JOC Note

# Ophiodilactones A and B, Cytotoxic Tetrameric Phenylpropanoids, from the Ophiuroid *Ophiocoma scolopendrina*

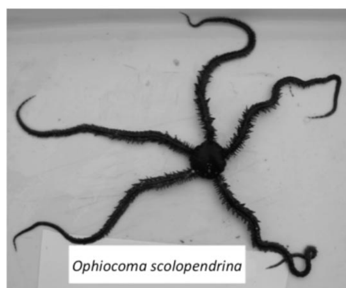
Reiko Ueoka,[†] Toshihiko Fujita,[‡] and Shigeki Matsunaga*,[†]

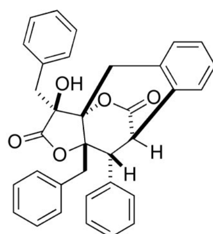
*Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo-ku, Tokyo 113-8657, Japan, and National Museum of Nature and Science, Shinjuku-ku, Tokyo 169-0073, Japan*

*assmats@mail.ecc.u-tokyo.ac.jp*

*Received February 20, 2009*

Downloaded by Jonathan Berry on September 10, 2009 | http://pubs.acs.org
Publication Date (Web): May 11, 2009 | doi: 10.1021/jo9003844




ophiodilactone B (**2**)

Two tetrameric phenylpropanoids, ophiodilactones A (**1**) and B (**2**), were isolated from the ophiuroid *Ophiocoma scolopendrina*. Their structures were determined on the basis of spectroscopic data. Ophiodilactone B has a novel carbon skeleton. They exhibit moderate cytotoxic activity against P388 murine leukemia cells.

*Ophiocoma scolopendrina* is a tropical and subtropical ophiuroid widely distributed in the Indo-Pacific. *O. scolopendrina* is microphagous feeder inhabiting in the intertidal zones.[1] Despite its wide occurrence, this echinoderm has been largely overlooked by marine natural product chemists.[2] In the course of our search for cytotoxic compounds from marine organisms, we found activity in the crude extract of *O. scolopendrina*, from which we have isolated ophiodilactones A and B (**1** and **2**, respectively). Their structures were elucidated by interpretation of spectroscopic data. Ophiodilactones exhibit moderate cytotoxic activity. Ophiodilactone B (**2**) has a novel carbon skeleton.

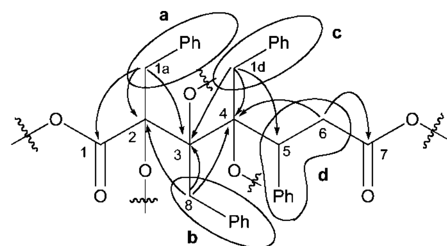
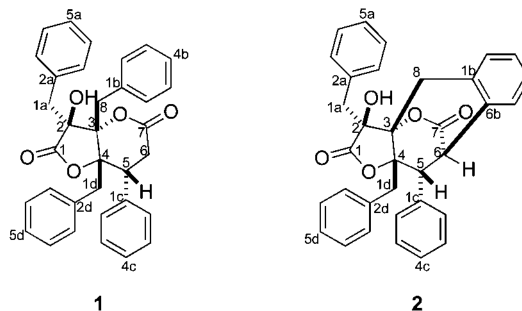



**FIGURE 1.** Structural units **a**−**d** and key HMBC correlations of ophiodilactone A (**1**).

The organic layer of the extract of the ophiuroid was subjected to a modification of Kupchan's solvent partitioning scheme[3] to yield 60% MeOH, $CHCl_3$, and *n*-hexane layers. The $CHCl_3$ layer was separated by ODS flash chromatography, and the cytotoxic fraction was purified by reversed-phase HPLC to give ophiodilactones A (**1**) and B (**2**).




