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Nakijiquinones C and D, New Sesquiterpenoid Quinones with a Hydroxy Amino Acid Residue from a Marine Sponge Inhibiting c-erbB-2 Kinase

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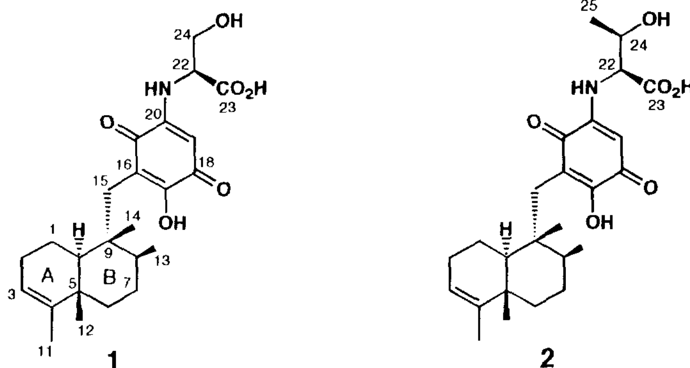
Abstract: Nakijiquinones C (**1**) and D (**2**), new sesquiterpenoid quinones with a serine or a threonine residue, respectively, have been isolated from an Okinawan marine sponge of the family Spongiidae and the structures were determined by spectroscopic data and chemical correlations with a known compound, isospongiaquinone (**5**). Nakijiquinones and synthetic related compounds exhibited inhibitory activity against c-erbB-2 kinase.

Marine sponge-derived sesquiterpenoid quinones have been extensively investigated because of their interesting biological activities.[1] In our continuing search for bioactive compounds from marine organisms,[2] we previously isolated new sesquiterpenoid quinones, nakijiquinones A (**3**) and B (**4**), from extracts of an Okinawan marine sponge of the family Spongiidae.[3] Further investigation of extracts of this sponge led to isolation of two new sesquiterpenoid quinones, nakijiquinones C (**1**) and D (**2**) containing a serine or a threonine residue, respectively, with inhibitory activity against c-erbB-2 kinase. In this paper we describe the isolation and structure elucidation of **1** and **2**, conversion of isospongiaquinone (**5**) into **1** ~ **4**, and inhibitory activities against tyrosine and protein C kinases and cytotoxicity of **1** ~ **4** and some synthetic related compounds (**6** ~ **10**).

Methanolic extract of the sponge collected off Nakijin, Okinawa Island, was partitioned between EtOAc and H_2O. *n*-BuOH soluble material of the aqueous phase was subjected to a silica gel column ($CHCl_3$/*n*-BuOH/AcOH/H_2O, 1.5:6:1:1) followed by Sephadex LH-20 ($CHCl_3$/MeOH, 1:1) and reversed-



phase columns ($CH_3CN/H_2O/CF_3CO_2H$, 85:15:0.1) to yield nakijiquinones C (**1**, 0.00028%, wet weight of the sponge) and D (**2**, 0.00028%) together with known compounds, nakijiquinones A (**3**) and B (**4**).[3] The EtOAc soluble portions were purified by silica gel column chromatography to give a known compound, isospongiaquinone (**5**, 0.22%)[4].

