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Nakijiquinones E and F, new dimeric sesquiterpenoid quinones from marine sponge

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ABSTRACT

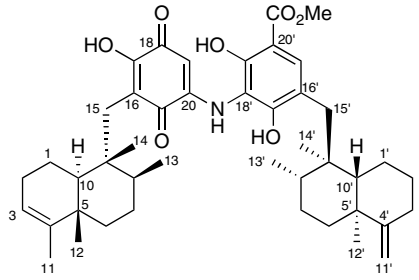
Two new dimeric sesquiterpenoid quinones, nakijiquinones E (**1**) and F (**2**), have been isolated from an Okinawan marine sponge, and the structures and relative stereochemistry of **1** and **2** were elucidated on the basis of the spectral data. Nakijiquinones E (**1**) and F (**2**) were the first dimeric sesquiterpenoid quinones possessing a 3-aminobenzoate moiety.

1. Introduction

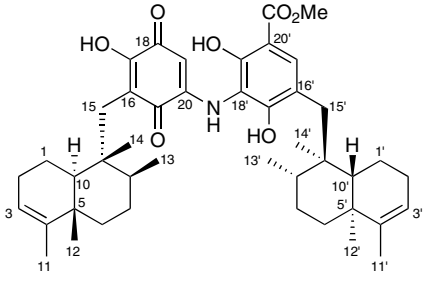
Marine sponges contain a number of unique secondary metabolites with a diversity of biological activities.[1] During our search for bioactive metabolites from marine organisms,[2] we recently isolated new sesquiterpenoid quinones, metachromins L–T, from an Okinawan sponge *Spongia* sp. (SS-1037).[3,4] Further investigation of extracts of another lot of the sponge resulted in the isolation of two new dimeric sesquiterpenoid quinones, nakijiquinones E (**1**) and F (**2**). Here, we describe the isolation and structure elucidation of **1** and **2**.

2. Results and discussion

The sponge collected off Unten Port, Okinawa, was extracted with MeOH. The extracts were partitioned between EtOAc and water. EtOAc-soluble materials were purified by silica gel C_{18} column chromatographies followed by C_{18} HPLC (Wakosil-II 5C18 AR, CH_3CN/H_2O/TFA) to afford nakijiquinones E (**1**, 0.0025%, wet weight) and F (**2**, 0.0009%) together with known related sesquiterpenoids, dictyoceratins A-C,[5,6] isospongiaquinone,[7] 6'-hydroxy-4'-methoxyavarone,[8] neoavarol,[9] nakijiquinones A–D,[10,11] and *endo*-olefin isomer at C-3 of smenospongine.[12]



1



2

Nakijiquinone E (**1**) was obtained as a red amorphous solid and the molecular formula was established to be $C_{44}H_{59}NO_7$ by HRESIMS data [*m*/*z* 736.42002 $(M+Na)^+$, Δ +1.1 mmu]. IR absorptions implied the presence of OH and/or NH (3280 cm^{-1}), carboxy (1670 cm^{-1}), and conjugated carbonyl (1640 and 1590 cm^{-1}) functionalities. UV

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absorptions (313 and 494 nm) suggested the presence of quinone chromophore. The HRESIMS data and the ^{1}H and ^{13}C NMR spectra suggested that **1** was a dimeric sesquiterpenoid quinone.

The ^{1}H NMR spectrum of **1** in $CDCl_3$ showed signals due to a secondary methyl (δ_H 0.99), three tertiary methyls (δ_H 1.55, 1.02, and 0.85), and a singlet olefinic proton (δ_H 5.15). These data suggested the presence of a tetramethyl decaline moiety with an endo olefin (C-1–C-14) in **1**, while signals due to a secondary methyl (δ_H 0.99), an exomethylene (δ_H 4.46 and 4.41), and two tertiary methyls (δ_H 1.07 and 0.85) implied the presence of a trimethyl decaline moiety with an exomethylene (C-1′–C-14′) in **1**. Inspection of the ^{1}H–^{1}H COSY and HMBC spectra of **1** revealed the presence of these two decaline moieties (C-1–C-14 and C-1′–C-14′) as shown in Figure 1.

