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Nakijinamines C–E, New Heteroaromatic Alkaloids from the Sponge *Suberites* Species

Yohei Takahashi,[†] Takaaki Kubota,[†] Azusa Shibazaki,[‡] Tohru Gonoi,[‡] Jane Fromont,[§] and Jun'ichi Kobayashi*,[†]

Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo 060-0812, Japan, Medical Mycology Research Center, Chiba University, Chiba 260-0856, Japan, and Western Australian Museum, Locked Bag 49, Welshpool DC, WA 6986, Australia

jkobay@pharm.hokudai.ac.jp

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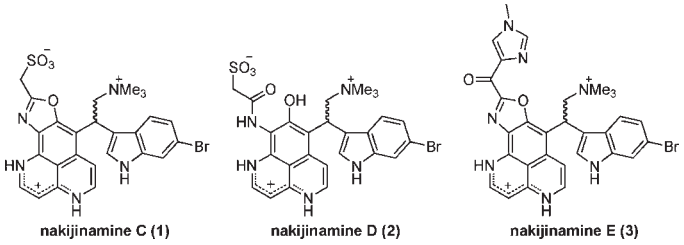
ABSTRACT



Three new heteroaromatic alkaloids, nakijinamines C–E (1–3), which are a hybrid of the aaptamine-type and bromoindole alkaloids possessing a taurine- or histidine-derived residue, have been isolated from an Okinawan marine sponge *Suberites* species. The structures of 1–3 were elucidated on the basis of spectroscopic data and chemical conversions. Nakijinamines C (1) and E (3) are the first natural products possessing a 1*H*-oxazolo[4′,5′:4,5]benzo[1,2,3-*de*][1,6]naphthyridine ring system.

Marine sponges have been recognized as a rich source of interesting bioactive metabolites with a unique structure.[1] During our continuing search for secondary metabolites possessing a unique structure from marine sponges,[2] we have investigated the extract of an Okinawan marine sponge *Suberites* sp. (SS-1084) and isolated three new heteroaromatic alkaloids, nakijinamines C–E (**1**–**3**), which are a hybrid of the aaptamine-type and bromoindole alkaloids possessing a taurine- or histidine-derived residue. In particular, nakijinamine C (**1**) and E (**3**) had a unique tetracyclic ring system containing a 2,4,5,6,7-pentasubstituted benzoxazole ring. Here we describe the isolation and structure elucidation of **1**–**3**.

The sponge *Suberites* sp. (SS-1084, 0.4 kg wet weight) was extracted with MeOH. The extract was partitioned between EtOAc and H_2O, and then the aqueous layer was extracted with *n*-BuOH. The *n*-BuOH-soluble materials were subjected to C_{18} column chromatography followed by repeated reversed-phase HPLC to afford nakijinamines C (**1**, 37.0 mg, 9.3×10^{-3}%, wet weight),[3] D (**2**, 11.2 mg,

[†] Hokkaido University.
[‡] Chiba University.
[§] Western Australian Museum.

(1) Blunt, J. W.; Copp, B. R.; Munro, M. H. G.; Northcote, P. T.; Prinsep, M. R. *Nat. Prod. Rep.* **2011**, *28*, 196–268.

(2) Takahashi, Y.; Iinuma, Y.; Kubota, T.; Tsuda, M.; Sekiguchi, M.; Mikami, Y.; Fromont, J.; Kobayashi, J. *Org. Lett.* **2011**, *13*, 628–631.

(3) Nakijinamine C (**1**): yellow amorphous solid; $[\alpha]^{21}_{D}$ −3 to +5 (*c* 0.15, MeOH); UV (0.1 M HCl aq, pH 1) λ_{max} 224 (log ε 4.6), 240 (4.5 sh), 256 (4.3), 284 (3.9 sh), 294 (3.9), 338 (4.1), 375 (3.9 sh), 406 (3.6 sh), and 432 nm (3.2 sh); IR (film) ν_{max} 3384, 2997, 2844, 1661, 1616, 1550, 1472, 1450, 1238, 1207, 1175, 1034, and 804 cm^{-1}; ^1H and ^{13}C NMR (DMSO-d_6) see Table 1; ESIMS (pos) *m*/*z* 582/584 (1:1, $[M]^+$); HRESIMS (pos) *m*/*z* 582.08073 ($[M]^+$, calcd for $C_{26}H_{25}{}^{79}BrN_5O_4S$, 582.08051).

