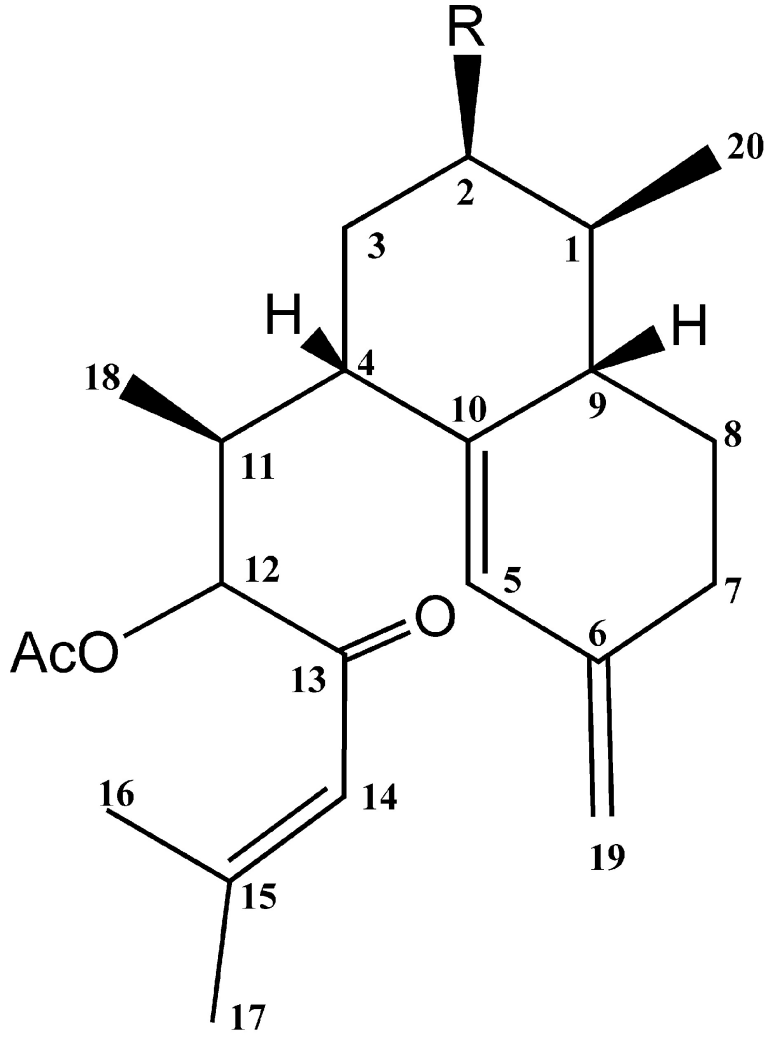
JOURNAL OF NATURAL PRODUCTS



Sinulodurin A (1) R = OAc
Sinulodurin B (2) R = H

More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

Journal of Natural Products is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Sinulodurins A and B, Antiproliferative and Anti-invasive Diterpenes from the Soft Coral *Sinularia dura*

Mohamed M. Radwan,[†] Susan P. Manly,[†] Khalid A. El Sayed,[§] Vikram B. Wali,[§] Paul W. Sylvester,[§] Bhushan Awate,[§] Girish Shah,[§] and Samir A. Ross*[,†,‡]

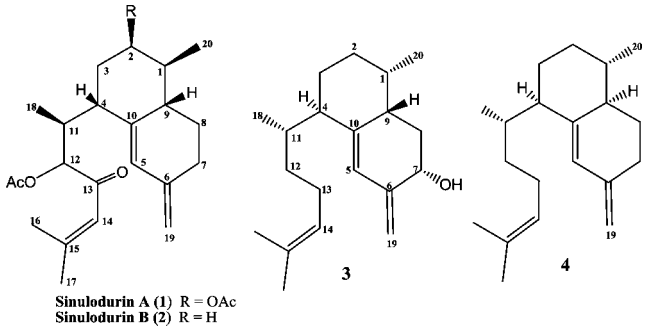
National Center for Natural Products Research and Department of Pharmacognosy, School of Pharmacy, University of Mississippi, University, Mississippi 38677-1848, and Department of Basic Pharmaceutical Sciences, College of Pharmacy, University of Louisiana at Monroe, 700 University Avenue, Monroe, Louisiana

