, C-8, C-9, and C-16) bearing one oxygen, one chlorine, one

Table 2. NMR Spectroscopic Data ($CDCl_3$) for Dankastatin A (**5**)

position	δ_C, multi.	δ_H[a]		*J*/Hz	HMBC[b]	NOESY
1	98.9, CH	4.59	s		OCH_3-1, 3, 9	OCH_3-1, 2, 10
2	47.8, CH	4.20	ddd	7.8 (10), 3.7 (3α), 2.7 (3*β*)	4	1, 3α, 3*β*, 10
3α	37.6, CH_2	2.26	dd	13.7 (3*β*), 3.7 (2)	4, 5, 9	2, 3*β*, 9
3*β*		2.38	dd	13.7 (3α), 2.7 (2)	1, 2, 4	1, 2, 3α, 5
4	67.6, qC					
5	144.5, CH	6.60	d	2.3 (9)	3, 7, 8, 9	3*β*, 10
6	130.7, qC					
7	182.6, qC					
8	60.7, CH	5.34	d	2.5 (9)	7	9
9	75.7, CH	4.39	dd	2.5 (8), 2.3 (5)	4, 5, 7, 8	OCH_3-1, 3α
10		5.55	br d	7.8 (2)	1, 2, 11	1, 2, 5, 13
11	166.4, qC					
12	116.4, CH	5.65	d	15.1 (13)	11, 13, 14	13, 23
13	147.8, CH	7.15	d	15.1 (12)	11, 12, 14, 15, 23	10, 12, 15
14	130.8, qC					
15	149.1, CH	5.65	dd	9.6 (16), 1.1 (23)	13, 14, 23, 24	13, 16, 24
16	33.3, CH	2.50	m			15, 23, 24
17A	37.2, CH_2	1.23	m		18	
17B		1.32	m		18	
18	27.5, CH_2	1.20	m		19	
19	29.4, CH_2	1.24	m			
20	31.8, CH_2	1.24	m			
21	22.7, CH_2	1.28	m		20	
22	14.1, CH_3	0.87	t	6.8 (21)	20, 21	
23	12.4, CH_3	1.74	d	1.1 (15)	13, 14, 15	12, 16
24	20.4, CH_3	0.96	d	6.6 (16)	15, 16, 17	15, 16
OCH_3-1	55.3, CH_3	3.48	s		1	1, 9

[a] As in Table 1. [b] HMBC correlations are from proton stated to the indicated carbon.

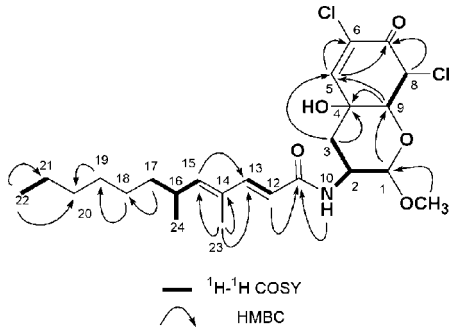


Figure 2. 2D NMR correlations for dankastatin A (**5**).

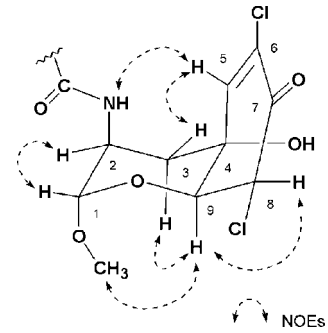
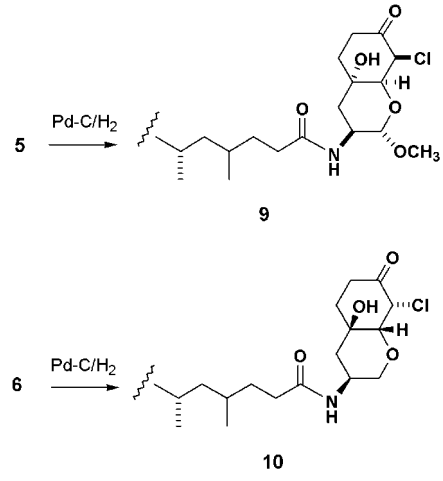


