Hyrtioerectines A−C, Cytotoxic Alkaloids from the Red Sea Sponge *Hyrtios erectus*

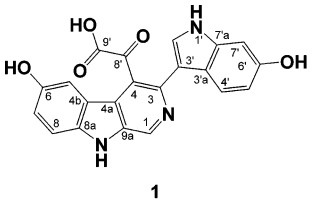
Diaa T. A. Youssef*

Department of Pharmacognosy, Faculty of Pharmacy, Suez Canal University, Ismailia 41522, Egypt

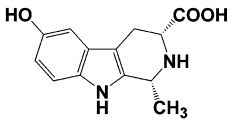
Received April 25, 2005

A Red Sea specimen of the marine sponge *Hyrtios erectus* was found to contain three new alkaloids, hyrtioerectines A−C (**1**−**3**). Hyrtioerectine A (**1**) possesses the carbon bond-linked moieties 6-hydroxy β-carboline and 6-hydroxyindole. The structure elucidation of **1**−**3** was based on intensive study of their spectral data including 1D (^{1}H and ^{13}C) and 2D (^{1}H−^{1}H COSY, HOHAHA, NOESY, ROESY, HMQC, and HMBC) NMR, together with high-resolution mass spectra. Hyrtioerectines A−C were moderately cytotoxic.

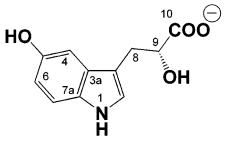
Marine sponges of the genus *Hyrtios* (family Thorectidae, order Dictyoceratida)[1] have proven to be a rich source of secondary metabolites, including terpenoids,[2−6] macrolides,[7] and tryptamine-derived alkaloids.[8,9,10a] The most important metabolites of the genus *Hyrtios* discovered to date include the powerful anticancer agents spongiastatins 1−3.[7c−e] In the course of our ongoing efforts to locate drug leads from Red Sea invertebrates, we have studied the extract of the Red Sea sponge *Hyrtios erectus*. The cytotoxic ethyl acetate fraction of the MeOH/CH_2Cl_2 extract of the sponge was subjected to successive fractionation using size exclusion chromatography on Sephadex LH-20 followed by final purification on reversed-phase HPLC to afford three Dragendorff positive compounds, named hyrtioerectines A−C (**1**−**3**).



1



2



3

Positive HRFABMS of hyrtioerectine A (**1**) established a molecular formula of $C_{21}H_{14}N_3O_5$ requiring 17 degrees of unsaturation. Interpretation of the NMR spectroscopic data [^{1}H NMR, ^{13}C NMR (Table 1), ^{1}H−^{1}H COSY, HOHAHA, HMQC, and HMBC] supported the presence of trisubstituted β-carboline and disubstituted indole moieties. The protons resonating at δ 7.30 (1H, d, H-8), 6.80 (1H, dd, H-7), and 8.07 (1H, d, H-5) ppm, together with a one-proton singlet at δ 9.64 (H-1), were indicative of the 3,4,6-trisubstituted β-carboline moiety.[10a] Moreover, the signals resonating at δ 7.60 (1H, d, H-7′), 7.13 (1H, dd, H-5′), 7.57 (1H, d, H-4′), and 8.87 (1H, s, H-2′) were characteristic of a 3,6-disubstituted indole moiety.[10b]

The ^{13}C NMR spectrum of **1** (Table 1) displayed resonances for 21 carbons including 8 methines and 13 quaternary carbons. Interpretation of the ^{1}H and ^{13}C NMR, ^{1}H−^{1}H COSY, and HMQC experiments allowed assignment of the protonated methines. The quaternary carbons were unambiguously assigned from HMBC data (Table 1 and Figure 1). The connection of the indole and β-carboline moieties through C-3 and C-3′ was secured from a $^3J_{CH}$ HMBC cross-peak between H-2′ (δ 8.87) and C-3 (δ 138.1). The two quaternary carbon resonances at δ 189.4 (C-8′) and 173.0 (C-9′) were assigned as an α-keto carboxylic acid on the β-carboline skeleton from negative ion FABMS