Ophiodilactone A (**1**) had a molecular formula of $C_{34}H_{30}O_5$ which was assigned by HR-ESIMS [*m/z* 541.1970 $(M + Na)^+$, $\Delta$ −2.1 mmu]. Analysis of the $^1H$ NMR data in conjunction with the HSQC spectrum revealed the presence of four monosubstituted benzene rings, four methylenes, and one methine. The $^{13}C$ NMR spectrum additionally showed the presence of two carboxyl (C-1 and C-7) and three oxygenated quaternary carbons (C-2, C-3, and C-4). Three benzyl groups (units **a**, **b**, and **c**) and a 1,2-disubstituted phenylethyl group (unit **d**) were deduced from the COSY and HMBC data (Figure 1). The mode of connection of the above partial structures was established by the HMBC data (Figure 1). The C-5 in unit **d** was attached to C-4, and C-4 was linked to C-1d as shown by the HMBC correlations, $H_2$-6/C-4 and $H_2$-1d/(C-4 and C-5). The HMBC cross peak between $H_2$-6 and C-7 showed that C-6 was connected to a carbonyl carbon. The HMBC correlations from $H_2$-8 to C-3 and C-4 together with the one from $H_2$-1d to C-3 indicated that unit **b** was connected to C-3 and C-3 was connected to C-4. Unit **a** was connected to C-2 to which was attached C-1 and C-3 as revealed by the HMBC cross-peaks between $H_2$-8 and C-2 and between $H_2$-1a and C-1, C-2, and C-3.

Because the resulting gross structure contained seven oxygen atoms, two of them should be shared by either ether or ester

* To whom correspondence should be addressed. Tel: +81-3-5841-5297.
[†] The University of Tokyo.
[‡] National Museum of Nature and Science.

(1) (a) Oak, T.; Scheibling, R. E. *Coral Reefs* **2006**, *25*, 213–222. (b) Fourgon, D.; Lepoint, G.; Eeckhaut, I. J. *Mar. Biol. Ass. U.K.* **2006**, *86*, 1443–1447. (c) Clark, A. M.; Rowe, F. W. E. In *Monograph of Shallow-Water Indo-West Pacific Echinoderms*; Trustees of the British Museum (Natural History): London, 1971.

(2) (a) Roccatagliata, A. J.; Maier, M. S.; Seldes, A. M.; Pujol, C. A.; Damonte, E. B. *J. Nat. Prod.* **1996**, *59*, 887–889. (b) Roccatagliata, A. J.; Maier, M. S.; Seldes, A. M. *J. Nat. Prod.* **1998**, *61*, 370–374. (c) Levina, E. V.; Andriyaschenko, P. V.; Kalinovsky, A. I.; Stonik, V. A. *J. Nat. Prod.* **1998**, *61*, 1423–1426. (d) Ivanchina, N. V.; Kicha, A. A.; Kalinovsky, A. I.; Dmitrenok, P. S.; Stonik, V. A. *J. Nat. Prod.* **2003**, *66*, 298–301. (e) Inagaki, M.; Shibata, M.; Isobe, R.; Higuchi, R. *Chem. Pharm. Bull.* **2001**, *49*, 1521–1525. (f) Sato, D.; Ando, Y.; Tsujimoto, R.; Kawasaki, K. *Lipids* **2001**, *36*, 1371–1375.

(3) Kupchan, S. M.; Britton, R. W.; Ziegler, M. F.; Sigel, C. W. *J. Org. Chem.* **1973**, *38*, 178–179.



10.1021/jo9003844 CCC: $40.75 © 2009 American Chemical Society
Published on Web 05/11/2009

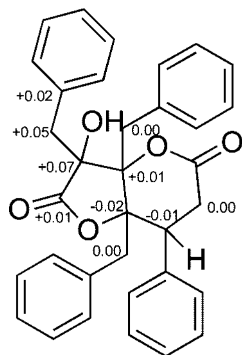



**FIGURE 2.** Deuterium shifts of ophiodilactone A (**1**).

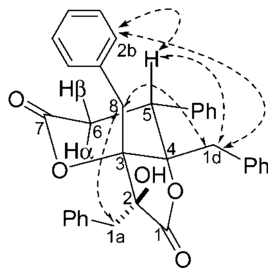



**FIGURE 3.** NOESY cross peaks in ophiodilactone A (**1**).

linkages and one of them should be a hydroxyl group. To assign the location of the hydroxyl group, deuterium-induced shifts were measured in the $^{13}$C NMR spectrum.[4] Significant shifts were observed at C-2 and C-1a indicating the presence of a hydroxyl group at C-2 (Figure 2). There are two possible modes of formation of lactone rings. One mode involves linkages between C-1 and C-3 and between C-7 and C-4 resulting in $\beta$- and $\gamma$-lactones, whereas the other mode involves linkages between C-1 and C-4 and between C-3 and C-7 resulting in $\gamma$- and $\delta$-lactones. The presence of a $\beta$-lactone was denied on the basis of the IR spectrum which exhibited absorptions at 1780 and 1730 cm$^{-1}$ but not at 1840 cm$^{-1}$. Therefore, C-1 and C-4 as well as C-3 and C-7 should be connected through an oxygen atom.