The presence of a 2-amino-5-hydroxy-benzoquinone moiety was deduced from the chemical shifts[10] of C-16–C-21 (δ_C 183.0, 180.0, 156.0, 148.9, 115.1, and 97.0) in **1**. This was confirmed by HMBC correlations of H-19 to C-17 and C-21, H_2-15 to C-16, C-17 and C-21, and 20-NH to C-19 and C-21. The 2-amino-5-hydroxy-benzoquinone moiety (C-16–C-23) and a decaline (C-l–C-14) were suggested to be connected between C-9 and C-16 through C-15 on the basis of HMBC correlations of H_2-15 to C-9 and C-10, while the chemical shifts of C-16′–C-23′ (δ_C 170.1, 156.6, 156.1, 133.2, 117.5, 110.6, 105.3, and 52.3) and HMBC correlations for H_2-15′ to C-16′, C-17′, and C-21′, H-21′ to C-17′, C-19′, C-21′, and C-22′, 17′-OH to C-17′, 20-NH to C-18′, 20-NH to C-18′, and H_3-23′ to C-22′ revealed the presence of methyl 3-amino-2,4-dihydroxybenzoate moiety (C-16′–C-22′ and C-23′) in **1** as shown in Figure 1. The connection between C-9′ and C-16′ through C-15′ was implied by HMBC cross-peaks for H_2-15 to C-9′ and C-10′. Thus, the gross structure of nakijiquinone E was elucidated to be **1**.

The relative stereochemistry of the two decaline moieties in nakijiquinone E (**1**) were elucidated on the basis of NOESY correlations as shown in Figures 2 and 3. The a-configuration of H-10 and β-configurations of C-12, C-13, and C-14 were deduced from NOESY correlations of H-8/H-10, H-10/H_2-15, and H_3-12/H_3-14, while NOESY correlations of H-8′/H-10′, H-10′/H_2-15′, and H_3-12′/H_3-14′ revealed that three methyl groups (Me-12′, Me-13′, and Me-14′) were all β-oriented and H-10 was α-oriented.

Nakijiquinone F (**2**) was obtained as a red amorphous solid and the molecular formula was established to be $C_{44}H_{59}NO_7$ by HRESIMS data [m/z 736.41828 $(M+Na)^+$, Δ −0.6 mmu]. IR and UV spectra of **2** were almost the same as those of **1**, suggesting that nakijiquinone F (**2**) was an analog of **1**. ^{1}H and ^{13}C NMR spectra of **2** differed from those of **1** in lacking of signals for an exomethylene present in **1**. The ^{1}H NMR spectrum of **2** showed signals for olefinic protons (δ_H 5.15, 2H) and olefinic methyls (δ_H 1.55, and 1.53, 3H each). These data suggested that nakijiquinone F (**2**) possessed two tetramethyl decaline rings with an endo olefin. Analysis

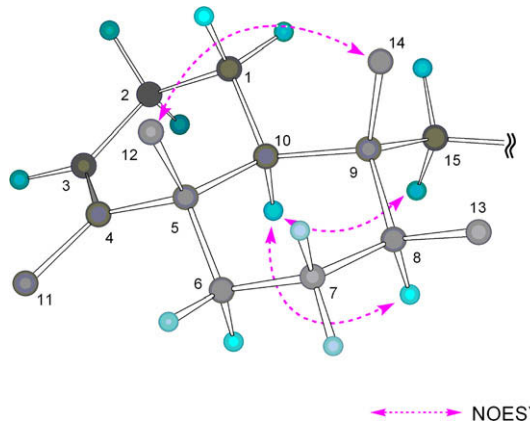

Figure 2. Selected NOESY correlations and relative stereochemistry for C-1–C-15 moiety of nakijiquinone E (**1**) (hydrogen atoms of methyl groups were omitted).