(4) Because of limited solubility, physicochemical properties of nakijinamine D (**2**) were obtained from a methylated derivative **6** and an elimination derivative **7** (see the Supporting Information).

(5) Nakijinamine E (**3**): orange amorphous solid; $[\alpha]^{21}_{D}$ −5 to +2 (*c* 0.15, MeOH); UV (0.1 M HCl aq, pH 1) λ_{max} 224 (log ε 4.4), 247 (4.2), 286 (4.0 sh), 294 (4.1), 306 (4.0 sh), 398 (3.7), and 456 nm (3.7); IR (film) ν_{max} 3400, 3222, 3086, 2884, 1656, 1525, 1449, 1299, 892, and 822 cm^{-1}; ^1H and ^{13}C NMR (DMSO-d_6) see Table 1; ESIMS (pos) *m*/*z* 596/598 (1:1, $[M-H]^+$); HRESIMS (pos) *m*/*z* 596.14061 ($[M-H]^+$, calcd for $C_{30}H_{27}{}^{79}BrN_7O_2$, 596.14041).

(6) (a) von Nussbaum, F.; Schumann, S.; Steglich, W. *Tetrahedron* **2001**, *57*, 2331–2335. (b) Herlt, A.; Mander, L.; Rombang, W.; Rumampuk, R.; Soemitro, S.; Steglich, W.; Tarigan, P.; von Nussbaum, F. *Tetrahedron* **2004**, *60*, 6101–6104.

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Table 1. ^{1}H, ^{13}C, and ^{15}N NMR Data of Nakijinamines C (**1**) and E (**3**) in DMSO-d_6[a]

1						**3**			
position	δ_C[c] (mult.)	δ_N[b,c]	δ_H[c] (mult., J in Hz)	$^{1}J_{CH}$[c] (Hz)	HMBC[c] (H to C or N)	position	δ_C[c] (mult.)	δ_H[c] (mult., J in Hz)	HMBC[d] (H to C)
1		145.5	13.73 (brs)			1			
2	141.1 CH		7.88 (d, 6.9)	179.4	1, 3a, 9a	2	141.5 CH	8.29 (d, 6.9)	3, 3a, 9a
3	101.2 CH		6.63 (d, 6.9)	169.2	1, 2, 9b	3	102.7 CH	6.91 (d, 6.9)	2, 9b
3a	151.1 C					3a	151.0 C		
4		143.5	13.00 (brs)			4		13.36 (brs)	
5	130.3 CH		7.54 (d, 7.3)	179.4	3a, 6, 6a	5	131.7 CH	7.72 (d, 7.6)	3a, 6, 6a
6	109.6 CH		7.59[g]	159.6	4	6	109.6 CH	7.82 (brs)[h]	5, 7, 9b
6a	130.1 C					6a	132.4 C		
7	110.9 C					7	111.8 C		
8	152.4 C					8	152.2 C		
9	125.8 C					9	125.4 C		
9a	131.8 C					9a	133.9 C		
9b	119.0 C					9b	119.2 C		
1′		135.8	11.38 (d, 2.0)		2′, 3′, 3′a, 7′a	1′		11.45 (brd, 1.8)	3′, 3′a, 7′a
2′	125.4 CH		7.57[g]	179.4	1′, 3′, 3′a, 7′a	2′	125.2 CH	7.69 (d, 1.8)	3′, 3′a, 7′a
3′	112.8 C					3′	112.9 C		
3′a	124.5 C					3′a	124.6 C		
4′	120.7 CH		7.83 (brd, 8.6)	159.0	6′, 7′a	4′	120.6 CH	7.60 (brs)[h]	3′, 6′, 7′a
5′	122.0 CH		7.14 (brd, 8.6)	159.0	3′a, 7′	5′	121.8 CH	7.07 (d, 8.8)	3′a, 6′, 7′
6′	114.3 C					6′	114.3 C		
7′	114.2 CH		7.51 (d, 1.3)	159.6	3′a, 5′, 6′	7′	114.2 CH	7.52 (d, 1.4)	3′a, 5′, 6′, 7′a
7′a	136.9 C					7′a	137.1 C		
1″-NMe	52.9 CH_3	51.0	3.08[e] (s)		NMe	1″-NMe	52.9 CH_3	3.15[e] (s)	1″
1″	67.1 CH_2		4.75 (dd, 13.8, 9.1)	149.4		1″	67.8 CH_2	4.55 (dd, 13.7, 4.5)	7, 2′, 2″, 1″-NMe
			4.26 (dd, 13.8, 4.0)	139.2	7			4.46 (dd, 13.7, 6.6)	7, 2′, 2″, 1″-NMe
2″	29.8 CH		5.43 (dd, 9.1, 4.0)	119.4	6a, 7, 8, 2′, 3′	2″	29.4 CH	5.48 (dd, 5.6, 5.6)	6a, 7, 8, 2′, 3′, 1″
1‴-N		238.0							
1‴	161.6 C					1‴	156.9 C		
2‴	51.2 CH_2		4.45 (d, 13.6)	140[g]	2‴, 3‴	2‴	170.2 C		
			4.39 (d, 13.6)	140[g]	2‴, 3‴				
						3‴	137.2 C		
						5‴	141.0 CH	8.07 (s)	
						7‴	132.9 CH	8.67 (s)	3‴, 6‴-Me
						6‴-Me	33.8 CH_3	3.85[f] (s)	5‴, 7‴