Received April 1, 2008

Two new diterpenes, sinulodurin A (**1**) and sinulodurin B (**2**), along with two known sterols, 24*S*-methyl cholesterol and 24-methylene cholesterol, were isolated from the Palau soft coral *Sinularia dura*. The structures of the new metabolites were determined on the basis of spectroscopic methods and by comparison of NMR data with those of related metabolites. Sinulodurin A (**1**) and sinulodurin B (**2**) showed antiproliferative activity against highly malignant +SA mammary epithelial cells with an IC_{50} range of 20−30 μM. They also displayed anti-invasive activity against human highly metastatic prostate cancer PC-3M-CT+ cells in the spheroid disaggregation assay. Furthermore, the antimicrobial activities of the isolates were tested.

Soft coral of the genus *Sinularia* has been found to be a rich source of diterpenes belonging to cembranoide,[1–5] norcembranoid,[6,7] and amphilectane skeletal classes.[8] Many of these compounds were reported to possess cytotoxic,[3,5,8] anti-inflammatory,[4,9,10] and antimicrobial[11] activities. The chemistry and the bioactivity of the genus *Sinularia* have been reviewed by Kamel et al.[12] Previous work on *Sinularia dura* resulted in the isolation of the known cembranoid epoxypukalide and sindurol, a hydroquinone derivative.[1]

In the course of our study of biologically active secondary metabolites from marine organisms,[13] we have undertaken the chemical examination of the soft coral *Sinularia dura*. In this paper the details of isolation and structure elucidation of two new diterpenes, sinulodurin A (**1**) and sinulodurin B (**2**), as well as two known sterols, 24*S*-methyl cholesterol and 24-methylene cholesterol, are discussed together with their biological properties. Sinulodurin A (**1**) and sinulodurin B (**2**) showed antiproliferative and anti-invasive activities.



Sinulodurin A (1) R = OAc
Sinulodurin B (2) R = H

Sinulodurin A (**1**) was obtained as a colorless oil, $[\alpha]_D$ +91.3 (*c* 0.25, $CHCl_3$), and showed a pseudomolecular ion peak at *m*/*z* 425.2326 $[M + Na]^+$ in the HRESIMS, indicating the molecular formula $C_{24}H_{34}O_5$ and eight degrees of unsaturation. The IR spectrum displayed a characteristic absorbance for an α,β-unsaturated ketone (1640 cm^{-1}), which was confirmed by a UV absorbance band at λ_{max} 239 nm (calcd 239 nm).[14] The IR spectrum also showed an ester carbonyl absorbance at ν_{max} 1736 cm^{-1}. The ^1H NMR spectrum (Table 1) showed the presence of two olefinic protons (δ_H 6.03 and 6.08, 1H each, s), an exocyclic methylene (δ_H 4.73, 2H, brs), two vinyl methyls (δ_H 1.94, 2.18, 3H each, s), two secondary methyls [δ_H 0.99 (3H, d, J = 7.0 Hz), 1.03 (3H, d, J = 7.0 Hz)], two oxymethines [δ_H 4.81 (1H, brs), 5.19 (1H, td, J = 4.0, 12.8 Hz)], and two acetoxy methyl singlets resonating at δ_H 2.05 and 2.14. The ^{13}C, DEPT-135, and HMQC NMR data (Table 1) displayed 24 resonances assigned to six methyl, four methylene, eight methine, and six quaternary carbons, of which three resonances are assigned to an α,β-unsaturated (δ_C 197.2 ppm) and two ester carbonyls (δ_C 170.6, 170.4, 22.0, 21.1 ppm). Taking into account the eight degrees of unsaturation inferred from the molecular formula and the six degrees of unsaturation corresponding to three olefinic bonds and three carbonyl groups, sinulodurin A had to have a bicyclic structure. From the 2D COSY and HMBC spectra (Figure 1) as well as comparison of the NMR data of **1** (Table 1) with those reported for compounds **3** and **4**, the related diterpenes previously isolated from *Pseudopterogorgia elisabethae*,[15,16] the structure of sinulodurin A (**1**) should be similar to **3** except for the absence of a hydroxyl group at C-7, the presence of a α,β-unsaturated ketone at C-13, and two acetoxy groups at C-2 and C-12. The configurations at C-1, C-4, and C-9 were determined by comparing their ^{13}C NMR and proton−proton coupling constants with **3** and **4** (Table 1) and confirmed by ROESY correlations.[15,16] The *S* configuration of C-9 was based on matching its ^{13}C NMR chemical shift data (δ_C 36.8) with C-9 of **3** (δ_C 38.7), unlike the C-9*R* in **4** (δ_C 49.9).[15,16] The β-oriented proton H-9 (δ_H 2.43) showed ROESY correlations with H-4 (δ_H 2.58) and H_3-20 (δ_H 0.99), suggesting similar stereo-orientation. Similarly, protons H-1 and H-2 were found to be α-oriented. Despite the β-substitution upfield shifting effect of the C-12 acetoxy, the downfield shifting of C-11 (δ_C 50.7) in **1** compared to those of **3** (δ_C 32.4) and **4** (δ_C 35.5) (Table 1) suggested opposite configurations. Therefore, C-11 in **1** was proved to be in the *S* configuration. The ^{13}C NMR data similarity of **1** with those reported for sinulobatin A (an amphilectane-type diterpene previously isolated from *Sinularia nanolobat*),[8] support the assumption that **1** could be a biosynthetic intermediate for the amphelectane sinulobatin A.[16] The configuration at C-12 was not determined due to insufficiency of the compound (0.8 mg) left after running the bioactivity assay.