Nakijiquinone C (**1**) was obtained as a red amorphous solid {$[\alpha]^{20}_D$ -73° (*c* 0.03, EtOH)}. HRFABMS analysis revealed the molecular formula to be $C_{24}H_{33}NO_6$ [*m/z* 432.2381, $(M+2H-H)^-$, Δ -0.5 mmu]. UV absorptions (λ_{max} 321 and 498 nm) were similar to those of nakijiquinone A (**3**), indicating the presence of a hydroxy quinone moiety. IR absorptions at 3400 and 1670 cm^{-1} implied that **1** possessed OH and/or NH and carboxyl groups, respectively. The ^1H NMR (Table 1) spectrum of **1** in DMSO-d_6 showed signals due to a secondary methyl (δ_H 0.90), three tertiary methyls (δ_H 0.78, 0.93, and 1.48), and an olefinic proton (δ_H 5.05). These data and the ^1H-^1H COSY spectra of **1** indicated the presence of a tetramethyldecaline moiety. The ^1H and ^{13}C NMR (Table 1) spectra of **1** were assigned on the basis of ^1H-^1H COSY, HMQC[5], and HMBC[6] data. HMBC correlations of H_3-11 to C-3, C-4, and C-5, H_3-12 to C-4, C-5, and C-6, H_3-13 to C-7 and C-9, and H_3-14 to C-8, C-9, and C-10 indicated that four methyl groups were attached at C-4, C-5, C-8, and C-9. Cross-peaks of H_2-15 to C-8, C-9, and C-10 in the HMBC spectrum allowed the connection between C-15 and C-9. The carbon chemical shifts of C-19 (δ_C 93.1) and C-20 (δ_C 147.1) and HMBC correlations of H-19 and H_2-15 to C-17 and C-21 indicated that substitution pattern of the quinone ring in **1** was the same as that of nakijiquinone A (**3**). Cross-peaks of NH-20/H-22 and H-22/H-24 in the ^1H-^1H COSY spectrum and HMBC correlations of H-24 to C-23 (δ_C 171.9) implied the presence of a serine residue. The serine residue was attached at C-20, based on HMBC correlations of NH-20 to C-19 and C-21. Thus the structure of nakijiquinone C was elucidated to be **1**. Relative stereochemistry of the decaline ring of nakijiquinone C (**1**) was elucidated by the NOESY data (Fig. 1) as followed. NOESY correlations of H-1'/H_3-12, H-2'/H-10, and H-6/H_3-11 indicated a twist-boat conformation of ring A, while a chair conformation of ring B was deduced from NOESY correlations of H-7/H_3-14 and H-8/H-10. NOESY correlations of H_3-14/H_3-12 and H_3-14/H_3-13 implied that three methyl groups (Me-12, Me-13, and Me-14) were all β-oriented. The presence of a serine residue in **1** was also confirmed by standard amino acid analysis of ozonolysis products of **1**. The serine residue was determined to be L-form by chiral HPLC analysis of the ozonolysis products. Absolute stereochemistry of

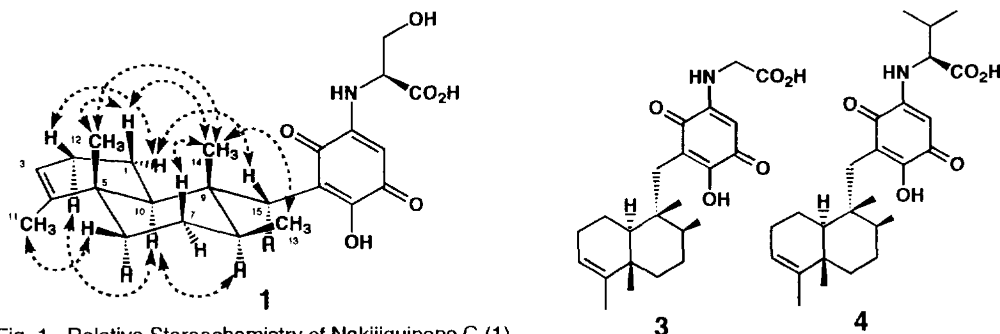


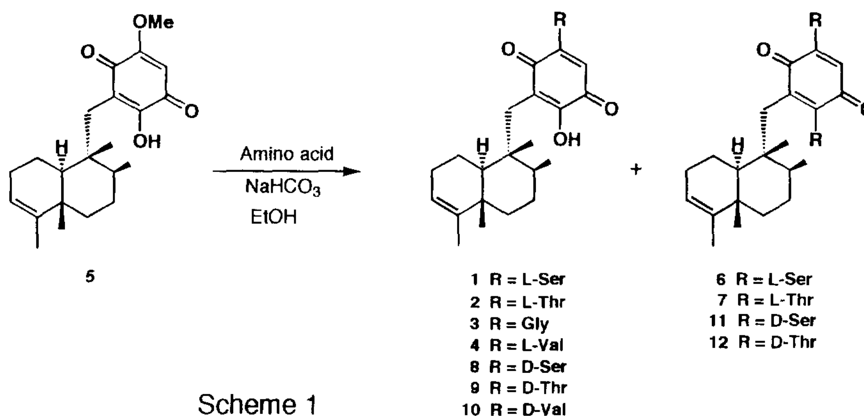
Fig. 1. Relative Stereochemistry of Nakijiquinone C (1). Dotted arrows denote NOESY correlations.