[a] ^{1}H and ^{13}C NMR spectra were recorded at 600 and 150 MHz, respectively. [b] Assigned from $^{1}H-^{15}N$ HMBC spectrum (500 MHz). [c] Recorded at 300 K. [d] Recorded at 350 K. [e] 9H. [f] 3H. [g] J values were not determined correctly because of overlap with other signal(s). [h] Observed as a broad signal because of restricted rotation.

2.8×10^{-3}%),[4] and E (**3**, 5.0 mg, 1.3×10^{-3}%)[5] together with known alkaloids, bisdemethylaaptamine,[6] and aaptamine.[7]

Nakijinamine C (**1**) was obtained as an optically inactive yellow amorphous solid with limited solubility, which was soluble in DMSO and slightly soluble in MeOH. The molecular formula, $C_{26}H_{24}BrN_5O_4S$, was established by HRESIMS (m/z 582.08073 $[M]^+$, Δ −0.22 mmu). IR absorptions (ν_{max} 3384, 1175, and 1034 cm^{-1}) implied the presence of OH and/or NH and sulfonate functionalities, and a conjugated aromatic chromophore was suggested by UV absorptions (λ_{max} 256 and 338 nm) under acidic conditions. ^{1}H NMR (Table 1) spectrum of **1** in DMSO-d_6 at 300 K included 3 D_2O-exchangeable, 8 sp^2, and 14 sp^3 protons, while 12 sp^2 quaternary carbons, 8 sp^2 methines, 1 sp^3 methine, 2 sp^3 methylenes, and 3 sp^3 methyls were observed in the ^{13}C NMR (Table 1) spectrum of **1**.

$^{1}H-^{1}H$ COSY, $^{1}H-^{13}C$ and $^{1}H-^{15}N$ HMBC, and ROESY spectra of nakijinamine C (**1**) and the comparison of ^{1}H and ^{13}C NMR spectra and $^{3}J_{HH}$ and $^{1}J_{CH}$ values of **1** with those of bisdemethylaaptamine[6] and aaptamine[7] revealed the presence of a 1*H*-benzo[*de*][1,6]naphthyridine (N-1−C-9b), a 3-substituted 6-bromoindole (N-1′−C-7′a),

(7) Nakamura, H.; Kobayashi, J.; Ohizumi, Y.; Hirata, Y. *Tetrahedron Lett.* **1982**, *23*, 5555–5558.