The relative stereochemistry of **1** was elucidated on the basis of the NOESY data (Figure 3). A coupling constant of 13.8 Hz between H-6α and H-5 suggested that H-5 was axial in the $\delta$-lactone ring. NOESY cross peaks H-5/(H-2b and H-1d) and H-1d/H-8 demonstrated that H-5, C-1d, and C-8 were on the same face of the $\delta$-lactone ring. The presence of a weak cross peak H-1a/H-8 and the absence of a cross peak H-1a/H-1d indicated that C-1d and C-1a were on the opposite faces of the $\gamma$-lactone ring.

The absolute stereochemistry of **1** was assigned by analysis of the CD data. In the CD spectrum of $\gamma$-lactones, the sign of the Cotton effect is known to be affected by the stereochemistry of the α-carbon.[5] Fortuitously, CD spectra of α-arylmethyl-α-hydroxy-$\gamma$-lactones have been reported[6] and demonstrate that the 2*S*-isomer gives a negative n→$\pi$* absorption, whereas the 2*R*-isomer gives a positive n→$\pi$* absorption.[6a] The CD spectrum of **1** gave a negative Cotton effect at 219 nm ($\Delta\varepsilon$ −9) in MeCN, indicating the 2*S*-stereochemistry. Therefore, the stereochemistry of **1** was assigned as 2*S*,3*S*,4*S*,5*R*.

**TABLE 1.** $^{1}$H and $^{13}$C NMR Data for Compounds 1 and 2 in $CD_3OD$

| no. | 1 $^{1}$H | 1 $^{13}$C | 2 $^{1}$H | 2 $^{13}$C |
|---|---|---|---|---|
| 1 | | 177.2 | | 177.0 |
| 2 | | 79.6 | | 79.1 |
| 3 | | 96.3 | | 91.3 |
| 4 | | 85.9 | | 87.4 |
| 5 | 2.24 (dd, 4.4, 13.8) | 46.4 | 3.87 s | 54.8 |
| 6 | 2.12 (dd, 4.4, 18.1) | 34.4 | 3.73 s | 54.4 |
| | 2.8 (dd, 13.8, 18.1) | | | |
| 7 | | 170.0 | | 172.2 |
| 8 | 2.53 (d, 15.4) | 35.8 | 2.53 (d, 19.0) | 34.5 |
| | 3.19 (d, 15.4) | | 3.49 (d, 19.0) | |
| 1a | 3.27 (d, 14.7) | 40.5 | 3.34 m | 39.6 |
| | 3.33 m | | 3.44 (d, 14.2) | |
| 2a | | 136.4 | | 136.5 |
| 3a | 7.69 (d, 7.6) | 133.3 | 7.6 (d, 6.9) | 133.0 |
| 4a | 7.37 (t, 7.6) | 129.1 | 7.33 (t, 6.9) | 129.1 |
| 5a | 7.28 (t, 7.6) | 128.2 | 7.29 m | 128.2 |
| 1b | | 136.6 | | 134.4 |
| 2b | 7.33 (d, 6.8) | 132.5 | 7.00 (d, 6.9) | 131.5 |
| 3b | 7.41 m | 130.0 | 7.30 m | 129.3 |
| 4b | 7.40 m | 128.9 | 7.30 m | 130.2 |
| 5b | 7.41 m | 130.0 | 7.20 m | 131.2 |
| 6b | 7.33 (d, 6.8) | 132.5 | | 134.0 |
| 1c | | 139.0 | | 139.3 |
| 2c | 6.67 br | 130.7 | 6.45 (d, 7.3) | 129.6 |
| 3c | 6.97 br | 129.7 | 7.05 (t, 7.3) | 129.3 |
| 4c | 7.10 (t, 7.6) | 129.1 | 7.11 (t, 7.3) | 128.4 |
| 1d | 2.87 (d, 14.0) | 41.0 | 2.35 (d, 14.2) | 46.9 |
| | 4.26 (d, 14.0) | | 4.01 (d, 14.2) | |
| 2d | | 136.4 | | 135.9 |
| 3d | 6.39 (d, 7.3) | 132.0 | 6.91 (d, 7.3) | 132.7 |
| 4d | 6.79 (t, 7.3) | 128.2 | 7.22 (d, 7.3) | 129.5 |
| 5d | 6.89 (t, 7.3) | 126.6 | 7.25 (t, 7.3) | 128.5 |