Table 1. ^{1}H and ^{13}C NMR Data of Nakijiquinone C (1) in DMSO-d_6

position	^{1}H^a		J(Hz)	^{13}C^a		H coupled with C^b
1	2.01	m		18.9	t	H-10
	1.39	m				H-10
2	1.92^c	m		25.8	t	
3	5.05	brs		121.0	d	H_3-11
4				142.8	s	H_3-11, H_3-12
5				37.2	s	H-3, H_3-11, H_3-12
6	1.53	m		34.9	t	H_3-12
	0.98	m				
7	1.33^c	m		26.9	t	H_3-13
8	1.30	m		36.8	d	H-6, H-10, H_3-14, H_2-15
9				41.2	s	H-10, H_3-13, H_3-14, H_2-15
10	1.02	m		46.5	d	H_3-12, H_3-14, H_2-15
11	1.48	s		17.5	q	H-3
12	0.93	s		19.3	q	
13	0.90	d	7.0	17.2	q	
14	0.78	s		16.5	q	H-10, H_2-15
15	2.43	d	13.6	31.4	t	H_3-14
	2.32	d	13.6			
16				113.5	s	H_2-15
17				158.5	s	H_2-15, H-19
18				178.8	s	H-19, NH-20
19	5.35	s		93.1	d	NH-20
20				147.1	s	
20-NH	7.15	d	8.0			
21				182.0	s	H_2-15, H-19, NH-20
22	4.20	m		56.5	d	
23				171.9	s	H_2-24
24	3.78	dd	11.4, 2.9	59.8	t	
	3.82	dd	11.4, 2.9			

a) δ in ppm b) HMBC correlations c) 2H

the decaline moiety in 1 was determined by comparison of spectral data of compound 1 derived from isospongiaquinone (5) with those of nakijiquinone C (Scheme 1). Reaction of 5 with L-Ser in EtOH



Scheme 1

Table 2. ^{1}H and ^{13}C NMR Data of Nakijiquinone D (2) in DMSO-d_6

position	$^{1}H^{a}$		J(Hz)	$^{13}C^{a}$		H coupled with C[b]
1	1.99	m		19.9	t	H-10
	1.35	m				H-10
2	1.88[c]	m		26.2	t	
3	5.05	brs		120.5	d	H_3-11
4				143.5	s	H_3-11, H_3-12
5				37.5	s	H-3, H_3-11, H_3-12
6	1.56	m		35.7	t	H_3-12
	0.95	m				
7	1.28[c]	m		28.5	t	H_3-13
8	1.25	m		37.2	d	H-10, H_3-13, H_3-14, H_2-15
9				41.9	s	H-10, H_3-13, H_3-14, H_2-15
10	0.98	m		46.9	d	H_3-12, H_3-14, H_2-15
11	1.48	s		17.9	q	H-3
12	0.94	s		20.0	q	
13	0.92	d	7.0	18.0	q	
14	0.78	s		17.1	q	H-10
15	2.47	d	13.7	31.7	t	H_3-14
	2.32	d	13.7			
16				114.1	s	H_2-15
17				158.1	s	H_2-15
18				179.1	s	H-19
19	5.33	s		93.0	d	
20				149.5	s	
20-NH	6.95	brd	7.0			
21				183.1	s	H_2-15, H-19
22	4.07	m		60.2	d	NH-20, H_3-25
23				171.0	s	
24	4.27	m		66.5	d	H-22
25	1.09	d	7.0	20.8	q	

a) δ in ppm b) HMBC correlations c) 2H

containing $NaHCO_3$ afforded **1**, whose optical rotation and other spectral data were coincident with those of natural nakijiquinone C (**1**). Compound **6** having two L-Ser residues at C-17 and C-20 also generated as a minor product in this reaction. Thus the absolute configurations at C-5, C-8, C-9, and C-10 of **1** were determined to be *S*, *S*, *R*, and *S*, respectively.

Nakijiquinone D (**2**) was obtained as a red amorphous solid {$[\alpha]^{20}_{D}$ -172° (*c* 0.2, EtOH)} and the molecular formula was established to be $C_{25}H_{35}NO_6$ by HRFABMS data [*m/z* 446.2524, $(M+2H-H)^-$, Δ -1.9 mmu]. The IR (ν_{max} 1630, 1590, and 1540 cm^{-1}) and UV (λ_{max} 317 and 490 nm) spectra of **2** indicated the presence of a hydroxy quinone chromophore. The ^{1}H and ^{13}C NMR data (Table 2) of **2** were similar to those of **1** except for an amino acid moiety. Detailed analyses of the ^{1}H-^{1}H COSY spectrum revealed connectivities of C-1 to C-3, C-6 to C-8, and C-10 to C-1. The connectivities around quaternary carbons, C-4, C-5, and C-9, of the decaline moiety were established by HMBC data (Table 2). These data suggested the presence of the same decaline moiety in **2** as that of **1**. Cross-peaks of NH-20/H-22, H-22/H-24, and H-24/H-25 in the ^{1}H-^{1}H COSY spectrum implied the presence of a threonine residue in place of the serine residue in **1**. Thus the structure of nakijiquinone D was assigned to be **2**. Relative