and a 2,2-disubstituted *N*,*N*,*N*-trimethylethanaminium moiety (Figure 1). H-2″ showed HMBC cross-peaks for C-6a, C-7, C-8, C-2′, and C-3′ and ROESY correlations for H-6 and H-4′, revealing that an N-1−C-9b portion was connected to an N-1′−C-7′a portion at C-7 and C-3′ via C-2″. On the other hand, the connectivity of 1‴-N−C-2‴ was deduced by HMBC correlations from H_2-2‴ to C-1‴ and 1‴-N. In addition, the chemical shifts of 1‴-N (δ_N 238.0), C-1‴ (δ_C 161.6), and CH_2-2‴ (δ_H 4.45 and 4.39; δ_C 51.2) suggested that C-1‴ and 1‴-N were connected to each other by a double bond and that an oxygen atom and a sulfonate group were attached to C-1‴ and C-2‴, respectively. Considering the molecular formula of **1**, it was implied that C-1‴ was connected to both C-8 and C-9 through an oxygen atom or a nitrogen atom, respectively. The ^{13}C chemical shift of C-8 in **1** was close to that of aaptamine, while the ^{13}C chemical shift of C-9 in **1** was observed approximately 5 ppm higher than that of aaptamine.[7] These comparisons showed that an oxygen atom and a nitrogen atom were connected to C-8 and C-9, respectively, to form a 2,4,5,6,7-pentasubstituted benzoxazole ring (C-6a, C-7, C-8, C-9, C-9a, C-9b, 8-O, C-1‴, and 1‴-N). Thus, the gross structure of nakijinamine C was elucidated to be **1**.

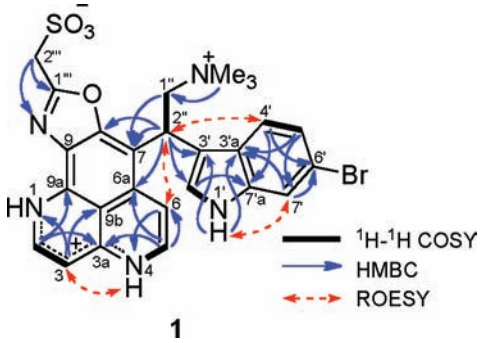

Figure 1. Selected 2D NMR correlations for nakijinamine C (**1**) in DMSO-d_6 at 300 K.

To confirm the gross structure of nakijinamine C (**1**), chemical conversions of **1** were carried out (Figure 2). Compound **1** was treated with methyl iodide under basic conditions to yield 1,4-dimethylnakijinamine C (**4**), and successively, **4** was heated under basic conditions to give an elimination derivative (**5**) of **4**. The ^1H NMR spectrum of **5** in DMSO-d_6 at 300 K gave sharp signals compared to those of **1** and **4**, implying that a part of C−C single bond rotations in **1** and **4** were slow due to interference by three bulky substituents attached to an sp^3 methine (CH-2″).

Nakijinamine D (**2**) was obtained as a pale yellow powder with very limited solubility. Since **2** was practically insoluble in general NMR solvents, the structure of **2** was elucidated using 1,4-dimethylnakijinamine D (**6**) and an dihydrofuran derivative (**7**) of **6**, which were obtained from methylation of **2** by methyl iodide and heating of **6** under basic conditions, respectively.

In the ^1H NMR spectrum of 1,4-dimethylnakijinamine D (**6**), very complicated signals were obtained in DMSO-d_6 at 300 K, while a part (H-5, H-6, H-4′, H-5′, H-1″, and H-2″) of the broadening signals were observed in CD_3OD at 300 K. On the other hand, the ^1H NMR spectrum of **7** in DMSO-d_6 at 300 K showed a part (H-6, H-4′, H-5′, and H-2″) of the broadening signals; however, all signals became sharp in DMSO-d_6 at 350 K. The structures of **6** and **7** were elucidated as shown in Figure 2 on the basis of spectroscopic data of **6** and **7**. Thus, the gross structure of nakijinamine D was elucidated to be **2**.