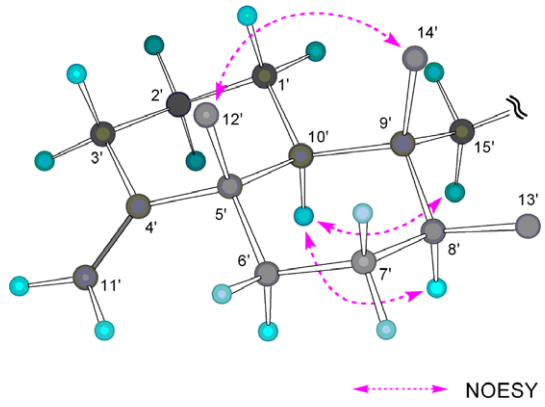

Figure 3. Selected NOESY correlations and relative stereochemistry for C-1′–C-15′ moiety of nakijiquinone E (**1**) (hydrogen atoms of methyl groups were omitted).

of ^{1}H–^{1}H COSY and HMBC spectra of nakijiquinone F indicated the gross structure to be **2** (Fig. 4). The relative stereochemistries of the two decaline rings in **2** were elucidated to be the same as those of C-1–C-15 moiety of **1** on the basis of the NOESY data (Fig. 5).

Nakijiquinones E (**1**) and F (**2**) were the first dimeric sesquiterpenoid quinones possessing a 3-aminobenzoate moiety, though some dimeric sesquiterpenoid quinones from the sponges *Dysidea* sp.[13–15] have been reported so far. Nakijiquinones E (**1**) and F (**2**) did not show cytotoxicity against murine leukemia P388 and L1210, and KB human epidermoid carcinoma cells (IC_{50} > 10 μg/mL).

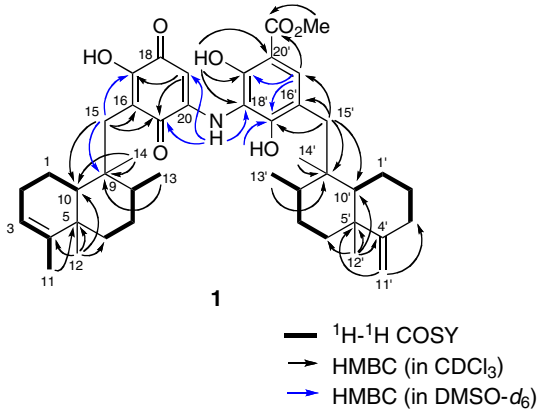

Figure 1. Selected 2D NMR correlations for nakijiquinone E (**1**).

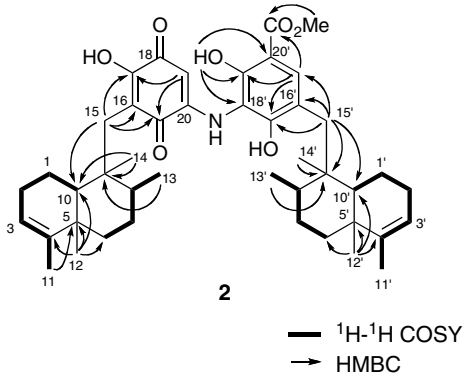

Figure 4. Selected 2D NMR correlations for nakijiquinone F (**2**).

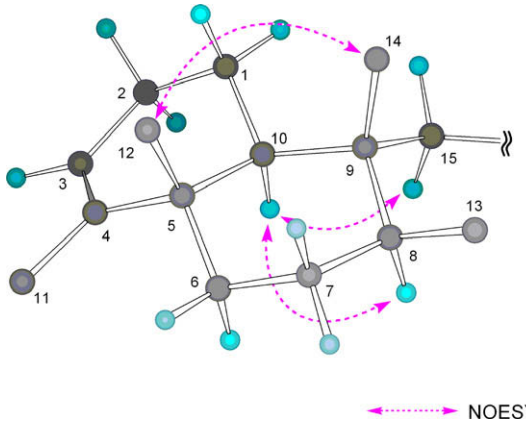

Figure 5. Selected NOESY correlations and relative stereochemistry for C-1–C-15 moiety of nakijiquinone F (**2**) (hydrogen atoms of methyl groups were omitted).

3. Experimental

3.1. General

Optical rotation was recorded on a JASCO P-1030 polarimeter. IR and UV spectra were recorded on JASCO FT/IR-230 and Shimadzu UV-1600PC spectrophotometer, respectively. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX-600 spectrometers using 2.5 mm micro cells (Shigemi Co., Ltd) for $CDCl_3$ and DMSO-d_6, respectively. The 7.26 and 77.0 ppm resonances of residual $CHCl_3$ and the 2.49 and 49.8 ppm resonances of residual DMSO were used as internal references for ^{1}H and ^{13}C NMR spectra, respectively. ESI mass spectra were obtained on a JEOL JMS-700TZ spectrometer. Molecular mechanics calculations were performed by using CS Chem 3D Ultra ver. 7.0 (MM2 force field).[16]