stereochemistry of the decaline moiety of nakijiquinone D (**2**) was elucidated to be the same as that of **1** on the basis of NOESY correlations. The presence of a threonine residue in **2** was also confirmed by standard amino acid analysis of ozonolysis products of **2**, while the threonine was determined to be L-form by chiral HPLC analysis. Absolute stereochemistry of the decaline moiety in nakijiquinone D (**2**) was established by derivatization of isospongiaquinone (**5**) to **2** under the same condition as described above (Scheme 1). Compound **2** derived from **5** and L-Thr gave the same spectral data and optical rotation as those of natural nakijiquinone D (**2**). This reaction also generated compound **7** having two L-Thr residues as a minor product. Thus the absolute configurations at C-5, C-8, C-9, and C-10 of **2** were determined to be *S*, *S*, *R*, and *S*, respectively.

Reaction of isospongiaquinone (**5**) with Gly or L-Val in EtOH containing $NaHCO_3$ at room temperature for 24 h afforded compounds **3** or **4**, respectively, whose optical rotation and other spectral data were consistent with those of natural nakijiquinones A (**3**) and B (**4**), while in these reactions no compounds with two amino acid residues were obtained (Scheme 1). Other related sesquiterpenoid quinones having D-Ser (**8**), D-Thr (**9**), and D-Val (**10**) at C-20, D-Ser (**11**) at C-17 and C-20, and D-Thr (**12**) at C-17 and C-20 were also prepared by reaction of **5** with D-Val, D-Ser, and D-Thr, respectively (Scheme 1).

Cytotoxic activities of nakijiquinones A ~ D (**1** ~ **4**) and the related compounds (**6** ~ **10**) were shown in Table 3. Nakijiquinones C (**1**) and D (**2**) exhibited cytotoxicity against L1210 murine leukemia cells with IC_{50} values of 5.8 and 8.1 μg/mL, respectively, and KB human epidermoid carcinoma cells with IC_{50} values of 6.2 and 1.2 μg/mL, respectively, *in vitro*. Interestingly, cytotoxicities of compounds **2** and **7** against KB cells showed more potent than those against L1210 cells, while other compounds (**1**, **3**, **4**, and **8** ~ **10**) showed less activity against KB cells. On the other hand, compounds **1** ~ **4** and **6** ~ **10** were tested for inhibitory activity against two tyrosine kinases, EGF receptor and c-erbB-2 kinases, and protein kinase C (Table 4). Nakijiquinones C (**1**) and D (**2**) exhibited inhibitory activity against c-erbB-2 kinase with IC_{50} values of 26 and 29 μM, respectively, EGF receptor kinase with those of 170 and >400 μM, respectively, and protein kinase C with those of 23 and 220 μM, respectively. Other compounds **3** ~ **4** and **6** ~ **10** also showed relatively potent inhibitory activity against c-erbB-2 kinase rather than EGF receptor kinase or protein kinase C.

Table 3. Cytotoxicity of Compounds **1**~**4** and **6**~**10** against L1210 and KB Tumor Cells.

	IC_{50} (μg/mL)								
Compound	**1**	**2**	**3**	**4**	**6**	**7**	**8**	**9**	**10**
L1210	5.8	8.1	3.8	2.8	>10	>10	3.5	0.87	3.9
KB	6.2	1.2	7.6	5.0	>10	0.6	>10	1.8	5.4

Table 4. Inhibitory Activities of Compounds **1**~**4** and **6**~**10** against Tyrosine and Protein C Kinases.

	IC_{50} (μM)								
Compound	**1**	**2**	**3**	**4**	**6**	**7**	**8**	**9**	**10**
EGF Receptor kinase	170	>400	>400	250	>400	200	>400	>400	200
c-erbB-2 kinase	26	29	30	95	62	100	120	22	17
Protein kinase C	23	220	270	200	>400	140	>400	150	150

Experimental Section

General Methods. Optical rotations were determined on a JASCO DIP-370 polarimeter. UV and IR spectra were obtained on JASCO Ubest-35 and JASCO IR report-100 spectrometers, respectively. ^1H and ^{13}C NMR spectra were recorded on JEOL EX-400 and Bruker ARX-500 spectrometers. The 2.49 and 7.26 ppm resonances of residual DMSO-d_5 and $CHCl_3$, respectively, and 39.5 and 77.0 ppm of DMSO-d_6 and $CDCl_3$, respectively, were used as internal references. EIMS and FABMS spectra were obtained on a JEOL DX-303 spectrometer operating at 70 eV and on a JEOL HX-110 spectrometer, respectively.