Sinulodurin B (**2**) was isolated as a colorless oil, $[\alpha]_D$ +110.7 (*c* 0.25, $CHCl_3$). Its molecular formula $C_{22}H_{32}O_3$ was determined

* To whom correspondence should be addressed. Tel: +1-662-915-1031. Fax: +1-662-915-7989. E-mail: sross@olemiss.edu.
[†] National Center for Natural Products Research.
[‡] Department of Pharmacognosy, University of Mississippi.
[§] University of Louisiana at Monroe.

10.1021/np800208k CCC: $40.75
Published on Web 07/17/2008

Table 1. ^{1}H and ^{13}C NMR Data of **1**[a] and **2**[a] Compared with **3** and **4**[a15,16]

	1		**2**		**3**		**4**[b]	
position	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	34.1, CH	2.16, m	34.7, CH	2.33, m	32.6, CH	1.96, m	31.9, CH	1.70, m
2	71.3, CH	5.19, td (4.0, 12.8)	29.0, CH_2	1.92, m	28.5, CH_2	1.35, m 1.75, m	23.2, CH_2	1.20, m 1.60, m
3	35.1, CH_2	1.66, m	29.3, CH_2	1.26, m	21.8, CH_2	1.60 m 1.65 m	27.5, CH_2	1.45, m 1.58, m
4	32.4, CH	2.58, dd (4.4, 12.4)	33.5, CH	2.32, m	47.8, CH	1.82, brd (10.0)	37.1, CH	2.24, m
5	131.4, CH	6.08, s	128.5, CH	5.94, s	125.6, CH	5.92, s	127.5, CH	6.12, d (2.0)
6	142.7, qC		143.2, qC		147.6, qC		144.0, qC	
7	26.4, CH_2	1.24, m 2.80 m	27.1, CH_2	1.35m 2.24, m	69.7, CH	4.20, m	29.8, CH_2	2.15, m 2.34, m
8	28.8, CH_2	2.14, m 2.26, m	29.0, CH_2	2.00 m 2.28, m	36.4, CH_2	1.52, q (12.0) 1.91, dt (12.0, 6.0)	35.2, CH_2	1.02, m 1.59, m
9	36.8, CH	2.43, dd (5.4, 14.2)	37.2, CH	2.47, dd (5.6, 14.2)	38.7, CH	2.56, m	49.9, CH	1.80, m
10	138.4, qC		141.6, qC		143.9, qC		143.9, qC	
11	50.7, CH	2.67, ddq (4.4, 6.8, 12.4)	46.8, CH	2.01, m	32.4, CH	1.65, m	35.5, CH	1.77, m
12	80.7, CH	4.81, brs	79.9, CH	4.81, d (1.6)	34.6, CH_2	1.31, 2H, m	29.6, CH_2	1.20, m 1.63, m
13	197.2, qC		196.9, qC		25.5, CH_2	2.02, 2H, m	25.9, CH_2	1.96, m 2.15, m
14	119.5, CH	6.03, s	119.6, CH	6.02, s	124.9, CH	5.01, t (7.1)	119.6, CH	5.19 m
15	159.4, qC		158.5, qC		131.2, qC		130.9, qC	
16	21.7, CH_3	2.18, 3H, s	21.1, CH_3	2.18, 3H, s	25.7, CH_3	1.67, 3H, s	25.9, CH_3	1.63, 3H, d (4.5)
17	28.7, CH_3	1.94, 3H, s	28.1, CH_3	1.93, 3H, s	17.7, CH_3	1.57, 3H, s	17.7, CH_2	1.57, 3H, s
18	14.8, CH_3	1.03, 3H, d (7.0)	14.6, CH_3	0.91, d (6.8)	17.5, CH_3	0.91, 3H, d (6.6)	15.1, CH_3	0.85, 3H, d (7.5)
19	111.2, CH_2	4.73, 2H, brs	109.5, CH_2	4.68, 2H, brs	105.0, CH_2	4.80 s 5.06 s	109.0, CH_2	4.76, dd (1.5, 1.0) 4.85, brs
20	15.7, CH_3	0.99, 3H, d (7.0)	11.9, CH_3	0.91, 3H, d (6.8)	14.6, CH_3	0.84, 3H, d (7.1)	17.6, CH_3	0.89, 3H, d (6.5)
2-OAc	22.0, CH_3 170.4, qC	2.05, 3H, s	20.8, CH_3 170.4, qC	2.16, 3H, s				
12-OAc	21.1, CH_3 170.6, qC	2.14, 3H, s						