Subsequently, nakijinamine C (**1**) and 1,4-dimethylnakijinamine D (**6**) were converted into **6** and 1,4-dimethylnakijinamine C (**4**), respectively (Figure 2), to confirm the structure relationship of nakijinamines C (**1**) and D (**2**). Compound **1** was treated under acidic conditions followed by methylation by methyl iodide to afford **6**, while **6** was treated with trifluoroacetic acid to give **4**. The ^1H NMR and MS data and retention times of **6** and **4** derived from **1** and **6** were identical with those derived from **1** and **2**, respectively; therefore, the structure of nakijinamine D was concluded to be **2**.

Nakijinamine E (**3**) was obtained as an orange amorphous solid with a molecular formula of $C_{30}H_{27}BrN_7O_2$ (*m*/*z* 596.14061 $[M-H]^+$, Δ +0.20 mmu). The existence of OH and/or NH, carbonyl group(s), and a conjugated aromatic chromophore was indicated from IR and UV absorptions of **3**, while ^1H and ^{13}C NMR spectra of **3** included 14 sp^2 quaternary carbons, 10 sp^2 methines, 1 sp^3 methine, 1 sp^3 methylene, and 4 sp^3 methyls.

$^1H-^1H$ COSY, TOCSY, HMBC, and ROESY spectra and the comparison of the chemical shifts of **3** with those of **1** indicated that **3** has the same partial structure (N-1−C-1‴) as **1** (Figure 3). In addition, the presence of a 4-substituted imidazole ring was deduced by the large $^1J_{CH}$ values of CH-5‴ (209 Hz) and CH-7‴ (190 Hz) and HMBC correlations of H-5‴/C-3‴, H-5‴/C-7‴, and H-7‴/C-3‴. A methyl group (N-6‴-Me, δ_H 3.85, δ_C 33.8) shifted to relatively low field showed HMBC cross-peaks to C-5‴ and C-7‴, suggesting the attachment of the methyl group at N-6‴. Considering the molecular formula of **3** and the existence of a carbonyl group (C-2‴, δ_C 170.2), it was indicated that C-1‴ was connected to C-3‴ through the carbonyl group (C-2‴). Thus, the gross structure of nakijinamine E was elucidated to be **3**.

Nakijinamines C−E (**1**−**3**) were obtained as an optically inactive mixture, although they have a chiral center (C-2″). Optical resolution of **1** by HPLC with a chiral column

(8) (a) Larghi, E. L.; Bohn, M. L.; Kaufman, T. S. *Tetrahedron* **2009**, *65*, 4257–4282 and references therein. (b) Utkina, N. K.; Denisenko, V. A.; Pushilin, M. A. *Tetrahedron Lett.* **2009**, *50*, 2580–2582. (c) Shaari, K.; Ling, K. C.; Rashid, Z. M.; Jean, T. P.; Abas, F.; Raof, S. M.; Zainal, Z.; Lajis, N. H.; Mohamad, H.; Ali, A. M. *Mar. Drugs* **2009**, *7*, 1–8. (d) Shubina, L. K.; Kalinovsky, A. I.; Federov, S. N.; Radchenko, O. S.; Denisenko, V. A.; Dmitrenok, P. S.; Dyshlovoy, S. A.; Krasokhin, V. B.; Stonik, V. A. *Nat. Prod. Commun.* **2009**, *4*, 1085–1088. (e) Shubina, L. K.; Makarieva, T. N.; Dyshlovoy, S. A.; Fedorov, S. N.; Dmitrenok, P. S.; Stonik, V. A. *Nat. Prod. Commun.* **2010**, *5*, 1881–1884.