Sponge Material. The sponge (order Dictyoceratida; family Spongiidae) was collected off Nakijin, Okinawa Island and kept frozen until used. Cavernous chocolate brown sponge. Same colour throughout ectosome and mesohyl. Thick membranous tissue occurs around cavities. Sponge texture is firm, compressible and spongy. Mesohyl consists of dense tissue with primary, secondary, and tertiary fibres forming a neat, small reticulation. All fibres are uncored. Primary fibres are 60 mm wide, secondary fibres are 24 mm wide, and tertiary fibres are 15 mm wide. The voucher specimen (SS-865) was deposited at the Faculty of Pharmaceutical Sciences, Hokkaido University.

Extraction and Separation. The sponge (0.4 kg, wet weight) was extracted with MeOH (600 mL x 2). The MeOH extract was partitioned between EtOAc (400 mL x 3) and H_2O (400 mL) and then the aqueous layer was extracted with *n*-BuOH (400 mL x 3). The *n*-BuOH soluble portions were evaporated under reduced pressure to give a residue (3.5 g), part of which (1.0 g) was subjected to a silica gel column eluted with $CHCl_3$/*n*-BuOH/AcOH/H_2O (1.5:6:1:1). The fraction eluting from 10 to 300 mL was separated by a Sephadex LH-20 column with $CHCl_3$/MeOH (1:1), in which the fraction eluting from 300 ~ 400 mL was purified by C_{18} reversed-phase HPLC (YMC-Pack AM323, YMC Co., Ltd., 1.0 x 25 cm; flow rate 2.5 mL/min; UV detection at 300 nm; eluent $CH_3CN/H_2O/CF_3CO_2H$, 85:15:0.1) to afford nakijiquinones C (**1**, 1.0 mg, t_R 10.8 min) and D (**2**, 1.0 mg, t_R 12.4 min).

Nakijiquinone C (1). A red amorphous solid; mp. 198-200°C; $[\alpha]^{20}_D$ -73° (*c* 0.03, EtOH); IR (KBr) ν_{max} 3400, 1670, 1630, 1590, 1540, 1380, and 1200 cm^{-1}; UV (MeOH) λ_{max} 321 (ε 12100) and 498 nm (920); ^1H and ^{13}C NMR (Table 1); FABMS (negative, diethanolamine matrix) *m/z* 432 $(M+2H-H)^-$; HRFABMS *m/z* 432.2381 $(M+2H-H)^-$, calcd for $C_{24}H_{34}NO_6$, 432.2386; NOESY correlations (DMSO-d_6, H/H) 1/1', 1/2, 1/10, 1/15, 1'/2, 1'/12, 1'/14, 2/3, 2'/10, 3/11, 6/6', 6/7, 6/11, 7/14, 8/10, 8/13, 8/15', 10/15, 12/14, 13/14, 13/15', 14/15, 14/15', 19/22, 22/24, 20-NH/22, and 20-NH/24.

Nakijiquinone D (2). A red amorphous solid; mp. 188-191°C; $[\alpha]^{20}_D$ -172° (*c* 0.2, EtOH); IR (KBr) ν_{max} 3400, 1670, 1630, 1590, 1540, 1380, and 1200 cm^{-1}; UV (MeOH) λ_{max} 317 (ε 12600) and 490 nm (1000); ^1H and ^{13}C NMR (Table 2); FABMS (negative, diethanolamine matrix) *m/z* 446 $(M+2H-H)^-$; HRFABMS *m/z* 446.2524 $(M+2H-H)^-$, calcd for $C_{25}H_{36}NO_6$, 446.2906; NOESY correlations (DMSO-d_6, H/H) 1/1', 1/2, 1/10, 1/15, 1'/2, 1'/12, 1'/14, 2/3, 2'/10, 3/11, 6/6', 6/7, 6/11, 7/14, 8/10, 8/13, 8/15', 10/15, 12/14, 13/14, 13/15', 14/15, 14/15', 19/22, 20-NH/22, and 20-NH/24.