3.2. Extraction and isolation

The sponge (0.6 kg, wet weight) was extracted with MeOH, and the extract (20.2 g) was partitioned between EtOAc and H_2O. EtOAc-soluble materials (2.2 g) was purified by a silica gel column (hexane/acetone), a C_{18} column (MeOH/H_2O) and silicagel columns (hexane/$CHCl_3$, and then hexane/EtOAc) followed by C_{18} HPLC (Wakosil-II 5C18 AR, Wako Pure Chemical Ind., Ltd, 10 × 250 mm; eluent CH_3CN/H_2O/TFA, 95:5:0.05; flow rate, 2.0 mL/min; UV detection at 300 nm) to afford nakijiquinones E (**1**) (**1**, 2.0 mg, t_R 36 min) and F (**2**, 0.7 mg, t_R 38 min).

3.3. Nakijiquinone E (1)

Red amorphous solid; $[\alpha]_D^{23}$ +54 (*c* 0.25, $CHCl_3$); IR (film) ν_{max} 3280, 1670, 1640, 1590, 1510, 1440, 1380, 1340, and 1210 cm^{-1};

Table 1
^{1}H and ^{13}C NMR Data of Nakijiquinone E (1) in $CDCl_3$

Position	δ_c	δ_H (m, *J* in Hz)	HMBC	Position	δ_c	$\delta_{H\ (m,\ J\ in\ Hz)}$	HMBC
1	20.0 CH_2	2.06 (m) 1.49 (m)		1′	23.1 CH_2	2.01 (m) 1.59 (m)	
2	27.1 CH_2	2.04 (m) 1.95 (m)		2′	27.9[c] CH_2	1.90 (m) 1.2–1.3 (m)	
3	120.9 CH	5.15 (br s)	2,[h] 5,[h] 11[h]	3′	33.0 CH_2	2.35 (ddd, 13.5, 13.5, 4.8)	
4	144.2 C					2.11 (m)	
5	38.5 C			4′	159.7 C		
6	35.9 CH_2	1.64 (m) 1.11 (m)		5′ 6′	40.2 C 36.5[f] CH_2	 1.50 (m)	
7	28.0 CH_2	1.38 (m) 1.34 (m)		 7′	 27.6 CH_2	1.21 (m) 1.42[a] (m)	
8	37.9 CH	1.32 (m)		8′	36.5[f] CH	1.25 (m)	
9	42.8 C			9′	41.9 C		
10	47.7 CH	1.11 (d, 12.0)		10′	48.1 CH	0.91 (d, 11.8)	
11	18.2 CH_3	1.55[b] (br s)	3, 4, 5	11′	103.1 CH_2	4.46 (s)	3′, 5′
12	20.2 CH_3	1.02[b] (s)	4, 5, 6, 10			4.41 (s)	
13	17.5[d] CH_3	0.99[b] (d, 6.3)	7, 8, 9	12′	20.5 CH_3	1.07[b] (s)	4′, 5′, 6′, 10′
14	17.3[e] CH_3	0.85[b] (s)	8, 9, 10, 15	13′	17.7[d] CH_3	0.99[b] (d, 6.3)	7′, 8′, 9′
15	32.6 CH_2	2.61 (d, 13.9) 2.47 (d, 13.9)	8,[h] 9,[h] 10, 16, 17,[h] 21 8,[h] 9,[h] 10, 16, 17,[h] 21	14′ 15′	17.4[e] CH_3 36.6[f] CH_2	0.85[b] (s) 2.59[a] (br s)	8′, 9′, 10′, 15′ 8′,[h] 9′, 10′, 14′, 16′, 17′, 21′
16	115.1 C			16′	117.5 C		
17	156.0 C			17′	156.6 C		
18	180.0 C			18′	110.6[g] C		
19	97.0 CH	5.21 (s)	17, 21	19′	156.1 C		
20	148.9 C			20′	105.3[g] C		
21	183.0 C			21′	133.2 CH	7.54 (s)	15′, 17′,[h] 19′, 22′
20-NH		5.8–6.4 (br s)	19,[h] 21,[h] 18′[h]	22′ 23′ 17′-OH 19′-OH	170.1 C 52.3 CH_3	 3.91[b] (s) 8.45 11.06 (s)	 22′ 16′[h] 18′, 19′, 20′

[a] 2H.
[b] 3H.
[c–g] Interchangeable.
[h] In DMSO-d_6.

