Pergamon

Tetrahedron Vol. 50, No. 28, pp. 8347-8354, 1994

0040-4020/94 $7.00+0.00

0040-4020(94)00464-1

Nakijiquinones A and B, New Antifungal Sesquiterpenoid Quinones with an Amino Acid Residue from an Okinawan Marine Sponge

Hideyuki Shigemori, Tatsushi Madono, Takuma Sasaki[a], Yuzuru Mikami[b], and Jun'ichi Kobayashi*

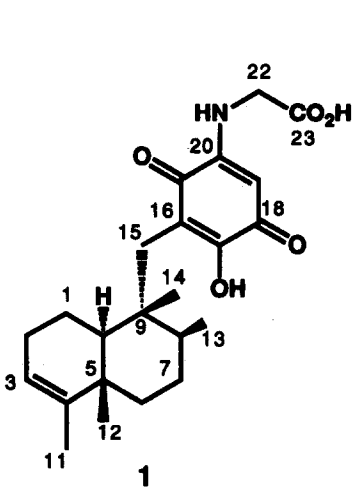
Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo 060, Japan, [a]Cancer Research Institute, Kanazawa University, Kanazawa 920, Japan, and [b]Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University, Chiba 280, Japan

Abstract: Two new sesquiterpenoid quinones with an amino acid residue, nakijiquinones A (**1**) and B (**2**), have been isolated from an Okinawan marine sponge (family Spongiidae) and the structures were determined by spectroscopic data, especially several types of 2D NMR spectra. The absolute configuration of nakijiquinone A (**1**) was established by chemical correlations with a known compound, isospongiaquinone (**3**).

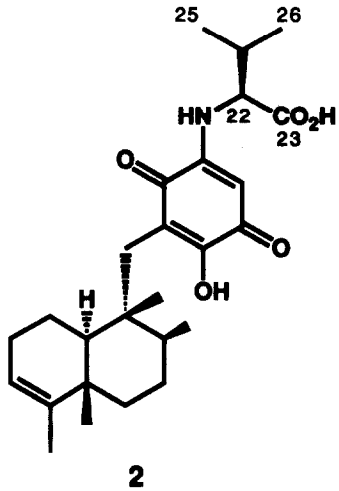
Many terpenoid quinones and phenols from marine sponges have been shown to exhibit interesting biological activities.[1] During our search for bioactive metabolites from marine organisms,[2] we examined extracts of an Okinawan marine sponge (family Spongiidae) and obtained two new sesquiterpenoid quinones with an amino acid residue, named nakijiquinones A (**1**) and B (**2**).[3] In this paper we describe the isolation and structure elucidation of **1** and **2**.

The sponge (family Spongiidae) was collected off Nakijin, Okinawa Island, and kept frozen until used. The methanolic extract of the sponge was partitioned between EtOAc and H_2O. The *n*-BuOH soluble material of the aqueous phase was subjected to silica gel column chromatography ($CHCl_3$/*n*-BuOH/AcOH/H_2O, 1.5:6:1:1) followed by Sephadex LH-20 ($CHCl_3$/MeOH, 1:1) and reversed-phase column chromatographies ($CH_3CN/H_2O/CF_3CO_2H$, 85:15:0.1) to yield nakijiquinones A (**1**, 0.009%, wet weight of the sponge) and B (**2**, 0.0007%). The EtOAc soluble portions were subjected to silica gel column chromatography to give two known compounds, isospongiaquinone (**3**, 0.22%)[4] and dictyoceratin A (0.03%).[5]

Nakijiquinone A (**1**) was obtained as a red solid {$[\alpha]^{20}_D$ -71.7° (*c* 1.0, MeOH)} and the molecular formula was established to be $C_{23}H_{31}NO_5$ by HRFABMS data [*m/z*



1



2

Table 1. ^{1}H and ^{13}C NMR Data of Nakijiquinone A (1) in CD_3OD

position	$^{1}H^{a}$		J(Hz)	$^{13}C^{a}$		H coupled with C^{b}
1	2.10	m		21.1	t	
	1.44	m				
2	1.93[c]	m		28.0	t	H-10
3	5.08	brs		121.9	d	H-11
4				144.9	s	H-11, H-12
5				39.6	s	H-10, H-11, H-12
6	1.63	m		37.4	t	H-12
	1.03	m				
7	1.36[c]	m		29.2	t	H-13
8	1.32	m		39.0	d	H-10, H-14, H-15
9				43.6	s	H-10, H-13, H-14, H-15
10	1.10	m		49.9	d	H-12, H-14, H-15
11	1.50	s		18.4	q	
12	1.00	s		20.7	q	H-10
13	0.97	d	7.0	18.4	q	
14	0.82	s		17.8	q	H-10, H-15
15	2.57	d	13.6	33.3	t	H-14
	2.42	d	13.6			
16				115.9	s	H-15
17				159.6	s	H-15, H-19
18				180.8	s	
19	5.28	s		93.8	d	
20				151.5	s	H-22
21				184.0	s	H-15, H-19
22	3.96	s		44.9	t	
23				171.9	s	H-22