Ophiodilactone B (**2**) had a molecular formula of $C_{34}H_{28}O_5$ as assigned by the HR-ESIMS [*m*/*z* 539.1837 (M + Na)$^+$, $\Delta$ 0.3 mmu]. Analysis of the $^{1}$H NMR data in conjunction with the HSQC spectrum revealed the presence of three monosubstituted benzene rings, one 1,2-disubstituted benzene ring, three methylenes, and two methines. The $^{13}$C NMR spectrum further showed the presence of two carboxyl and three oxygenated quaternary carbons (Table 1). Two benzyl groups (unit **a** and **c**), a 2-substituted benzyl group (unit **b**), and a 1,2,2-trisubstituted phenylethyl group (unit **d**) were deduced from the COSY and HMBC data (Figure 4); in order to facilitate the analysis of the crowded region of the HMBC spectrum by enhancing the resolution of the f1 axis, the HMBC spectrum was measured within a window of 65 ppm. The connection between C-6 and

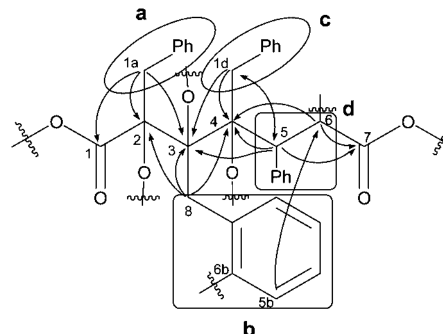



**FIGURE 4.** Structural units **a**−**d** and key HMBC correlations of ophiodilactone B (**2**).

(4) Newmark, R. A.; Hill, J. R. *Org. Magn. Reson.* **1980**, *13*, 40–44.
(5) (a) Beecham, A. F. *Tetrahedron Lett.* **1968**, 2355–2360. (b) Novák, J. J. K. *Collect. Czech. Chem. Commun.* **1974**, *39*, 869–882.
(6) (a) Kato, A.; Hashimoto, Y.; Kidokoro, M. *J. Nat. Prod.* **1979**, *42*, 159–162. (b) Sefkow, M. *J. Org. Chem.* **2001**, *66*, 2343–2349. (c) Inagaki, I.; Hisada, S.; Nishibe, S. *Chem. Pharm. Bull.* **1972**, *20*, 2710–2718.

Downloaded by Jonathan Berry on September 10, 2009 | http://pubs.acs.org
Publication Date (Web): May 11, 2009 | doi: 10.1021/jo9003844

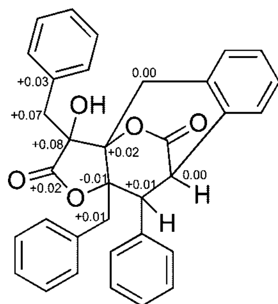



**FIGURE 5.** Deuterium shifts of ophiodilactone B (**2**).

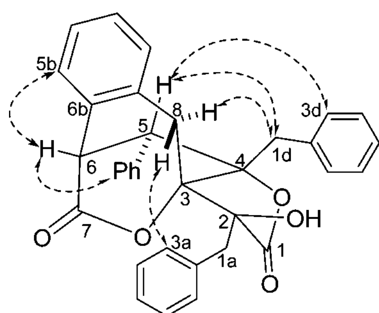



**FIGURE 6.** NOESY cross peaks in ophiodilactone B (**2**).

C-6b was indicated by the HMBC data. The connections of the remaining partial structures were unambiguously established on the basis of the HMBC data (Figure 4), resulting in the gross structure identical with that of **1** except for the presence of an additional C−C bond formed between C-6 and C-6b. The location of a hydroxyl group was determined by a deuterium exchange experiment in the $^{13}$C NMR spectrum (Figure 5) and the sizes of the lactone rings were assigned by IR bands at 1780 and 1770 cm$^{-1}$ which demonstrated the absence of a $\beta$-lactone.

The relative stereochemistry of **2** was elucidated on the basis of NOESY data (Figure 6). The absence of coupling between H-5 and H-6 suggested a dihedral angle of 90° between these protons. NOESY cross peaks H-5/(H-1d and 3d), H-6/H-2c, and H-8/H-3a indicated that C-8, C-1d, H-5, and C-6b were on the same face of the $\delta$-lactone ring. The absence of NOESY cross peaks from H$_2$-1a to either H$_2$-1d or H$_2$-8 suggested that C-1d and C-1a were on the opposite face of the $\gamma$-lactone ring.