Figure 3. Observed NOEs and conformation for dankastatin A (**5**).

nitrogen, and one methyl each, and a methyl ketal group (C-1). Additional functionalized carbons included a quaternary sp^3-carbon (C-4) linked to a hydroxy group, one disubstituted (C-12 and C-13) and two trisubstituted (C-14 and C-15; C-5 and C-6) double bonds, a secondary amide (C-11 and N-10), and a conjugated ketone (C-7). The $^1H-^1H$ COSY analysis of **5** led to five partial structural units as shown by boldfaced lines in Figure 2, which were supported by HMBC correlations (Table 2). The connection of these units and the remaining functional groups was determined on the basis of the key HMBC correlations summarized in Figure 2, and the planar structure of **5** was elucidated.

The relative stereochemistry of **5** was established by analysis of NOESY data (Table 2). The observation of NOEs from H-9 to H-3α and OCH_3-1 (Figure 3) suggested that the tetrahydropyran ring (C-1−C-4−C-9) exists in a chair conformation in $CDCl_3$, with H-3α, H-9, and OCH_3-1 in coaxial arrangements. NOEs from H-5 to NH-10 and H-3*β* implied that the C-2−N and C-4−C-5 bonds are in a coaxial arrangement and oriented *trans* to H-3α, H-9, and OCH_3-1. The appearance of the H-1 signal as a singlet suggested that the H-1/H-2 dihedral angle is approximately 90°. Since the stereochemistry of H-8 was not deduced from the NOE and the coupling constant between H-8 and H-9, compound **5** was hydrogenated in the presence of Pd−C to yield derivative **9**, in which one of two chlorines in **5** was replaced by hydrogen (Scheme 1). Derivative **9** showed an NOE between H-8 and H-6α and a coupling constant of 14 Hz between H-5*β* and H-6α, implying that the cyclohexanone

Scheme 1. Hydrogenation of Dankastatins A (**5**) and B (**6**)



ring of **9** exists in a chair conformation with H-6α and H-8 in a coaxial arrangement. This result clarified that H-9 and Cl-8 are oriented *trans* in compound **5**. The geometry of the dienes in the side chain of **5** was deduced from the large coupling constant between H-12 and H-13 (*J* 15.1 Hz), the chemical shift of the ^{13}C NMR signal of the vinylic methyl group (δ_C 12.4),[10] and NOEs

Table 3. NMR Spectroscopic Data ($CDCl_3$) for Dankastatin B (**6**)

position	δ_C, multi.	δ_H[a]		*J*/Hz	HMBC[b]	NOESY
1α	69.9, CH	4.13	ddd	10.8 (1*β*), 4.8 (2), 2.1 (3α)	2, 9	1*β*
1*β*		3.21	t	10.8 (1α, 2)	2, 9	1α, 9
2	43.9, CH	4.06	ddddd	12.1 (3*β*), 10.8 (1*β*), 8.0 (10), 4.8 (1α), 4.1 (3α)		1α, 3α, 3*β*, 10
3α	43.8, CH_2	2.47	ddd	12.1 (3*β*), 4.1 (2), 2.1 (1α)	1, 2, 5, 9	2, 3*β*, 5
3*β*		1.74	t	12.1 (2, 3α)	1, 2, 4, 5, 9	3α, 9
4	69.6, qC					
5	142.7, CH	6.83	d	2.5 (9)	3, 6, 7, 9	2, 3α
6	133.4, qC					
7	183.2, qC					
8	61.4, CH	5.35	d	2.3 (9)	7, 9	OH-4, 9
9	83.8, CH	3.96	dd	2.5 (5), 2.3 (8)	4, 5, 7, 8	1*β*, 3*β*, 8
10		5.43	br d	8.0 (2)	2, 11	12
11	166.7, qC					
12	116.6, CH	5.71	d	15.3 (13)	11, 14	10, 13, 23
13	147.8, CH	7.20	d	15.3 (12)	11, 12, 14, 15, 23	12, 15
14	130.7, qC					
15	149.0, CH	5.67	dd	9.6 (16), 1.1 (23)	15, 16, 17, 23, 24	13, 16, 24
16	33.3, CH	2.50	m			15, 23, 24
17A	37.2, CH_2	1.25	m			
17B		1.34	m			
18	27.5, CH_2	1.21	m			
19	29.4, CH_2	1.23	m			
20	31.8, CH_2	1.23	m			
21	22.6, CH_2	1.27	m			
22	14.1, CH_3	0.87	t	7.1 (21)	20, 21	
23	12.5, CH_3	1.75	d	1.1 (15)	13, 14, 15, 24	12, 16
24	20.5, CH_3	0.97	d	6.6 (16)	15, 16, 17	15, 16, 24
OH-4		3.85	br s			8