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

404.2461, $(M+2H+H)^+$, Δ +2.4 mmu], requiring nine degrees of unsaturation. The IR spectrum indicated the presence of OH and/or NH (ν_{max} 3300 cm^{-1}), carboxyl (ν_{max} 1720 cm^{-1}), and conjugated carbonyl (ν_{max} 1640 and 1580 cm^{-1}) groups. UV absorptions at λ_{max} 317 and 488 nm suggested the presence of a quinone chromophore. The ^1H NMR spectrum of **1** in CD_3OD showed signals due to a secondary methyl (δ_H 0.97), three tertiary methyls (δ_H 0.82, 1.00, and 1.50), and a singlet olefinic proton (δ_H 5.28). These data suggested the presence of a tetramethyl decaline moiety in **1**. The EIMS fragment ion peak at *m/z* 343 $(M-CH_2CO_2H+H)^+$ of **1** suggested the presence of a carboxymethyl group. The ^1H and ^{13}C NMR spectra of **1** were assigned on the basis of several types of 2D NMR data including ^1H-^1H COSY, HMQC[6], and HMBC[7] spectra. Detailed analysis of the ^1H-^1H 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 the following HMBC data (Table 1). HMBC correlations of H_3-11 to C-3, C-4, and C-5 revealed that Me-11 was attached at C-4. Me-12 and Me-14 were attached at C-5 and C-9, respectively, based on HMBC correlations of H_3-12 to C-4, C-5, C-6, and C-10 and H_3-14 to C-8, C-9, and C-10. A cross peak of H-10 to C-5 in the HMBC spectrum indicated the presence of a bond between C-5 and C-10 to form a decaline skeleton, while a prominent fragment ion peak characteristic of fission of the decaline moiety ($C_{14}H_{23}$) from **1** was observed at *m/z* 191 in the EIMS spectrum.[4] The chemical shifts of a methylene (δ_H 3.96, 2H, s; δ_C 44.9, t) and a carboxyl group (δ_C 171.9, s) implied the presence of a glycine residue. This was also supported by standard amino acid analyses of the ozonolysis products of **1**. HMBC correlations of H_2-22 to C-20 and C-23 indicated that the glycine residue was attached at C-20. The presence of a 2-amino-5-hydroxy-benzoquinone moiety[8] was deduced from the chemical shifts of C-19 (δ_C 93.8) and C-20 (δ_C 151.5) in **1**. This was confirmed by

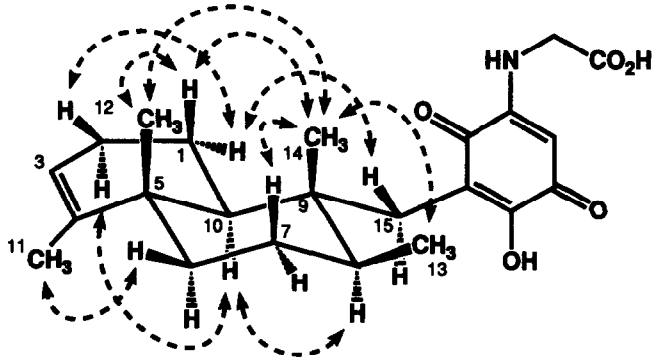


Fig. 1 Relative Stereochemistry of Nakijiquinone A (**1**). Dotted arrows denote NOESY correlations.