Determination of the Stereochemistry of Serine and Threonine Residues in 1 and 2. A stream of O_3 was bubbled into a MeOH solution (0.5 mL) of nakijiquinone C (1, 0.05 mg) at room temperature for 1 min. After evaporation, the residue was subjected to chiral HPLC analysis using SUMICHIRAL OA-5000 column [Sumitomo Chemical Industry, 4 x 150 mm; flow rate: 0.2 mL/min; eluent: H_2O containing 2.0 mmol $CuSO_4$; detection: UV at 254 nm]. Retention times of standard L- and D-serine were 26.1 and 28.1 min, respectively, and that of serine contained in ozonolysis products of 1 was found to be 26.1 min.

According to essentially the same procedure as descrived above, nakijiquinone D (2, 0.05 mg) afforded ozonolysis products, which was subjected to chiral HPLC analysis using SUMICHIRAL OA-5000 column [flow rate: 0.25 mL/min; eluent: H_2O containing 0.5 mmol $Cu(AcO)_2$; detection: UV at 254 nm]. Retention times of standard L- and D-threonine were 23.0 and 25.5 min, respectively, and that of threonine contained in ozonolysis products of 2 was found to be 23.0 min.

Nakijiquinones C (1) and D (2) Derived from Isospongiaquinone (5). A mixture of 5 (3.0 mg, 8.4 μmol) and L-serine (1.3 mg, 10 μmol) in EtOH (1 mL) was stirred at 40°C for 24 h in the presence of $NaHCO_3$ (27 mg, 34 μmol). After filtration, the filtrate was evaporated to dryness, and the residue was purified by C_{18} reversed-phase HPLC (YMC-Pack AM-323, 1.0 x 25 cm; flow rate 2.5 mL/min; UV detection at 300 nm; eluent $CH_3CN/H_2O/CF_3CO_2H$, 85:15:0.1) to afford nakijiquinone C (1.7 mg, 46%, $[\alpha]^{17}_D$ -71° (*c* 0.73, EtOH)) and 6 (0.9 mg, 21%).

According to essentially the same procedure as descrived above, isospongiaquinone (5, 3.0 mg, 8.4 mmol), L-threonine (1.3 mg, 13 μmol), and $NaHCO_3$ (11 mg, 130 μmol) afforded nakijiquinone D (2, 1.3 mg, 35%, $[\alpha]^{17}_D$ -183° (*c* 1.0, EtOH)) and 7 (0.6 mg, 20%).

Compound 6: a red amorphous solid; $[\alpha]^{20}_D$ -327° (*c* 0.2, EtOH); IR (KBr) ν_{max} 3300, 1640, 1580, 1380, and 1200 cm^{-1}; UV (MeOH) λ_{max} 350 (ε 12100) and 498 nm (920); ^1H NMR (DMSO-d_6) δ 0.84 (3H, s, H-14), 1.02 (3H, d, *J* = 7.0 Hz, H-13), 1.05 (3H, s, H-12), 1.26 (1H, m, H-10), 1.36 (2H, m, H-7), 1.38 (1H, m, H-8), 1.56 (3H, s, H-11), 1.65 (1H, m, H-6), 2.09 (2H, m, H-2), 2.25 (1H, m, H-1), 2.46 (1H, d, *J* = 13.6 Hz, H-15), 2.57 (1H, d, *J* = 13.6 Hz, H-15'), 4.10 (4H, m, H-24 and H-27), 4.67 (2H, m, H-22 and H-25), 5.15 (1H, brs, H-3), 5.66 (1H, s, H-19), and 8.54 (2H, d, *J* = 8.0 Hz, NH-17 and NH-20); ^{13}C NMR (DMSO-d_6) δ 16.5 (q, C-14), 17.2 (q, C-13), 17.5 (q, C-11), 20.2 (t, C-1), 19.3 (q, C-12), 25.8 (t, C-2), 26.9 (t, C-7), 31.4 (t, C-15), 34.9 (t, C-6), 36.8 (d, C-8), 37.2 (s, C-5), 41.2 (s, C-9), 46.5 (d, C-10), 56.5 (d, C-22 and C-25), 59.8 (t, C-24 and C-27), 93.1 (d, C-19), 113.5 (s, C-16), 121.0 (d, C-3), 142.8 (s, C-4), 147.1 (s, C-20), 158.5 (s, C-17), 171.2 (s, C-23 and C-26), 178.8 (s, C-18), and 182.0 (s, C-21); FABMS (negative, glycerol matrix) *m/z* 519 $(M+2H-H)^-$; HRFABMS *m/z* 519.2725 $(M+2H-H)^-$, calcd for $C_{27}H_{39}N_2O_8$, 519.2706.