Ophiodilactone B (**2**) exhibited an intense negative cotton effect at 218 nm ($\Delta\varepsilon$ −28) in MeCN. At the moment we are not able to rationalize the intense absorption, but the sign of the Cotton effect and biogenetic consideration imply the 2*S*,3*S*,4*S*,5*R*,6*R*-stereochemistry.

Ophiodilactones A and B exhibited moderate cytotoxic activity against P388 murine leukemia cells with IC$_{50}$ values of 5.0 and 2.2 $\mu$g/mL, respectively.

Ophiodilactones are new members of phenylalanine-derived $\gamma$-lactones, which have been isolated from fungi, tunicates, cyanobacteria, and plants.[7] A radical reaction mechanism was proposed for the condensation of monomeric units in the biosynthesis of butylolactones in fungi of the genus *Aspergillus* and in plants.[7c,d] It is interesting to note that the conversion from **1** to **2** has apparently proceeded through a radical reaction. Ophiodilactones are most closely related to maculalactones C and E, which have a furanocyclohexane ring system.[7j] The carbon skeleton of ophiodilactone A is identical with that of maculalactone M,[7l] whereas the carbon skeleton of ophiodilactone B is new. Because *O. scolopendrina* inhabits the rocky shore, ophiodilactones are suspected to originate in the dietary cyanobacteria colonizing on the surface of rocks.[8]

## Experimental Section

**Animal Material.** The ophiuroid *O. scolopendrina* was collected by hand at Amami-Oshima, Kagoshima Prefecture, Japan, in 2007, frozen after collection, and kept at −20 °C until used. A voucher specimen was deposited at the Laboratory of Aquatic Natural Products Chemistry with a reference number E07-001.

**Extraction and Isolation.** The sample (280 g) was extracted with MeOH (3 × 1 L) and CHCl$_3$/MeOH (1:1) (1 × 1 L), and the extracts were combined and concentrated in vacuo. The residue was suspended in H$_2$O (200 mL) and extracted with CHCl$_3$ (3 × 200 mL) and *n*-BuOH (2 × 200 mL). The CHCl$_3$ extract was partitioned between 90% MeOH and *n*-hexane. The 90% MeOH layer was diluted with H$_2$O to yield a 60% MeOH solution and then extracted with CHCl$_3$. The CHCl$_3$ layer was concentrated and separated by ODS flash chromatography (eluents: 50% MeOH, 70% MeOH, 70% MeCN, 85% MeCN, 100% MeOH, and CMW (7:3: 0.5)) to give six fractions (A−F). The active fraction D (85% MeCN fraction) was separated by reversed-phase HPLC (C$_{18}$-stationary phase, 20 × 250 mm) with a gradient elution from 80% MeOH to 100% MeOH to give 14 fractions (A′−N′). The cytotoxic fraction I′ was further purified by reversed-phase HPLC (phenylhexyl stationary phase, 10 × 250 mm) with 85% MeOH to give 1.4 mg of ophiodilactone A (**1**). The cytotoxic fraction D′ was further purified by reversed-phase HPLC (phenylhexyl stationary phase, 10 × 250 mm) with a stepwise elution of 75% MeOH, 85% MeOH, and 100% MeOH to give 0.9 mg of ophiodilactone B (**2**).

**Ophiodilactone A (1):** yellow powder; $[\alpha]^{21}_{D}$ −67 (*c* 0.068, MeOH); UV (MeOH) 205 nm ($\varepsilon$ 34700); IR (film) 3390, 1780, 1730 cm$^{-1}$; $^1$H NMR (CD$_3$OD) and $^{13}$C NMR (CD$_3$OD) data, see Table 1; HRESIMS *m/z* 541.1970 (calcd for C$_{34}$H$_{30}$O$_5$Na, 541.1991).

**Ophiodilactone B (2).** yellow powder; $[\alpha]^{20}_{D}$ −222 (*c* 0.043, MeOH); UV (MeOH) 259 nm ($\varepsilon$ 1,800), 206 nm ($\varepsilon$ 35700); IR (film) 3420, 1780, 1770 cm$^{-1}$; $^1$H NMR (CD$_3$OD) and $^{13}$C NMR (CD$_3$OD) data, see Table 1; HRESIMS *m/z* 539.1837 (calcd for C$_{34}$H$_{28}$O$_5$Na, 539.1834).