[a] As in Table 2. [b] As in Table 2.

(H-12/H-23 and H-13/H-15). Furthermore, the relative configuration of the chiral center (C-16) in the side chain was deduced from the fact that the NMR chemical shifts of C-16 and C-24 in **5** were completely identical with those of gymnastatins A (**1**) and F (**2**) and the other gymnastatins.[5,6]

The absolute configurations of C-16 and C-2 in **5** were not established independently, but were assumed to be the same as those for its co-metabolites, gymnastatins A (**1**), D, and E. This was supported by a consideration of the following biosynthesis for compound **5** via compound **8** corresponding to the oxidative product of gymanastatins A (**1**). It is quite plausible that gymnastatin S (**5**) is synthesized biogenetically via dienone **8** that is derived from L-tyrosine and 4,6 *R*-dimethyldodeca-2*E*,4*E*-dienoic acid (**7**) (Scheme S1, Supporting Information), as shown in Scheme S2 (Supporting Information). The summary of these considerations led to absolute stereostructure **5** for dankastatin A.

Dankastatin B (**6**) was shown to have the molecular formula $C_{23}H_{33}Cl_2NO_4$ by HREIMS. The IR spectrum showed absorption bands characteristic of a hydroxyl group, a ketone, an amide, and a double bond. The general features of the ^1H and ^{13}C NMR spectra (Table 3) of **6** closely resembled those of **5** except that the signals for methoxymethine of acetal in **5** were replaced by those of methylene [δ_H 4.13 (H-1α), 3.21 (H-1*β*); δ_C 69.9 (C-1)] in **6**. The planar structure of **6** deduced from this evidence was confirmed by analysis of $^1H-^1H$ COSY (H-1α/H-2, H-2/H-3*β*, H-8/H-9, and others) and HMBC (Table 3) correlations. The observation of NOEs from H-9 to H-1*β* and H-3*β*, coupling constants between vicinal protons ($J_{1\beta,2}$ 10.8 Hz and $J_{2,3\beta}$ 12.1 Hz), and *W*-type long-range couplings ($J_{1\alpha,3\alpha}$ 2.1 Hz) in **6** implied that the tetrahydropyran ring of **6** in $CDCl_3$ exists in a chair conformation with H-2 in an axial arrangement that is oriented *trans* to H-1*β*, H-3*β*, and H-9 in coaxial arrangements (Figure 4). In addition, NOEs from H-5 to H-2 and H-3α showed that OH-4 in an equatorial arrangement is oriented *trans* to H-2. Furthermore, an NOE between H-8 and OH-4 implied that the cyclohexenone ring (C-4−C-9) of **6** in $CDCl_3$ exists in a half-chair conformation, with H-8 and OH-4 in a copseudoaxial arrangement. This result was confirmed by NOESY experiments of the derivative **10**, as obtained by hydrogenation of compound **6** in the presence of Pd−C (Scheme 1). The observation of an NOE

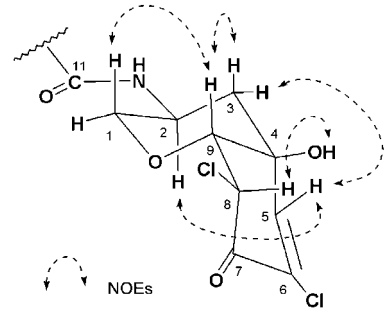


Figure 4. Observed NOEs and conformation for dankastatin B (**6**).