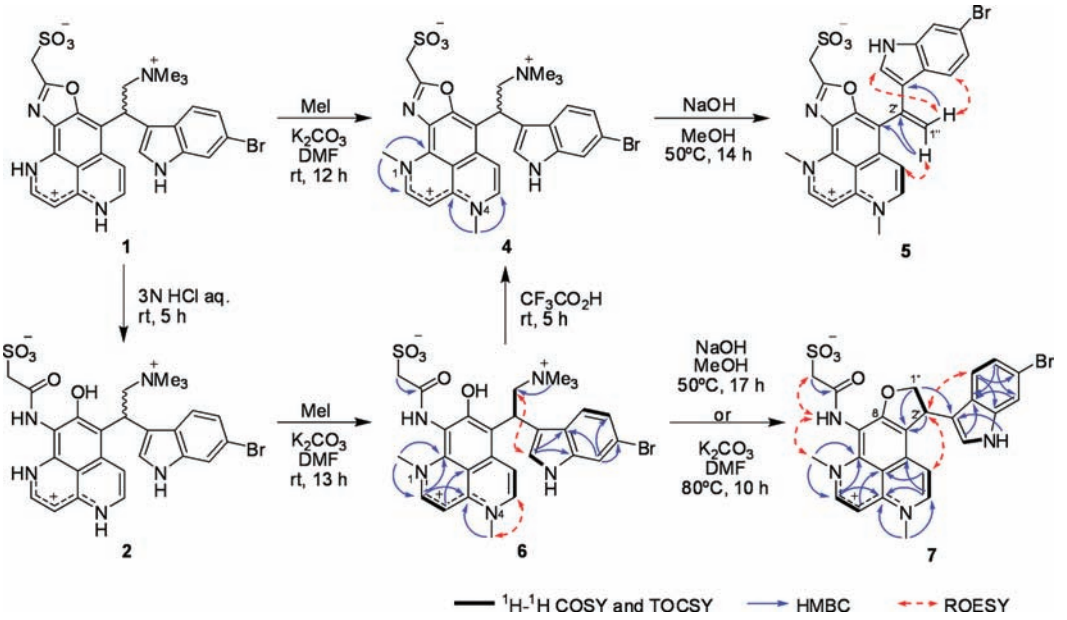

Figure 2. Chemical conversions of nakijinamines C (**1**) and D (**2**) into **4**–**7** and key 2D NMR correlations for **4**–**7**.

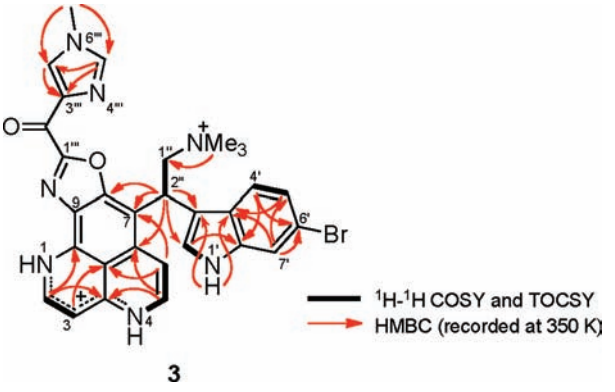

Figure 3. Selected 2D NMR correlations for nakijinamine E (**3**) in DMSO-d_6.

resulted in the separation of two enantiomers, the ratio of which was approximately 1:1. Furthermore, each enantiomer of **1** showed opposite $[\alpha]_D$ signs and close magnitudes. Thus, **1** was suggested to be a racemate. In addition, the enantiomers of **2** and **3** could be separated by chiral HPLC, indicating both **2** and **3** were racemates as well as **1**.

To the best of our knowledge, nakijinamines C (**1**) and E (**3**) are the first natural products possessing a 1*H*-oxazole-[4′,5′:4,5]benzo[1,2,3-*de*][1,6]naphthyridine ring system. In addition, nakijinamines C–E (**1**–**3**) are the first aaptamine-type alkaloids possessing an indole moiety, although about 23 aaptamine-type alkaloids have been isolated so far.[8]

Both nakijinamines C and E (**1** and **3**) showed antifungal activity against *Aspergillis niger* with MIC values of 16 μg/mL. Nakijinamines C–E (**1**–**3**) did not show cytotoxicity (IC_{50} > 10 μg/mL) against murine leukemia P388 and L1210 cells and human epidermoid carcinoma KB cells in vitro. Further biological investigations of **1**–**3** are in progress.

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Supporting Information Available. Detailed experimental section and 1D and 2D NMR data for nakijinamines C and D and their derivatives. This material is available free of charge via the Internet at http://pubs.acs.org.