[a] In $CDCl_3$, 100 MHz for ^{13}C and 400 MHz for ^{1}H. Carbon multiplicities were determined by DEPT135° experiment. qC = quaternary, CH = methine, CH_2 = methylene, CH_3 = methyl carbons. Coupling constants (J) are in Hz. [b] In C_6D_6.

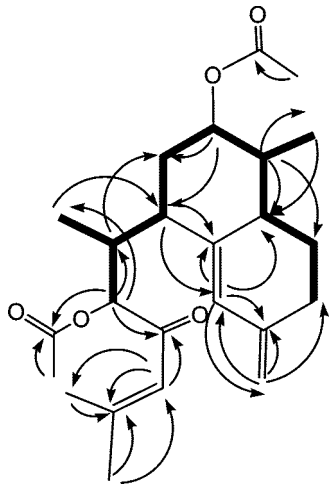


Figure 1. COSY (bold lines) and HMBC (plain arrow) correlations of **1**.

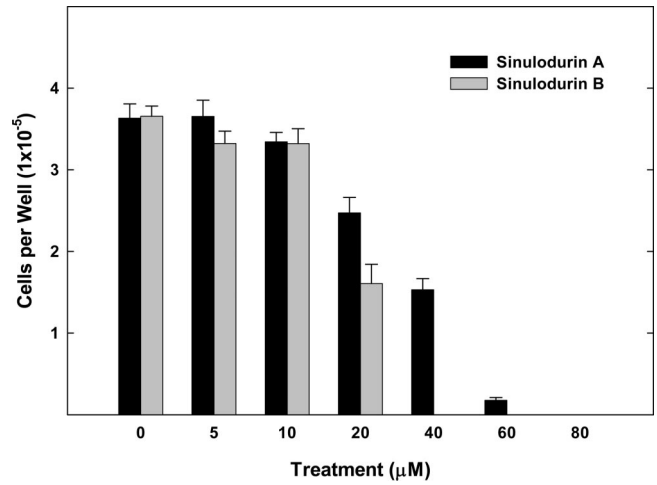


Figure 2. Effects of various doses of sinulodurin A (**1**) and sinulodurin B (**2**) on malignant +SA mammary epithelial cell proliferation.

by HRESIMS [$(M + H)^+$ *m/z* 345.2436, $(M + Na)^+$ *m/z* 367.2259, $(2M + Na)^+$ *m/z* 711.4606] and ^{13}C NMR data. The ^{1}H and ^{13}C NMR data (Table 1) of **2** were similar to those of **1** except for the absence of the C-2 acetoxy group. The structure of **2** was confirmed by 2D NMR analysis.