between H-8 and H-6*β* and the large coupling constant ($J_{5\alpha,6\beta}$ 13.7 Hz) in derivative **10** showed that the cyclohexanone ring of **10** exists in a chair conformation with H-8, H-6*β*, and OH-4 in coaxial arrangements. The stereochemistry of the side chain was determined by comparison of the NMR data of the side chain in **6** with those of **5**. The above-mentioned evidence allowed assignment of the relative stereostructure **6** to dankastatin B. As described above for dankastatin A (**5**), the absolute chemistry of **6** has not been established independently, but is assumed to be the same as for its co-metabolites, gymnastatins A (**1**), D, and E. This was supported by a consideration of the biosynthetic pathway of **6** via dienone **8** (Scheme S2, Supporting Information) as described above for **5**.

The cancer cell growth inhibitory properties of the isolated metabolites (**3**–**6**) were examined using the murine P388 lymphocytic leukemia cell line. Gymnastatins Q (**3**) and R (**4**) and dankastatins A (**5**) and B (**6**) exhibited growth inhibition against the P388 cell line (ED_{50} 1.7, 2.8, 0.15, and 0.16 *μ*g/mL, respectively). It is possible that their growth inhibitory activities are due to a conjugated ketone system. The inhibitory activities of compounds **5** and **6** were more potent than those of **3** and **4**, suggesting that a tetrahydropyran system is important for enhancement of the activity in these analogues. The cancer cell growth inhibitory property of gymnastatin Q (**3**) was also evaluated using a disease-oriented panel of 39 human cancer cell lines (HCC panel)

in the Japanese Foundation for Cancer Research.[11] The mean value (MG-MID) of log GI_{50} over all cell lines tested was −4.81, suggesting that the growth inhibitory activity of gymnastatin Q (**3**) is weak on average. However, compound **3** exihibited appreciable growth inhibition against BSY-1 (breast) and MKN7 (stomach) cell lines (log GI_{50} −5.47 and −5.17, respectively).

As described above, the sponge-derived fungus *G. dankaliensis* OUPS-N134 produced gymnastatins Q (**3**) and R (**4**) and dankastatins A (**5**) and B (**6**) as cancer cell growth inhibitory metabolites in the malt extract medium (type A) containing soluble starch. Interestingly, dankastatins A (**5**) and B (**6**) among these compounds possess an unprecedented skeleton and are a new class of natural products.

Experimental Section

General Experimental 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 × 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.[5,6,8]

Culture Conditions. The fungal strain was grown in a liquid medium (type A, 100 L) containing 1% malt extract, 1% soluble starch, and 0.05% peptone in artificial seawater adjusted to pH 7.5 for 28 days 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 (76.6 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; 61.2 g), in which the activity was concentrated, was chromatographed on a silica gel column with an *n*-hexane−CH_2Cl_2−MeOH gradient, to give four fractions [F2 (3.922 g), F3 (417 mg), and F4 (269 mg) eluted with MeOH−CH_2Cl_2 (1:99) and F5 (550 mg) eluted with MeOH−CH_2Cl_2 (1:49)]. Fraction F3 was further separated by silica gel column chromatography with a CH_2Cl_2−MeOH gradient as eluent, affording two fractions [F6 (155 mg) and F7 (27 mg)] eluted with MeOH−CH_2Cl_2 (1:199). Fraction F6 was purified by HPLC (ODS) using acetone−H_2O (17:3 and 3:1, respectively) then acetonitrile−H_2O (17:3) as eluents to afford dankastatin B (**6**) (3.5 mg). Fraction F7 was purified by HPLC using acetone−H_2O (4:1) then acetonitrile−H_2O (17:3) as eluent, to give dankastatin A (**5**) (1.8 mg). The MeOH−CH_2Cl_2 (1:199) eluate (135.5 mg) from the silica gel column chromatography of F4 was separated by HPLC using acetone−H_2O (17:3) as eluent, affording two fractions [F8 (48.3 mg) and F9 (20.9 mg)]. Fraction F8 was purified by HPLC using MeOH−H_2O (17:3), then acetonitrile−H_2O (3:1) as eluent, to afford gymnastatin Q (**3**) (15.8 mg). Fraction F9 afforded gymnastatin R (**4**) (5.6 mg) after purification by HPLC using acetonitrile−H_2O (4:1) as eluent.