Table 1. ^{1}H and ^{13}C NMR Data for Hyrtioerectine A (**1**) (CD_3OD)

no.	δ_C (mult.)	δ_H [mult., *J*(Hz)]	HMBC[c]
1	140.8 (CH)	9.64 (s)	C-3, C-4a, C-9a
3	138.1 (C)		
4	138.8 (C)		
4a	115.9 (C)		
4b	130.1 (C)		
5	108.2 (CH)	8.07 (d, 2.0)	C-7, C-8a
6	154.3 (C)		
HO-6		9.60 (br s)[a,b]	
7	113.5 (CH)	6.80 (dd, 8.7, 2.0)	C-8a
8	113.1 (CH)	7.30 (d, 8.7)	C-4b, C-6
8a	132.2 (C)		
9a	132.2 (C)		
1′		11.73 (s)[a]	C-3′, C-3′a, C-7′a
2′	119.6 (CH)	8.87 (s)	C-3, C-3′, C-3′a, C-7′a
3′	123.2 (C)		
3a′	137.5 (C)		
4′	114.1 (CH)	7.57 (d, 8.7)	C-3′a, C-7′a
5′	119.7 (CH)	7.13 (dd, 8.7, 2.2)	C-3′a
6′	153.0 (C)		
HO-6′		9.00 (br s)[a,b]	
7′	106.7 (CH)	7.60 (d, 2.0)	C-7′a, C-6′, C-3′a
7a′	132.6 (C)		
8′	189.4 (C)		
9′	173.0 (C)		
HO-9′		12.44 (s)[a]	

[a] Data from spectrum in DMSO-d_6. [b] Exchangeable signals. [c] Protons that correlate with carbons.

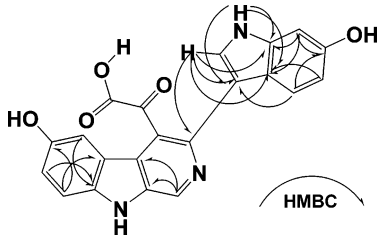


Figure 1. Selective HMBC correlations for **1**.

* To whom correspondence should be addressed. Tel: +20-64-3381593. Fax: +20-64-3355741. E-mail: youssefdiaa@hotmail.com.

10.1021/np050142c CCC: $30.25
Published on Web 08/16/2005

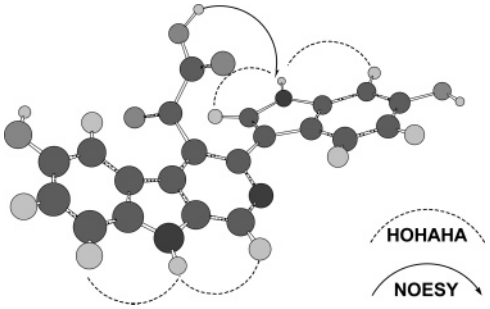


Figure 2. Energy-minimized (Chem3D-MM2) representation of **1** showing selective 2D NOESY and HOHAHA correlations (in DMSO-d_6).

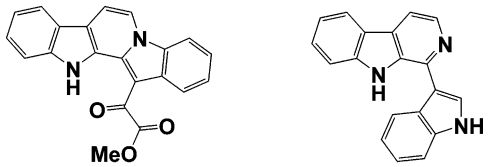


Figure 3. Structures of homofascaplysin B[11] and eudistomin U.[12]

fragments at *m/z* 386 $[M - H]^+$, 341 $[M - H - COOH]^+$, 313 $[M - H - COCOOH]^+$, and 255 $[M - C_8H_6NO]^+$ and by an IR absorption band at 1610−1570 cm^{-1}. In DMSO-d_6, the ^1H NMR spectrum of **1** (Table 1) showed two more singlets at δ 12.44 and 11.73 for the exchangeable protons of the COO*H* and N*H*-1′ moieties, respectively. The low-field shift of the N*H*-1′ of the indole moiety indicated that it was hydrogen bonded with the α-keto carboxylic acid functionality. Moreover, a NOESY correlation between the COO*H* and N*H*-1′ supported the placement of the α-keto carboxylic acid moiety at C-4 of the β-carboline skeleton, as shown on the MM2-energy-minimized representation of **1** (Figure 2). The occurrence of an α-keto carboxylic acid moiety has been reported previously in the marine-derived alkaloid homofascaplysin B (Figure 3), which was isolated from the sponge *Fascaplysinopsis reticulata*.[11]

The presence of the two phenolic OH moieties at C-6 and C-6′ was evident from the ^1H NMR (DMSO-d_6) chemical shifts at δ 9.00 and 9.60 and ^{13}C NMR chemical shifts at δ 154.3 (C-6) and 153.0 (C-6′), respectively. HMBC correlations of H-8/C-6, H-4′/C-6′, and H-7′/C-6′ (Table 1 and Figure 1) further supported the assignment. The assignment of the overlapped resonances C-8a and C-9a at δ 132.2 was supported by $^3J_{CH}$ and $^2J_{CH}$ HMBC cross-peaks of H-5/C-8a, H-7/C-8a, and H-1/C-9a, respectively.