Table 2. ^{1}H and ^{13}C NMR Data of Nakijiquinone B (**2**) in $CDCl_3$

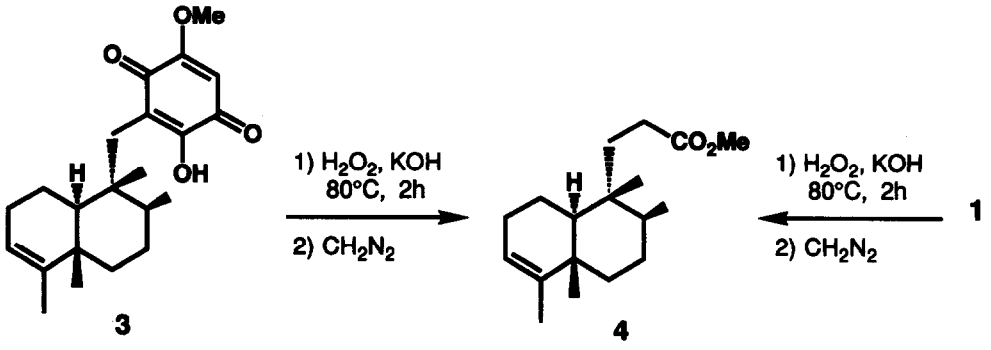
position	$^{1}H^{a}$		*J*(Hz)	$^{13}C^{a}$		H coupled with C[b]
1	2.04	m		20.2	t	H-3, H-10
	1.42	m				H-10
2	2.01	m		27.1	t	H-10
	1.83	m				
3	5.11	brs		120.7	d	H-11
4				144.2	s	H-11, H-12
5				38.5	s	H-3, H-10, H-11, H-12
6	1.60	m		36.0	t	H-12
	1.03	m				
7	1.32[c]	m		27.9	t	H-13
8	1.28	m		37.8	d	H-6, H-13, H-14, H-15
9				42.8	s	H-10, H-13, H-14, H-15
10	1.10	m		47.8	d	H-6, H-12, H-14, H-15
11	1.54	s		17.7	q	H-3
12	1.00	s		19.9	q	H-10
13	0.97	d	7.0	18.3	q	
14	0.83	s		17.3	q	H-10
15	2.58	d	13.8	32.5	t	H-14
	2.45	d	13.8			
16				114.6	s	H-15
17				156.7	s	H-15, H-19
18				179.2	s	
19	5.41	s		93.0	d	
20				149.6	s	H-22
20-NH	6.76	brd	7.0			
21				188.6	s	H-15, H-19
22	3.86	m		63.0	d	H-25, H-26
23				172.3	s	
24	2.34	m		30.9	d	H-22
25	1.09	d	6.5	18.2	q	
26	1.05	d	6.7	18.8	q	
27				172.3	s	

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

HMBC correlations of H-19 to C-17 and C-21, and H_2-15 to C-17 and C-21. The aminobenzoquinone moiety (C-16 ~ C-23) and the decaline (C-1 ~ C-14) were suggested to be connected between C-9 and C-16 through an isolated methylene (δ_H 2.42 and 2.47; δ_C 33.3), based on HMBC correlations of H_2-15 to C-8, C-9, C-10, C-16, C-17, and C-21. Thus the planar structure of nakijiquinone A was concluded to be **1**.

Relative stereochemistry of nakijiquinone A (**1**) was elucidated on the basis of NOESY correlations as shown in Fig. 1. 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 revealed that three methyl groups (Me-12, Me-13, and Me-14) were all β-oriented. Furthermore the

absolute stereochemistry of nakijiquinone A (**1**) was established by the following chemical correlation with isospongiaquinone (**3**) (Scheme 1): compound (**1**) was treated with H_2O_2 and then CH_2N_2[9] to give a degradation product (**4**). All spectral data ($[\alpha]_D$, ^1H NMR, IR, and EIMS) of **4** were identical with those of a degradation product obtained from compound **3** by the same procedure as described above. Thus absolute configurations at C-5, C-8, C-9, and C-10 of **1** were determined to be *S*, *S*, *R*, and *S*, respectively.[4]



Scheme 1

Nakijiquinone B (**2**) was obtained as a red amorphous solid {$[\alpha]^{20}_D$ -282.3° (*c* 0.13, $CHCl_3$)}. HRFABMS analysis revealed the molecular formular to be $C_{26}H_{40}NO_5$ [*m/z* 446.2933 $(M+2H+H)^+$, Δ +2.7 mmu]. UV absorptions (λ_{max} 320 and 492 nm) were similar to those of nakijiquinone A (**1**), indicating the presence of a quinone moiety. IR absorptions at 3300 and 1720 cm^{-1} implied that **2** possessed OH and/or NH and carboxyl groups, respectively. The ^1H NMR (Table 2) and ^1H-^1H COSY spectra of **2** indicated the presence of a tetramethyl decaline moiety in **2** and this was supported by the prominent EIMS fragment ion peak at *m/z* 191 ($C_{14}H_{23}$). HMBC correlations (Table 2) 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, C-8, 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 93.0) and C-20 (δ_C 149.6) and HMBC correlations of H-19 and H_2-15 to C-17 and C-21 indicated that the substitution pattern of the benzoquinone ring in **2** was the same as that of **1**. A cross peak between NH-20 and H-22 in the ^1H-^1H COSY spectrum and HMBC correlations of H-22 and H-24 to C-23 (δ_C 172.3) implied the presence of a valine

residue. The valine residue was attached at C-20, based on HMBC correlation of H-22 to C-20. Thus the planar structure of nakijiquinone B was elucidated to be **2**.