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

Position	δ_C	δ_H (m, *J* in Hz)	HMBC	Position	δ_C	δ_H (m, *J* in Hz)	HMBC
1	20.0[c] CH_2	2.08 (m) 1.49 (m)		1′	19.7 CH_2	1.99 (m) 1.64 (m)	
2	27.1 CH_2	2.04 (m) 1.92 (m)		2′ 3′	26.0 CH_2 120.4 CH	2.11[a] (m) 5.15 (br s)	
3	120.9 CH	5.15 (br s)		4′	144.4 C		
4	144.2 C			5′	38.3 C		
5	38.5 C			6′	35.9[d] CH_2	1.60 (m)	
6	35.9[d] CH_2	1.65 (m) 1.11 (m)		7′	27.7 CH_2	0.94 (m) 1.37[a] (m)	
7	28.0 CH_2	1.37[a] (m)		8′	36.2 CH	1.22 (m)	
8	37.9 CH	1.32 (m)		9′	41.5 C		
9	42.8 C			10′	45.6 CH	1.17 (d, 11.8)	
10	47.7 CH	1.11 (d, 11.6)		11′	18.1 CH_3	1.53[b] (s)	3′, 4′, 5′
11	18.2 CH_3	1.55[b] (br s)	3, 4, 5	12′	20.0[c] CH_3	1.03[b,g] (s)	4′, 5′, 6′, 10′
12	20.2 CH_3	1.02[b,g] (s)	4, 5, 6, 10	13′	17.6[e] CH_3	1.00[b] (d, 6.3)	7′, 8′, 9′
13	17.7[e] CH_3	1.00[b] (d, 5.7)	7, 8, 9	14′	17.3[f] CH_3	0.86[b] (s)	8′, 9′, 10′, 15′
14	17.5[f] CH_3	0.86[b] (s)	8, 9, 10, 15	15′	36.7 CH_2	2.70 (d, 14.5)	9′, 10′, 16′, 17′, 21′
15	32.6 CH_2	2.62 (d, 13.7) 2.49 (d, 13.7)	8, 9, 10, 16, 17, 21 9, 10, 16, 17, 21	16′	115.1 C	2.61 (d, 14.5)	8′, 9′
16	115.1 C			17′	156.6 C		
17	155.9 C			18′	105.4 C		
18	180.0 C			19′	156.2 C		
19	97.1 CH	5.24 (s)	17, 21	20′	105.4 C		
20	148.9 C			21′	133.4 CH	7.60 (s)	15′, 17′, 19′, 22′
21	183.0 C			22′	170.2 C		
20-NH		5.88 (br)		23′ 19′-OH	52.2 CH_3	3.92[b] (s) 11.07 (s)	22′ 18′, 19′, 20′

[a] 2H.
[b] 3H.
[c–g] Interchangeable.

UV (MeOH) λ_{max} 265 (ε 14,300, sh), 313 (17,500), and 494 nm (1700); ^{1}H and ^{13}C NMR data see Table 1; ESIMS *m*/*z* 736 $(M+Na)^+$ and 1449.8 $(2M+Na)^+$; HRESIMS *m*/*z* 736.42002 [$(M+Na)^+$, calcd for $C_{44}H_{59}NO_7Na$, 736.41892, error +1.49 ppm].

3.4. Nakijiquinone F (2)

Red amorphous solid; $[\alpha]_D^{25}$ +44 (*c* 0.2, CHCl3); IR (film) ν_{max} 3270, 1670, 1640, 1590, 1500, 1440, 1380, 1340, and 1210 cm^{-1}; UV (MeOH) λ_{max} 265 (ε 17,300, sh), 313 (21,500), and 490 nm (2200); ^{1}H and ^{13}C NMR data see Table 2; ESIMS (positive) *m*/*z* 714 $(M+H)^+$ and 736 $(M+Na)^+$; HRESIMS *m*/*z* 736.41828 [$(M+Na)^+$, calcd for $C_{44}H_{59}NO_7$, 736.41892, error −0.87 ppm].

Acknowledgments

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