The above discussion supports the proposed structure for hyrtioerectine A (**1**) as 6-hydroxy-3-(6-hydroxyindolyl)-4-(α-oxoacetic acid)-9*H*-pyrido[3,4-*b*]indole. The C−C linked alkaloids of the β-carboline and indole moieties are not common in marine organisms. To the best of our knowledge, this class is represented by only eudistomin U (Figure 3), an alkaloid of the Caribbean ascidian *Lissoclinum fragile*.[12] However, the linkage of the β-carboline and indole moieties in eudistomin U occurs between C-1 and C-3, respectively. In the case of hyrtioerectine A, the linkage occurs between C-3 and C-3, respectively.

Hyrtioerectine B (**2**) was purified as a light gray solid. Its positive HRFABMS displayed a molecular ion peak at *m/z* 247.1089, indicating a molecular formula of $C_{13}H_{15}N_2O_3$ for the $[M + H]^+$ and suggesting 8 degrees of unsaturation. The ^1H NMR spectrum (DMSO-d_6) (Table 2) displayed resonances for 13 protons. The ^{13}C NMR spectrum of **2** showed resonances for 13 carbons including five methines, one methylene, one methyl, and six quaternary carbons

Table 2. ^1H and ^{13}C NMR Data for Hyrtioerectine B (**2**) (DMSO-d_6)

no.	δ_C (mult.)	δ_H [mult., *J*(Hz)]	HMBC[b]
1	49.1 (CH)	4.52 (m)	
3	57.6 (CH)	3.56 (m)	
4	23.2 (CH_2)	3.03 (dd, 15.5, 3.7)	C-3
		2.68 (dd, 15.5, 14.3)	
4a	105.8 (C)		
4b	126.7 (C)		
5	102.0 (CH)	6.72 (d, 1.5)	C-8a
6	150.7 (C)		
*H*O-6		8.78 (s)	
7	111.4 (CH)	6.58 (dd, 8.6, 1.5)	C-5, C-8a
8	111.5 (CH)	7.10 (d, 8.6)	C-4b, C-4
8a	130.7 (C)		
9		10.70 (s)	C-4a, C-4b
9a	132.3 (C)		
1′	16.7 (CH_3)	1.56 (d, 6.8)	C-1
2′	169.4 (C)	*a*	

[a] Signal not observed. [b] Protons that correlate with carbons.

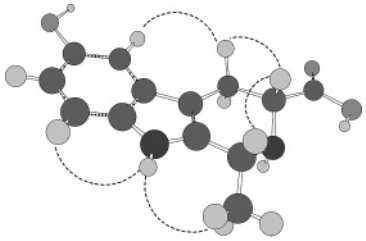


Figure 4. Energy-minimized (Chem3D-MM2) representation of **2** showing selective 2D ROESY correlations.

(Table 2). Interpretation of the ^1H, ^{13}C, COSY, HMQC, and HMBC NMR experiments led to the assembly of three spin systems within **2**. The first spin coupling system is an ABC system including signals at δ 6.72 (d, H-5), 6.58 (dd, H-7), and 7.10 (d, H-8). The second spin system comprised H_3-1′/H-1(J = 6.8 Hz). The remaining coupling system was H-3 and H_2-4 ($J_{4\alpha,4\beta}$ = 15.5 Hz, $J_{3\beta,4\alpha}$ = 14.3 Hz, and $J_{3\beta,4\beta}$ = 3.7 Hz).

The placement of the methyl and carboxylic moieties at C-1 and C-3, respectively, was supported by the HMBC experiments, which showed H_3-1′/C-1 and H_2-4/C-3 connectivity. Similarly, the assignment of all quaternary carbons within **1** was secured from HMBC correlations (Table 2).