The structures of the two sterols 24*S*-methyl cholesterol and 24-methylene cholesterol were determined by comparing their NMR data with literature.[17]

The effect of various concentrations of the diterpenes **1** and **2** was studied on the proliferation of highly malignant mice +SA mammary epithelial cells.[18] Sinulodurin B (**2**) showed antiproliferative activity against +SA mammary epithelial cells at a dose of 20 μM, while sinulodurin A (**1**) was less active and showed a comparable activity at 30 μM compared to their respective vehicle controls (Figure 2). This is a relevant activity compared with the positive drug control δ-tocotrienol, which showed an IC_{50} of 7 μM under the same assay conditions.[18]

Spheroid disaggregation provides the measure of cell disaggregation as well as cell migration, and this model simulates the process of tissue disaggregation and invasion of cells *in vivo*.[19,20] This assay is based on disaggregation of cancer cell spheroids and radial migration of released cells on extracellular matrix (ECM).[19–21] Current evidence has shown that tumor cells from primary tumors *in vivo* are generally released in clumps, which attach to a favorable

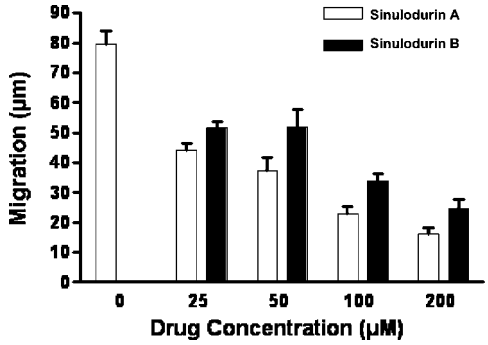


Figure 3. Spheroid disaggregation of PC-3M-CT+ prostate cells using different doses of sinulodourin A (**1**) and sinulodourin B (**2**) versus no treatment control.

ECM and are later released gradually to migrate in all directions.[21] Therefore, the spheroid disaggregation model is closer to *in situ* tumor metastasis than the linear invasion assays. Figure 3 demonstrates that sinulodurins A and B (**1** and **2**, respectively) effectively decreased the disaggregation and cell migration of PC-3M-CT+ spheroids at 25, 50, 100, and 200 μM doses compared to vehicle control. Sinulodurin A was more active than sinulodurin B especially at the lower doses (Figure 3).

All compounds were also tested for antimicrobial activity against *Candida albicans*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Cryptococcus neoformans*, *Mycobacterium intracellulare*, *Aspergillus fumigatus*, and methicillin-resistant *Staphylococcus aureus* (MRSa).[13] All compounds displayed no activity up to 50 μg/mL against all test microbes. They also lacked cytotoxicity against Vero cells (African green monkey kidney fibroblast).

Experimental Section

General Experimental Procedures. Optical rotations were measured at ambient temperature using a Rudolph Research Analytical Autopol IV automatic polarimeter. UV spectra were obtained on a Varian Cary 50 Bio UV−visible spectrophotometer. IR spectra were recorded on a Bruker Tensor 27 spectrophotometer. 1D and 2D NMR spectra were recorded in $CDCl_3$ on a Varian AS 400 spectrometer. HRESIMS was obtained using a Bruker Bioapex FTMS in ESI mode. TLC was performed using Merck precoated plates (Si gel 60 F254). HPLC was performed on a Waters Delta Prep 4000 preparative chromatography system using a Phenomenex Luna C18 column (250 × 10.0 mm, 5 μm).

Animal Material. The soft coral *Sinularia dura* was collected (coll. No. C016103) from Palau (07°28′10″ N; 134°37′62″ E) at a depth of 12 m using scuba in June 1996. A voucher specimen was deposited at the Smithsonian Institution in Washington D.C.