Compound 7: a red amorphous solid; $[\alpha]^{20}_D$ -137° (*c* 0.6, EtOH); IR (KBr) ν_{max} 3300, 1720, 1640, 1590, 1540, 1380, and 1200 cm^{-1}; UV (MeOH) λ_{max} 351 (ε 18300) and 525 nm (360); ^1H NMR (DMSO-d_6) δ 0.74 (3H, s, H-14), 0.91 (3H, s, H-12), 0.97 (3H, d, *J* = 7.0 Hz, H-13), 1.02 (6H, d, *J* = 7.0 Hz, H-25 and H-29), 1.07 (1H, m, H-10), 1.20 (2H, m, H-7), 1.25 (1H, m, H-8), 2.25 (1H, m, H-1), 2.28 (1H, d, *J* = 13.6 Hz, H-15), 2.41 (1H, d, *J* = 13.6 Hz, H-15'), 4.32 (2H, m, H-22 and H-26), 4.49 (2H, m, H-24 and H-28), 5.00 (1H, brs, H-3), 5.73 (1H, s, H-19), and 8.58 (2H, d, *J* = 8.0 Hz, NH-17 and NH-20); ^{13}C NMR (DMSO-d_6) δ 17.1 (q, C-14), 17.9 (q, C-13), 18.0 (q, C-11), 19.9 (t, C-1), 20.0 (q, C-12), 20.8 (q, C-25 and C-29), 26.2 (t, C-2), 28.5 (t, C-7), 31.7 (t, C-15), 35.7 (t, C-6), 37.2 (d, C-8), 37.5 (s, C-5), 41.9 (s, C-9), 46.9 (d, C-10), 60.2 (d, C-22 and C-26), 66.5 (d, C-24 and C-28), 93.0 (d, C-19), 114.1 (s, C-16), 120.5 (d, C-3), 143.5 (s, C-4), 149.5 (s, C-20), 158.1 (s, C-17), 171.0 (s, C-23 and C-27), 179.1 (s, C-18), and 183.1 (s, C-21); FABMS (negative, glycerol matrix) *m/z* 547 $(M+2H-H)^-$; HRFABMS *m/z* 547.3028 $(M+2H-H)^-$, calcd for $C_{29}H_{43}N_2O_8$, 547.3019.

According to essentially the same procedure as descrived above, isospongiaquinone (5, 3.0 mg, 8.4 mmol), D-serine (1.3 mg, 13 μmol), and $NaHCO_3$ (11 mg, 130 μmol) afforded 8 (1.4 mg, 40%) and 11 (0.5 mg, 12%) and isosponjiaquinone (5, 3.0 mg, 8.4 μmol), D-threonine (1.5 mg, 13 μmol), and $NaHCO_3$ (11 mg, 130 μmol) afforded 9 (1.5 mg, 40%) and 12 (0.8 mg, 17%).

Compound 8: a red amorphous solid; $[\alpha]^{20}_D$ -561° (*c* 0.7, EtOH); IR (KBr) ν_{max} 3300, 1720, 1640, 1580, 1380, and 1200 cm^{-1}; UV (MeOH) λ_{max} 321 (ε 12100) and 498 nm (920); ^1H NMR (DMSO-d_6) δ 0.78 (3H, s, H-14), 0.92 (3H, d, *J* = 7.0 Hz, H-13), 0.97 (3H, s, H-12), 0.98 (1H, m, H-6'), 1.00 (1H,

m, H-10), 1.30 (1H, m, H-8), 1.31 (2H, m, H-7), 1.39 (1H, m, H-1'), 1.48 (3H, s, H-11), 1.90 (2H, brs, H-2), 2.03 (1H, m, H-1), 2.32 (1H, d, J = 13.6 Hz, H-15), 2.43(1H, d, J = 13.6 Hz, H-15), 3.78 (1H, dd, J = 11.4 and 3.9 Hz, H-24), 3.82 (1H, dd, J = 11.4 and 3.9 Hz, H-24), 4.20 (1H, m, H-22), 5.07 (1H, brs, H-3), 5.35 (1H, s, H-19), and 7.11 (1H, brd, J = 8.0 Hz, NH-20); FABMS (negative, glycerol matrix) *m/z* 432 $(M+2H-H)^-$; HRFABMS *m/z* 432.2381 $(M+2H-H)^-$, calced for $C_{24}H_{34}NO_6$, 432.2386.