The relative configuration of the methyl and carboxylic acid moieties was established by a 2D ROESY experiment (Figure 4), which showed correlations of H-5/H-4β (δ 3.03), H-4β/H-3β (δ 3.56), and H-3β/H-1β (δ 4.52). From the above discussion, compound **2** was assigned as 6-hydroxy-1-methyl-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole-3-carboxylic acid.

The spectral properties of compound **3** indicated that it is 5-hyrdroxy-3-(2-hydroxypropionate)-1*H*-indole. This compound has been previously isolated as a microbial metabolite of 5-hydroxy-L-tryptophan produced by *Chromobacterium violaceum*.[13] This is the first report of compound **3** from a marine invertebrate.

Tryptophan derivatives have been previously reported from the saltwater culture of *Aspergillus niger* derived from the sponge *Hyrtios proteus*,[14,15] and an indole trimer, trisindoline, was found to be a product of a bacterium of the genus *Vibrio* isolated from the sponge *Hyrtios altum*.[16] The presence of such metabolites in the Red Sea sponge *Hyrtios erectus* suggests that hyrtioerectines A−C could be of symbiotic origin.

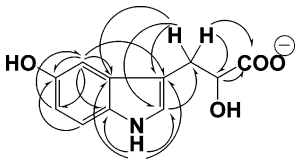


Figure 5. Observed HMBC correlations for **3**.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Jasco-DIP-370 digital polarimeter using MeOH at 25 °C at the sodium D line (589 nm). UV spectra were recorded on a Hitachi 300 spectrometer. IR spectra were recorded on a Perkin-Elmer 1310/84 spectrometer. NMR spectra were determined on a Bruker 600 instrument (600 MHz for ^{1}H and 150 MHz for ^{13}C NMR). Homonuclear ^{1}H connectivities were determined by using the 2D double-quantum-filtered COSY. One-bond heteronuclear ^{1}H−^{13}C connectivities were determined by 2D proton-detected HMQC experiment. Two- and three-bond ^{1}H−^{13}C connectivities were determined by 2D proton-detected HMBC experiment. FABMS spectra were determined on a Finnigan MAT-312 using 3-NBA and TEA as matrix for positive and negative modes, respectively. HPLC purification was performed on a semipreparative C_{18} HPLC column (Cosmosil ARII, 250 × 10 mm, 5 μm, Waters).

Biological Material. The sponge material was collected by hand using scuba at depths between 10 and 25 m off Hurghada at the Red Sea, Egypt, in 2001. The sponge forms an erect, thickly lobate mass with a conulose surface. The individual lobes are 5 cm high and 4 cm in diameter. In life, the color is black outside and beige to brown inside; the preserved fragment has discolored the alcohol to dark brown. The surface conules are densely distributed, about 2 mm high and 1−2 mm apart. The oscules spread evenly over the surface of the lobes, measuring 2−5 mm in diameter. The organic membrane is unarmored and darkly pigmented. The skeleton is formed by an irregular reticulation of debris-filled laminated spongin fibers. There is little distinction in primary and secondary fibers. All fibers have an irregular outline and measure 70−200 μm in diameter, and they enclose meshes of 120−550 μm in diameter. Coring of the fibers is variable; the debris is occasionally light or absent. The voucher, which was identified by Dr. Rob van Soest, was incorporated in the collections of the Zoological Museum of Amsterdam under the registration number 16632. It conforms in all details with the type specimen. Another voucher of the sponge was deposited at our Red Sea Invertebrates collection at the Faculty of Pharmacy, Suez Canal University, under the registration number DY-20.

Extraction and Isolation. The frozen sponge materials (1.32 kg, wet wt) were extracted three times (3 × 1000 mL) with a mixture of MeOH/CH_2Cl_2 (1:1) at room temperature. The combined organic extracts were concentrated under reduced pressure and suspended in 600 mL of MeOH/H_2O (9:1). The resulted mixture was extracted with *n*-hexanes (3 × 400 mL) to give 2.1 g of *n*-hexanes residue. The remaining methanolic layer was diluted with H_2O to (3:2) MeOH/H_2O and then extracted successively with CH_2Cl_2 (3 × 400 mL), EtOAc (3 × 400 mL), and finally n-BuOH (3 × 400 mL). Each of the extracts was concentrated under reduced pressure to give 1.3 g of CH_2Cl_2 extract, 1.1 g of EtOAc extract, and 3.4 g of n-BuOH extract, respectively. All fractions were evaluated for cytotoxic activity against HeLa cells. The ethyl acetate extract showed cytotoxicity with IC_{50} = 10 μg/mL.