Extraction and Isolation. The organism was ground in dry ice, then allowed to thaw to 4 °C, and stirred with 1 L of H_2O. The entire mixture was centrifuged, and the supernatant solution freeze-dried to give the aqueous extract. The pellet was also freeze-dried and then extracted at room temperature with a 50/50 mixture of CH_2Cl_2 and MeOH overnight. The organic layer was removed, taken to dryness below 4 °C, and then high vaccuum-dried to give the organic extract. Three grams of the residue was subjected to fractionation by vacuum liquid chromatography over a silica gel column (100 g, 160−200 mesh) eluting with *n*-hexane−EtOAc (100:0 to 0:100) followed by EtOAc−MeOH (80:20, 60:40), yielding 11 fractions (A−K). Fraction B (269 mg, eluted with *n*-hexane−EtOAc, 90:10) was chromatographed over a silica gel column (7 g, 1 × 50 cm) eluted with CH_2Cl_2−MeOH (100:0−85:15) to afford 40 subfractions (B_1−B_{40}, 20 mL each). Subfraction B_{3-4} (86 mg) was purified on a C_{18} SPE column (2 g), eluted with MeOH−H_2O (90:10), to give 24*S*-methyl cholesterol (9.4 mg) and 24-methylene cholesterol (13.5 mg). Subfraction B_{15-40} (8.3 mg) was purified by C_{18} HPLC using MeOH−H_2O (80:20) as an eluent to give **1** (2.5 mg, t_R 15.5 min) and **2** (1.9 mg, t_R 11.4 min).

Antiproliferative Assay. The antiproliferative effects of **1** and **2** were tested in culture of the highly malignant +SA mouse mammary epithelial cell line maintained on serum-free media and containing 10 ng/mL EGF and 10 μg/mL insulin as mitogens, as described previously in detail.[18] Cells were plated at a density of 5 × 10^4 cells/well (6 wells/group) in 24-well culture plates and fed media containing various concentrations (0.01−1000 μM) of each compound. After a 4-day culture period, the viable +SA cell number was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) colorimetric assay as described previously.[18]

Spheroid Disaggregation Assay. Spheroids were prepared from single cell suspension of prostate cell lines as described before.[19−21] In brief, 5 × 10^4 cells/mL in RPMI 1640 serum-free medium was placed on 96-well low-attachment tissue culture plates. The plates were rocked on a gyrorotatory shaker in a CO_2 incubator at 37 °C for 2 days, at the end of which spheroids measuring 150−300 μm in diameter (~4 × 10^4 cells/spheroid) were formed. A single spheroid was then placed in the center of each well of an ECM-coated 24-well microplate in 200 μL of serum-free medium.[19−21] From previous studies, it was determined that 1 h is an appropriate time for spheroids to begin adhering to an ECM. Thus $t = 0$ was set as 1 h from initial plating, so that if the plate was not disturbed, the spheroids would not move from their location at the time of plating. Spheroids were photographed digitally at $t = 0$, cultured at 37 °C for 48 h, and then rephotographed. The spheroids were then fixed, stained with Diff-Quik (Dade Behring, Newark, DE), and examined under light microscopy. The diameter of the area covered with cells migrated from the spheroids was measured in a microscope calibrated with a stage and ocular micrometer. The radial distance of migration was calculated after subtraction of the mean initial spheroidal diameter at $t = 0$. Values shown represent the average percent increase in surface area of spheroids.[19−21]

Sinulodurin A (1): colorless oil; $[\alpha]_D^{24}$ +91.3 (*c* 0.25, $CHCl_3$); UV (EtOH) λ_{max} 236, 239 nm; IR ν_{max} 1736, 1640, 1620 cm^{-1}; ^1H NMR and ^{13}C NMR, see Table 1; HRESIMS *m*/*z* 425.2326 $[M + Na]^+$ (calcd for $C_{24}H_{34}O_5Na$, 425.2304).

Sinulodurin B (2): colorless oil; $[\alpha]_D^{24}$ +110.7 (*c* 0.25, $CHCl_3$); UV (EtOH) λ_{max} 236, 239 nm; IR ν_{max} 1736, 1640, 1620 cm^{-1}; ^1H NMR and ^{13}C NMR, see Table 1; HRESIMS *m*/*z* 367.2259 $[M + Na]^+$ (calcd for $C_{22}H_{32}O_3Na$, 367.2249).