Gymnastatin Q (3): colorless powder; mp 105–108 °C; $[\alpha]^{23}_D$ −34.3 (*c* 0.26, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 264 (4.45) nm; CD (EtOH) λ_{max} ($\Delta\epsilon$) [*c* 6.63 × 10^{-5} M] 386 (0), 339 (−1.27), 296 (−0.72), 276 (−3.49), 264 (0), 251 (+3.85), 231 (0), 220 (−0.85); IR (KBr) ν_{max} 3384 (OH, NH), 1713 (C═C−C═O), 1652 (CONH), 1611 (C═C) cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and S1 (Supporting Information); EIMS *m/z* 487 $[M]^+$ (6.3), 402 $[M - C_6H_{13}]^+$ (5.0), 374 $[M - C_8H_{17}]^+$ (10.6), 207 $[C_{14}H_{23}O]^+$ (59.9), 179 $[C_{13}H_{23}]^+$ (87.2), 95 $[C_5H_5NO]^+$ (100); HREIMS *m/z* 487.1895 $[M]^+$ (calcd for $C_{24}H_{35}Cl_2NO_5$, 487.1881).

Gymnastatin R (4): colorless powder; mp 79–82 °C; $[\alpha]^{24}_D$ −104.5 (*c* 0.48, EtOH); UV (EtOH) λ_{max} (log ϵ) 264 (4.47) nm; CD (EtOH) λ_{max} ($\Delta\epsilon$) [*c* 5.35 × 10^{-5} M] 386 (0), 341 (−2.06), 300 (−0.52), 271 (−8.25), 257 (0), 248 (+428), 231 (0), 211 (−1.11); IR (KBr) ν_{max} 3410 (OH, NH), 1706 (C═C−C═O), 1651 (CONH), 1610 (C═C) cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and S1 (Supporting Information); EIMS *m/z* 457 $[M]^+$ (12.7), 372 $[M - C_6H_{13}]^+$(12.0), 344 $[M - C_8H_{17}]^+$(9.1), 207 $[C_{14}H_{23}O]^+$ (26.4), 179 $[C_{13}H_{23}]^+$ (75.3), 95 $[C_5H_5NO]^+$ (100); HREIMS *m/z* 457.1789 $[M]^+$ (calcd for $C_{23}H_{33}Cl_2NO_4$, 457.1776).

Dankastatin A (5): colorless powder; mp 169–171 °C; $[\alpha]^{22}_D$ +114.4 (*c* 0.176, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 266 (4.45) nm; CD (EtOH) λ_{max} ($\Delta\epsilon$) [*c* 6.69 × 10^{-5}M] 306 (0), 292 (+0.62), 282 (0), 260 (−3.49), 222 (−0.77), 211 (−1.37); IR (KBr) ν_{max} 3382 (OH, NH), 1724 (C═C−C═O), 1656 (CONH), 1610 (C═C) cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 2; EIMS *m/z* 487 $[M]^+$ (2.6), 402 $[M - C_6H_{13}]^+$ (1.8), 374 $[M - C_8H_{17}]^+$ (4.3), 207 $[C_{14}H_{23}O]^+$ (100.0), 179 $[C_{13}H_{23}]^+$ (81.3), 95 $[C_5H_5NO]^+$ (64.7); HREIMS *m/z* 487.1893 $[M]^+$ (calcd for $C_{24}H_{35}Cl_2NO_5$, 487.1881).

Dankastatin B (6): colorless powder; mp 90–92.5 °C; $[\alpha]^{22}_D$ −157.4 (*c* 0.177, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 265 (4.45) nm; CD (EtOH) λ_{max} ($\Delta\epsilon$) [*c* 6.26 × 10^{-5}M] 340 (0), 311 (−0.40), 297 (0), 268 (−6.22), 240 (+3.09), 220 (0), 211 (−1.56); IR (KBr) ν_{max}3393 (OH, NH), 1720 (C═C−C═O), 1652 (CONH), 1611 (C═C) cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 3; EIMS *m/z* 457 $[M]^+$ (9.5), 344 $[M - C_8H_{17}]^+$ (8.7), 207 $[C_{14}H_{23}O]^+$ (100.0), 179 $[C_{13}H_{23}]^+$ (92.6), 95 $[C_5H_5NO]^+$ (62.1); HREIMS *m/z* 457.1782 $[M]^+$ (calcd for $C_{23}H_{33}Cl_2NO_4$, 457.1776).