The EtOAc residue (1.1 g) was subjected to size exclusion chromatography on a Sephadex LH-20 column equilibrated with MeOH. Fractions of 10 mL were collected and monitored by TLC. Similar fractions were combined to give nine major fractions. Fractions 4 and 5 showed similar TLC and were moderately cytotoxic (IC_{50} = 7.0 μg/mL). The combined fractions 4 and 5 (330 mg) were partitioned on a Sephadex LH-20 column using MeOH as an eluting solvent. Fractions of 3 mL were collected and monitored by TLC. Similar fractions were combined to give five major fractions. Fraction 3 (22 mg) was purified on a semipreparative C_{18} HPLC column using 10% MeCN at a flow rate of 2 mL/min to give compound **1** (3.5 mg). Fraction 5 (21 mg) was subjected to final purification on a semipreparative C_{18} HPLC column using 20% MeCN at a flow rate of 2 mL/min to afford compounds **2** (3.1 mg) and **3** (2.5 mg).

Table 3. ^{1}H and ^{13}C NMR Data for Hyrtioerectine C (**3**) (DMSO-d_6)

no.	δ_C (mult.)	δ_H [mult., *J*(Hz)]	HMBC[a]
1		10.60 (s)	C-2, C-3, C-3a, C-7a
2	124.4 (CH)	7.10 (s)	C-3, C-3a, C-8
3	108.8 (C)		
3a	127.9 (C)		
4	102.4 (CH)	6.91 (d, 1.5)	C-3, C-3a, C-5, C-6, C-7a
5	150.4 (C)		
6	111.3 (CH)	6.59 (dd, 8.5, 2.0)	C-4, C-5, C-7a
7	111.6 (CH)	7.13 (d, 8.5)	C-3a, C-5
7a	130.8 (C)		
8	27.6 (CH_2)	3.20 (dd, 15.0, 3.0) 2.85 (dd, 15.0, 9.6)	C-2, C-3, C-3a, C-9, C-10
9	54.7 (CH)	3.42 (dd, 9.6, 3.0)	C-8, C-10
10	170.7 (C)		

[a] Protons that correlate with carbons.

Cytotoxicity Testing. Cytotoxicity evaluation of compounds **1**−**3** against HeLa cells was carried out according to Fukuzawa's method.[17] Compounds **1**−**3** showed moderate cytotoxicity with IC_{50} = 10, 5.0, and 4.5 μg/mL, respectively.

Hyrtioerectine A (1): yellow amorphous solid; UV (MeOH) λ_{max} (log ϵ) 387 nm (4.05); NMR data, see Table 1; positive HRFABMS *m/z* 388.0945 (calcd for $C_{21}H_{14}N_3O_5$ $[M + H]^+$, 388.0933).

Hyrtioerectine B (2): light gray solid; $[\alpha]^{25}_D$ −19.2° (*c* 0.18, MeOH); UV (MeOH) λ_{max} (log ϵ) 357 (1.822), 276 (3.74), 231 nm (3.81); NMR data, see Table 2; positive HRFABMS *m/z* 247.1089 (calcd for $C_{13}H_{15}N_2O_3$ $[M + H]^+$, 247.1083).

Hyrtioerectine C (3): light gray solid; $[\alpha]^{25}_D$ −31.3° (*c* 0.13, MeOH); UV (MeOH) λ_{max} (log ϵ) 301 (3.44), 276 (3.56), 229 nm (3.76); NMR data, see Table 3; positive HRFABMS *m/z* 221.0685 (calcd for $C_{11}H_{11}NO_4$, $[M + H]^+$, 221.0688).

Acknowledgment. This research was supported by the Committee on Scientific and Technological Cooperation of the Organization of Islamic Conference, Islamabad, Pakistan, and the International Foundation for Science, Sweden, through a grant (F/3116-2) to D.T.A.Y. We thank Prof. Dr. R. van Soest for the taxonomic identification of the sponge, the EEAA and the Red Sea Protectorate of Egypt for permission to make collections, and M. Idelbi for the NMR and MS data.