Acknowledgment. Materials were provided from the Open and Active Repository of the Natural Products Branch at the National Cancer Institute under the code no. C016103. We are grateful to B. Avula for assistance with the HRESIMS, and M. Jacob and M. Wright for conducting the antimicrobial testing. The authors also acknowledge the United States Department of Agriculture, Agriculture Research Services Specific Cooperation Agreement No. 58-6408-2-0009, and the NIH, NIAID, Division of AIDS, grant no. AI 27094, for partial support of this work.

References and Notes

(1) Goldshlager, G. K.; Klein, P.; Rudi, A.; Banyahu, Y.; Schleyer, M.; Kashman, Y. *J. Nat. Prod.* **1996**, *59*, 262–266.
(2) Bowden, B. F.; Coll, J. C.; Mitchell, S. J.; Kazlauskas, R. *Aust, J. Chem.* **1981**, *34*, 1551–1556.
(3) Li, G.; Zhang, Y.; Deng, Z.; Ofwegen, L.; Proksch, P.; Lin, W. *J. Nat. Prod.* **2005**, *68*, 649–652.
(4) Radhika, P.; Rao, P. R.; Archana, J.; Rao, N. K. *Biol. Pharm. Bull.* **2005**, *28*, 1311–1313.
(5) Zhang, C. X.; Yan, S. J.; Zhang, G. W.; Lu, W. G.; Su, J. Y.; Zeng, L. M. *J. Nat. Prod.* **2005**, *68*, 1087–1089.
(6) Rudi, A.; Shmul, G.; Banyahu, Y.; Kashman, Y. *Tetrahedron Lett.* **2006**, *47*, 2937–2939.
(7) Tseng, Y. J.; Ahmed, A. F.; Dai, C. F.; Chiang, M. Y.; Sheu, J. H. *Org. Lett.* **2005**, *7*, 3813–3816.
(8) Yamada, K.; Ujiie, T.; Yoshida, K.; Miyamoto, T.; Highuchi, R. *Tetrahedron* **1997**, *53*, 4569–4578.
(9) Buckle, P. J.; Baldo, B. A.; Taylor, K. M. *Agents Actions* **1980**, *10*, 361–367.
(10) Takaki, H.; Koganemaru, R.; Iwakawa, Y.; Higuchi, R.; Miyamato, T. *Biol. Pharm. Bull.* **2003**, *26*, 380–382.
(11) Aceret, T. L.; Coll, J. C.; Uchio, Y.; Sammarco, P. W. *Comp. Biochem Physiol. C* **1998**, *120*, 121–126.
(12) Kamel, H. N.; Slattery, M. *Pharm. Biol.* **2005**, *43*, 253–269.
(13) Radwan, M. M.; Manly, S. P.; Ross, S. A. *Nat. Prod. Commun.* **2007**, *2*, 901–904.
(14) Pretsch, E., Clerc, T., *Spectral Data for Structure Determination of Organic Compounds, ^{13}C-NMR, ^1H-NMR, IR, MS, UV/VS*, 2nd ed.; Springer-Verlag: Berlin, 1989; p U20.

(15) Duque, C.; Puyana, M.; Castellanos, L.; Arias, A.; Correa, H.; Osorno, O.; Asai, T.; Hara, N.; Fujimoto, Y. *Tetrahedron* **2006**, *62*, 4205–4213.
(16) Coleman, A. C.; Kerr, R. G. *Tetrahedron* **2000**, *56*, 9569–9574.
(17) Kingston, J., F.; Benson, E.; Gregory, B.; Fallis, A. G. *J. Nat. Prod.* **1979**, *42*, 528–531.
(18) McIntyre, B. S.; Briski, K. P.; Gapor, A.; Sylvester, P. W. *PSEBM* **2000**, *224*, 292–30.
(19) Byers, H. R.; Etoh, T.; Doherty, J. R.; Sober, A. J.; Mihm, M. C. *Am. J. Pathol.* **1991**, *139*, 423–435.
(20) Hotulainen, P.; Paunola, E.; Vartiainen, M. K.; Lappalainen, P. *Mol. Biol. Cell* **2005**, *16*, 649–664.
(21) Hoevel, T.; Macek, R.; Swisshelm, K.; Kubbies, M. *Int. J. Cancer* **2004**, *108*, 374–383.

NP800208K