Compound 9: a red amorphous solid; $[\alpha]^{20}_D$ -100° (c 0.8, EtOH); IR (KBr) ν_{max} 3300, 1720, 1640, 1580, 1380, and 1210 cm^{-1}; UV (MeOH) λ_{max} 317 (ε 12600) and 490 nm (1000); ^1H NMR (DMSO-d_6) δ 0.81 (3H, s, H-14), 0.91 (3H, d, J = 7.0 Hz, H-13), 0.98 (3H, s, H-12), 0.98 (1H, m, H-6'), 1.00 (1H, m, H-10), 1.07 (3H, d, J = 7.0 Hz, H-25), 1.28 (1H, m, H-8), 1.29 (2H, m, H-7), 1.39 (1H, m, H-1'), 1.50 (3H, s, H-11), 1.88 (2H, brs, H-2), 1.99 (1H, m, H-1), 2.34 (1H, d, J = 13.6 Hz, H-15), 2.47(1H, d, J = 13.6 Hz, H-15), 4.07 (1H, m, H-22), 4.24 (1H, m, H-24), 5.05 (1H, brs, H-3), 5.38 (1H, s, H-19), and 6.87 (1H, brd, J = 7.0 Hz, NH-20); FABMS (negative, glycerol matrix) *m/z* 446 $(M+2H-H)^-$; HRFABMS *m/z* 446.2524 $(M+2H-H)^-$, calcd for $C_{25}H_{36}NO_6$, 446.2543.

Nakijiquinones A (3) and B (4) Derived from Isospongiaquinone (5). A mixture of **5** (3.0 mg, 8.4 μmol) and glycine (0.8 mg, 10 μmol) in EtOH (1 mL) was stirred at room temperature for 24 h in the presence of $NaHCO_3$ (11 mg, 130 μmol). After filtration, the filtrate was evaporated to dryness, and the residue was purified by C_{18} reversed-phase HPLC (YMC-Pack AM-323, 1.0 x 25 cm; flow rate 2.5 mL/min; UV detection at 300 nm; eluent $CH_3CN/H_2O/CF_3CO_2H$, 85:15:0.1) to afford nakijiquinone A (**3**, 1.6 mg, 47%).

According to essentially the same procedure as descrived above, isospongiaquinone (**5**, 3.0 mg, 8.4 mmol), L-valine (1.2 mg, 10 μmol), and $NaHCO_3$ (11 mg, 130 μmol) afforded nakijiquinone B (**4**, 1.3 mg, 35%), while isospongiaquinone (**5**, 3.0 mg, 8.4 mmol), D-valine (1.5 mg, 13 μmol), and $NaHCO_3$ (11 mg, 130 μmol) afforded **10** (2.6 mg, 69%).

Compound 10: a red amorphous solid; $[\alpha]^{20}_D$ +106° (c 0.2, $CHCl_3$); IR (KBr) ν_{max} 3300, 1720, 1640, 1590, 1380, and 1210 cm^{-1}; UV (MeOH) λ_{max} 320 (ε 12000) and 492 nm (910); ^1H-NMR ($CDCl_3$) δ 0.86 (3H, s, H-14), 0.97 (3H, d, J = 7.0 Hz, H-13), 1.02 (3H, s, H-12), 1.03 (1H, m, H-6'), 1.05 (3H, d, J = 6.5 Hz, H-25), 1.09 (3H, d, J = 6.7 Hz, H-26), 1.13 (1H, m, H-10), 1.28 (1H, m, H-8), 1.30 (2H, m, H-7), 1.42 (1H, m, H-1'), 1.54 (3H, s, H-11), 2.01 (2H, brs, H-2), 2.04 (1H, m, H-1), 2.42 (1H, d, J = 13.8 Hz, H-15), 2.58 (1H, d, J = 13.8 Hz, H-15), 3.86 (1H, m, H-22), 5.11 (1H, brs, H-3), 5.43 (1H, s, H-19), and 6.73 (1H, brd, J = 7.0 Hz, NH-20); FABMS (negative, glycerol matrix) *m/z* 446 $(M+2H-H)^-$; HRFABMS *m/z* 446.2933 $(M+2H-H)^-$, calcd for $C_{26}H_{40}NO_5$, 446.2906.

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