References and Notes

(1) Hooper, J. N. A.; van Soest, R. W. M. In *Systema Porifera: A Guide to the Classification of Sponges*; Hooper, J. N. A., van Soest, R. W. M., Eds.; Kluwer Academic/Plenum Publishers: New York, 2002; Vol. 1, pp 1028−1050.
(2) Youssef, D. T. A.; Yamaki, R. K.; Kelly, M.; Scheuer, P. J. *J. Nat. Prod.* **2002**, *65*, 2−6.
(3) Ryu, G.; Matsunaga, S.; Fusetani, N. *J. Nat. Prod.* **1996**, *59*, 515−517.
(4) Williams, D. E.; Tahir, A.; Andersen, R. J. *J. Nat. Prod.* **1999**, *62*, 653−654.
(5) Nasu, S. S.; Yeung, B. K. S.; Hamann, M. T.; Scheuer, P. J.; Kelly-Borges, M.; Goins, K. *J. Org. Chem.* **1995**, *60*, 7290−7292.
(6) Salmoun, M.; Devijver, C.; Daloze, D.; Braekman, J. C.; Gomez, R.; de Kluijver, M.; van Soest, R. W. M. *J. Nat. Prod.* **2000**, *63*, 452−456.
(7) (a) Kobayashi, M.; Aoki, S.; Sakai, H.; Kawazoe, N.; Kihara, N.; Sasaki, T.; Kitagawa, I. *Tetrahedron Lett*. **1993**, *34*, 2795−2798. (b) Kobayashi, M.; Aoki, S.; Sakai, H.; Hihara, N.; Sasaki, T.; Kitagawa, I. *Chem. Pharm. Bull.* **1993**, *41*, 989−991. (c) Pettit, G. R.; Cichacz, Z. A.; Gao, F.; Herald, C. L.; Boyd, M. R. *J. Chem. Soc., Chem. Commun*. **1993**, 1166−1168. (d) Pettit, G. R.; Cichacz, Z. A.; Gao, F.; Herald, C. L.; Boyd, M. R.; Schmidt, J. M.; Hooper, J. N. A. *J. Org. Chem*. **1993**, *58*, 1302−1304. (e) Pettit, G. R. *J. Nat. Prod.* **1996**, *59*, 812−821.
(8) Kobayashi, J.; Murayama, T.; Ishibashi, M.; Kosuge, S.; Takamatsu, M.; Ohizumi, Y.; Kobayashi, H.; Ohta, T.; Nozoe, S.; Sasaki, T. *Tetrahedron* **1990**, *46*, 7699−7702.

(9) Bourguet-Kondracki, M. L.; Martin, M. T.; Guyot, M. *Tetrahedron Lett*. **1996**, *37*, 3457−3460.
(10) (a) Salmoun, M.; Devijver, C.; Daloze, D.; Braekman, J.-C.; van Soest, R. W. M. *J. Nat. Prod*. **2002**, *65*, 1173−1176. (b) Sato, H.; Tsuda, M.; Watanabe, K.; Kobayashi, J. *Tetrahedron* **1998**, *54*, 8687−8690.
(11) Jiminez, C.; Quinaoa, E.; Adamczeski, M.; Hunter, L. M.; Crews, P. *J. Org. Chem*. **1991**, *56*, 3403−3410.
(12) Badre, A.; Boulanger, A.; Abou-Mansour, E.; Banaigs, B.; Combaut, G.; Francisco, C. *J. Nat. Prod*. **1994**, *57*, 528−533.
(13) Hoshino, T.; Yamamoto, M.; Uchiyama, T. *Biosci. Biotech. Biochem*. **1993**, *57*, 1609−1610.
(14) Varoglu, M.; Corbett, T. H.; Valeriote, F. A.; Crews, P. *J. Org. Chem.* **1997**, *62*, 7078−7079.
(15) Varoglu, M.; Crews, P. *J. Nat. Prod.* **2000**, *63*, 41−43.
(16) Kobayashi, M.; Aoki, S.; Gato, K.; Matsunami, K.; Kurosu, M.; Kitagawa, *I. Chem. Pharm. Bull.* **1994**, *42*, 2449−2451.
(17) Fukuzawa, S.; Matsunaga, S.; Fusetani, N. *J. Org. Chem*. **1995**, *60*, 608−614.

NP050142C