Hydrogenation of Dankastatin A (5). To a solution of dankastatin A (**5**) (3.8 mg) in MeOH (2 mL) was added 10% Pd/C (6 mg), and the reaction mixture was stirred under hydrogen atmosphere (1 atm) at room temperature overnight. The catalyst was filtered off and the solvent evaporated under reduced pressure. The residue was purified by HPLC using acetonitrile−H_2O (17:3) as eluent to afford compound **9** (0.4 mg, 11.2%) as a colorless powder. Compound **9**: ^{1}H NMR ($CDCl_3$) δ 0.81 (3H, d, *J* = 6.6 Hz, H-24), 0.84 (3H, d, *J* = 6.4 Hz, H-23), 0.88 (3H, t, *J* = 6.6 Hz, H-22), 1.31 (2H, m, H-21), 1.42 (1H, m, H-16), 1.49 (1H, m, H-14), 1.87 (1H, ddt, *J* = 14.0, 5.9, 2.1 Hz, H-5α), 2.08 (2H, m, H-3), 2.38 (1H, td, *J* = 14.0, 4.6 Hz, H-5*β*), 2.48 (1H, ddd, *J* = 14.0, 4.6, 2.1 Hz, H-6*β*), 2.90 (1H, td, *J* = 14.0, 5.9 Hz, H-6α), 3.44 (3H, s, OCH_3-1), 4.12 (1H, dt, *J* = 7.3, 3.4 Hz, H-2), 4.33 (1H, dd, *J* = 4.1, 2.1 Hz, H-9), 4.60 (1H, s, H-1), 5.16 (1H, d, *J* = 4.1 Hz, H-8), 5.54 (1H, br d, *J* = 7.3 Hz, H-10); EIMS *m/z* 459 $[M]^+$.

Hydrogenation of Dankastatin B (6). Using the same procedure as above with compound **5**, a solution of dankastatin B (**6**) (3.8 mg) in MeOH (2 mL) was hydrogenated under the presence of 10% Pd/C (6 mg), and the resulting product was purified by HPLC using acetone−H_2O (3:1) as eluent to afford compound **10** (0.8 mg, 22.3%) as a colorless powder. Compound **10**: ^{1}H NMR (acetone-d_6) δ 0.85 (3H, dd, *J* = 6.4 Hz, H-24), 0.87 (3H, dd, *J* = 6.4 Hz, H-23), 0.91 (3H, t, *J* = 6.8 Hz, H-22), 1.35 (2H, m, H-21), 1.52 (1H, m, H-16), 1.58 (1H, m, H-14), 1.75 (1H, t, *J* = 12.4 Hz, H-3*β*), 1.87 (1H, ddt, *J* = 13.7, 6.6, 2.1 Hz, H-5*β*), 2.19 (1H, m, H-3α), 2.34 (1H, ddd, *J* = 13.7, 5.0, 2.1 Hz, H-6α), 2.41 (1H, td, *J* = 13.7, 5.0 Hz, H-5α), 2.91 (1H, tdd, *J* = 13.7, 6.6, 0.7 Hz, H-6*β*), 3.07 (1H, t, *J* = 11.0 Hz, H-1*β*), 3.76 (1H, dd, *J* = 3.2, 2.1 Hz, H-9), 4.05 (1H, ddd, *J* = 11.0, 5.4, 1.8 Hz, H-1α), 4.14 (1H, m, H-2), 5.10 (1H, br s, H-10), 5.29 (1H, dd, *J* = 3.2, 0.7 Hz, H-8); EIMS *m/z* 430 $[M + H]^+$.

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Supporting Information Available: HMBC and NOESY correlations for compounds **3** and **4** (Table S1) and biosynthetic pathways for compounds **36** (Schemes S1 and S2). This material is available free of charge via the Internet at http://pubs.acs.org.

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