Relative stereochemistry of the decaline ring of nakijiquinone B (**2**) was shown to be the same as that of **1** (Fig. 1) by the NOESY data. 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 revealed that three methyl groups (Me-12, Me-13, and Me-14) were all β-oriented. The presence of a valine residue in **2** was indicated by TLC analysis of ozonolysis products of **2**. The valine residue was determined to be L-form by chiral HPLC analysis of the ozonolysis products.

Aminoquinone compounds such as nakijiquinones A (**1**) and B (**2**) are very rare from natural sources, although only a few examples from marine sponges are known.[8,10,11] To our knowledge, this is the first isolation of sesquiterpenoid quinones with a normal amino acid residue from natural origin. Nakijiquinone A (**1**) and B (**2**) exhibited cytotoxicity against L1210 murine leukemia cells with IC_{50} values of 3.5 and 4.0 μg/mL, respectively, and KB human epidemoid carcinoma cells with IC_{50} values of 4.0 and 3.2 μg/mL, respectively, *in vitro*. Compounds **1** and **2** also showed antifungal activity against fungi *Candida albicans* (MIC values, 2.5 and 33 μg/mL, respectively) and *Aspergillus niger* (MIC values, 5.0 and 133 μg/mL, respectively). In addition Nakijiquinone A (**1**) was found to inhibit protein tyrosine kinase (IC_{50} value, 20 μg/mL).

Experimental Section

General Methods. Optical rotations were determined on a JASCO DIP-370 polarimeter. ^1H and ^{13}C NMR spectra were recorded on JEOL EX-400 and Bruker ARX-500 spectrometers. The 3.35 and 7.26 ppm resonances of residual CD_2HOD and $CHCl_3$, respectively, and 49.0 and 77.0 ppm of CD_3OD 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 μm wide, secondary fibres are 24 μm wide, and tertiary fibres are 15 μm 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 150 ~ 200 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 nakijiquinone A (**1**, 32.7 mg, t_R 13.6 min). The fraction eluting from 300 ~ 400 mL of the Sephadex LH-20 column was purified by C_{18} reversed-phase HPLC (YMC-Pack AM323, 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 B (**2**, 2.5 mg, t_R 21.6 min). The EtOAc soluble portions were evaporated under reduced pressure to give a residue (2.9 g), part of which (1.0 g) was subjected to a silica gel column eluted with hexane/EtOAc (3:1) to give isospongiaquinone (**3**, 300 mg) and dictyoceratin A (**4**, 37.6 mg).

Nakijiquinone A (1). A red amorphous solid; mp. 156 - 158 °C; $[\alpha]^{20}_D$ -71.7° (*c* 1.0, MeOH); IR (KBr) ν_{max} 3300, 1720, 1640, 1580, 1370, and 1200 cm^{-1}; UV (MeOH) λ_{max} 317 (ε 11800) and 488 nm (860); ^1H and ^{13}C NMR (Table 1); EIMS *m/z* (%) 401 (M^+, 1), 385 (1), 357 (4), 343 (3), 211 (20), 191 (25), and 95 (100); FABMS (positive) *m/z* 404 $(M+2H+H)^+$; HRFABMS *m/z* 404.2461 $(M+2H+H)^+$, calcd for $C_{23}H_{34}NO_5$, 404.2437; NOESY correlations (CD_3OD, 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, and 14/15'.

Nakijiquinone B (2). A red amorphous solid; $[\alpha]^{20}_D$ -282.3° (*c* 0.13, $CHCl_3$); 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 and ^{13}C NMR (Table 2); EIMS *m/z* (%) 427 (M-OH+H, 1)$^+$, 399 (1), 253 (6), 207 (40), 191 (17), and 95 (100); FABMS (positive) *m/z* 446 $(M+2H+H)^+$; HRFABMS *m/z* 446.2933 $(M+2H+H)^+$, calcd for $C_{26}H_{40}NO_5$, 446.2906; NOESY correlations ($CDCl_3$, 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, and 14/15'.