**Assay for the Cytotoxicity against P388 Cells.** P388 murine leukemia cells were cultured in RPMI-1640 medium containing 10% fetal bovine serum, 100 $\mu$g/mL of kanamycin, and 10 $\mu$g/mL of 2-hydroxyethyl disulfide at 37 °C under an atmosphere of 5% CO$_2$. To each well of the 96-well microplate containing 100 $\mu$L of tumor cell suspension (1 × 10$^4$ cells/mL) was added 100 $\mu$L of test solution dissolved in RPMI-1640 medium, and the plate was incubated in a CO$_2$ incubator at 37 °C for 96 h. After addition of 50 $\mu$L of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) saline solution (1 mg/mL) to each well, the plate was incubated for 3 h under the same condition to stain live cells. After the incubation, the plate was centrifuged, the supernatants

(7) (a) Mason, C. P.; Edwards, K. R.; Carlson, R. E.; Pignatello, J.; Gleason, F. K.; Wood, J. M. *Science* **1982**, *215*, 400–402. (b) Pignatello, J. J.; Porwoll, J.; Carlson, R. E.; Xavier, A.; Gleason, F. K.; Wood, J. M. *J. Org. Chem.* **1983**, *48*, 4035–4038. (c) Kiriyama, N.; Nitta, K.; Sakaguchi, Y.; Taguchi, Y.; Yamamoto, Y. *Chem. Pharm. Bull.* **1977**, *25*, 2593–2601. (d) Nitta, K.; Fujita, N.; Yoshimura, T.; Arai, K.; Yamamoto, Y. *Chem. Pharm. Bull.* **1983**, *31*, 1528–1533. (e) Braña, M. F.; García, M. L.; López, B.; de Pascual-Teresa, B.; Ramos, A.; Pozuelo, J. M.; Domínguez, M. T. *Org. Biomol. Chem.* **2004**, *2*, 1864–1871. (f) Miao, S.; Andersen, R. J. *J. Org. Chem.* **1991**, *56*, 6275–6280. (g) Yang, X.; Shimizu, Y.; Steiner, J. R.; Clardy, J. *Tetrahedron Lett.* **1993**, *34*, 761–764. (h) Smith, C. J.; Hettich, R. L.; Jompa, J.; Tahir, A.; Buchanan, M. V.; Ireland, C. M. *J. Org. Chem.* **1998**, *63*, 4147–4150. (i) Tsui, W. Y.; Williams, G. A.; Brown, G. D. *Phytochemistry* **1996**, *43*, 1083–1085. (j) Lee, S. C.; Brown, G. D. *J. Nat. Prod.* **1998**, *61*, 29–33. (k) Lee, S. C.; Williams, G. A.; Brown, G. D. *Phytochemistry* **1999**, *52*, 537–540. (l) Wong, H. F.; Williams, G. A.; Brown, G. D. *Phytochemistry* **2002**, *60*, 425–429. (m) Brown, G. D.; Wong, H. F.; Hutchinson, N.; Lee, S. C.; Chan, B. K. K. *Phytochem. Rev.* **2004**, *3*, 381–400. (o) Ward, R. S. *Nat. Prod. Rep.* **1999**, *16*, 75–96.

(8) Wang, W.; Hong, J.; Lee, C. O.; Cho, H. Y.; Shin, S.; Jung, J. H. *Nat. Prod. Sci.* **2004**, *10*, 253–261.

Downloaded by Jonathan Berry on September 10, 2009 | http://pubs.acs.org
Publication Date (Web): May 11, 2009 | doi: 10.1021/jo9003844

were removed, and the cells were dissolved in 150 μL of DMSO to determine the $IC_{50}$ values.

**Acknowledgment.** We thank the officers and crew of T/S Toyoshio-maru of Hiroshima University for their assistance in collecting the ophiuroid specimen. We acknowledge S. Otsuka, Hiroshima University, for the arrangement of the cruise. We are indebted to H. Watanabe and H. Ozawa for the measurement of IR and CD spectra, respectively.

**Supporting Information Available:** NMR spectra for compounds **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO9003844

Downloaded by Jonathan Berry on September 10, 2009 | http://pubs.acs.org
Publication Date (Web): May 11, 2009 | doi: 10.1021/jo9003844