Oxidation of 1 with H_2O_2. Nakijiquinone A (**1**, 4.7 mg) in 5N KOH (0.3 mL) was stirred at 80 °C for 1 h, and then 5N KOH (0.1 mL) and 30% H_2O_2 (0.1 mL) were added. After stirring for 1 h at 80 °C, 12N HCl (0.2 mL) was added and then the mixture was stirred for 1 h. The reaction mixture was extracted with $CHCl_3$ and then the $CHCl_3$ layer was treated with CH_2N_2 in Et_2O at room temperature for 10 min. After evaporation, the residue was subjected to a silica gel column (hexane/EtOAc, 10:1) to give compound **4** (0.7 mg, 21%). **4:** a colorless oil; $[\alpha]^{20}_D$ +13.7° (*c* 0.1, MeOH); IR

(neat) ν_{max} 1730 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.74 (3H, s, H-14), 0.81 (3H, d, J = 7.0 Hz, H-13), 0.98 (3H, s, H-12), 1.14 (1H, m, H-6'), 1.20 (1H, m, H-10), 1.35 (1H, m, H-1'), 1.40 (1H, m, H-8), 1.42 (2H, m, H-7), 1.46 (1H, m, H-1), 1.55 (3H, s, H-11), 1.66 (1H, m, H-15), 1.72 (1H, m, H-6), 1.76 (1H, m, H-15), 2.03 (2H, m, H-2), 2.13 (1H, m, H-16'), 2.19 (1H, m, H-16), 3.66 (3H, s, MeO), and 5.18 (1H, s, H-3); EIMS *m/z* 278 (M^+); HREIMS *m/z* 278.2236 (M^+, calcd for $C_{18}H_{30}O_2$, 278.2246)

According to essentially the same procedure as described above, isospongiaquinone (**3**, 10.5 mg) afforded compound **4** {4.2 mg, 40%, $[\alpha]^{20}_D$ +14.1° (*c* 0.5, MeOH)}.

Determination of the stereochemistry of a valine residue in 2. A stream of O_3 was bubbled into a MeOH solution (0.5 mL) of nakijiquinone B (**2**, 0.2 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: 2.0 mL/min; eluent: MeOH/H_2O (1:9) containing 2.0 mmol $CuSO_4$; detection: UV at 254 nm]. Retention times of standard L- and D-valine were 4.0 and 6.3 min, respectively, and that of valine contained in ozonolysis products of **2** was found to be 4.0 min.

Acknowledgements: We thank Dr. J. Fromont of James Cook University for identification of the sponge, Mr. Z. Nagahama for his help with collecting the sponge, and Banyu Pharmaceutical Co., Ltd., for tyrosine kinase assay. This work was partly supported by a Grant-in-Aid from the Akiyama Foundation and a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture of Japan.

References and Notes

1. Rodriguez, J.; Quinoa, E.; Riguera, R.; Peters, B. M.; Abrell, L. M.; Crews, P. *Tetrahedron* **1992**, *48*, 6667-6680.
2. Kobayashi, J.; Kondo, K.; Ishibashi, M.; Wälchli, M. R.; Nakamura, T. *J. Am. Chem. Soc.* **1993**, *115*, 6661-6665 and references cited therein.
3. This sponge was collected off "Nakijin" in Okinawa Island, after which the compounds are named.
4. Kazulauskas, R.; Murphy, P. T.; Warren, R. G.; Wells, R. J.; Blount, J. F. *Aust, J. Chem.* **1978**, *31*, 2685.
5. Nakamura, H.; Deng, S.; Kobayashi, J.; Ohizumi, Y.; Hirata, Y. *Tetrahedron* **1986**, *42*, 4197-4201.
6. Bax, A.; Subramanian, S. *J. Mag. Reson.* **1986**, *67*, 565-569.
7. Bax, A.; Summers, M. F. *J. Am. Chem. Soc.* **1986**, *108*, 2093-2094.
8. The 2-amino-5-hydroxy-benzoquinone moiety was contained in smenospongin. Kondracki, M.-L.; Guyot, M. *Tetrahedron* **1989**, *45*, 1995-2004.
9. Luibrand, R. T.; Erdman, T. R.; Vollmer, J. J.; Scheuer, P. J.; Finer, J.; Clardy, J. *Tetrahedron* **1979**, *35*, 609-612.
10. Kobayashi, J.; Naitoh, K.; Sasaki, T.; Shigemori, H. *J. Org. Chem.* **1992**, *57*, 5772-5776.
11. Alvi, K. A.; Diaz, M. C.; Crews, P.; Slate, D. L.; Lee, R. H.; Moretti, R. *J. Org. Chem.* **1992**, *57*, 6604-6607.

(*Received in Japan* 25 *April* 1994; *accepted* 23 *May* 1994)