Three New Cyclic Peroxides from the Marine Sponge *Plakortis aff simplex*

Amira Rudi,[†] Rania Afanii,[†] Lola Garcia Gravalos,[#] Maurice Aknin,[‡] Emile Gaydou,[§] Jean Vacelet,[⊥] and Yoel Kashman*[,†]

School of Chemistry, Tel-Aviv University, Ramat Aviv 69978, Israel, Université de Droit, d'Economie et des Sciences d'Aix Marseille III, France, PharmaMar S.A. Madrid, Spain, Laboratoire de Chimîe des Substances Naturelles et des Aliments, Faculté des Sciences et Techniques, Université de la Réunion, 15 Avenue Cassin, B.P. 7151, 97715, Saint-Denis, Cedex 9, France, and Centre d'Oceanologie de Marseille, Station Marine d'Endoume, Université de la Méditerranée, UMR 6540, 13007 Marseille, France

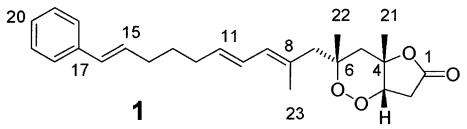
Received December 18, 2002

In our continuing program to identify bioactive compounds from marine invertebrates, the MeOH−EtOAc (1:1) extract of the sponge *Plakortis aff simplex*, collected in Madagascar, was found to be cytotoxic to a series of human tumor cells. From this sponge, three new compounds and one known one, two new 1,2-dioxane peroxylactones named plakortolides H (**1**) and I (**2**), and one new 1,2-dioxolane, designated andavadoic acid (**3**), have been isolated and their structures elucidated. In addition, the known N(3)-methyladenine (**4**) was isolated, to the best of our knowledge, for the first time from a marine source. Andavadoic acid (**3**) showed significant activity against 13 tumor cells with GI_{50} values in the submicromolar range.

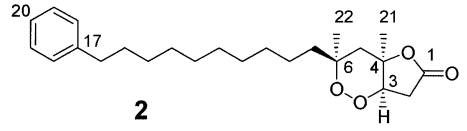
Cyclic peroxides have been previously reported from a number of marine organisms, especially sponges of the family Plakinidae.[1,2] The majority of these cyclic peroxides possesses 1,2-dioxanes, and others contain the more rare 1,2-dioxolane ring system.[3−7] Already in 1979, we reported one of the first 1,2-dioxanes, muqubilin, from the sponge *Prianos* sp.,[8] and since then we have isolated several other cyclic peroxides from several sponges.[9−11]

Herein we report the structures of three additional cyclic peroxides isolated from the massive black anthracite colored sponge *Plakortis aff simplex* (Schulze, 1880), collected by hand using scuba at a depth of 10−20 m in three locations of Andavadoc, 100 km north of Tulear, Madagascar (see Experimental Section). The MeOH−EtOAc (1:1) extract of the sponge exhibited cytotoxic activity against several tumor cells, vide infra, and was partitioned between aqueous MeOH and petrol ether, CCl_4, and $CHCl_3$, affording after additional chromatography compounds **1**−**4**. Compounds **1**−**3**, three new cyclic peroxides, designated plakortolide H (**1**), plakortolide I (**2**), and andavadoic acid (**3**), were obtained from the petrol ether phase, while the more polar compound **4**, N(3)-methyladenine, was obtained from the $CHCl_3$ fraction.

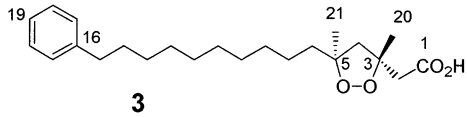
Compound **1** was isolated as a colorless oil (2 mg, 0.006% yield of dry weight) by VLC of the petrol ether partition on a silica gel stationary phase eluted with petrol ether−EtOAc (9:1). The ^{13}C NMR spectrum and HREIMS suggested a molecular formula of $C_{25}H_{32}O_4$. Analysis of the NMR spectra suggested that compound **1** was closely related to plakortolide,[12] which possesses a dimethylperoxy (1,2-dioxane) lactone on one terminus of the molecule, a disubstituted double bond (C11−C12) in the center of the molecule, and a styrene group on the other terminus. The presence of the 1,2-dioxane functionality is supported by the observation of two characteristic carbon resonances at δ 80.7 (CH) and 80.8 (C) on both sides of the peroxy group.



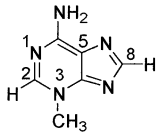
1



2



3



4

The carbons observed at δ 173.0 (C) and 82.5 (C) and an absorption at 1780 cm^{-1} in the IR spectrum suggest a γ-lactone. Resonances at the proton and carbon aromatic region (Table 1) support the existence of the vinylbenzene. The NMR data of both termini were in full agreement with those found in plakortolide[12] (an ABX system for H-2,2′,3, an AB system for H-5,5′ and an *AB*X_2 system for H-15,16). Furthermore, the good agreement of the chemical shifts and coupling constants between these two compounds (Table 1) suggested that **1** possessed the same relative stereochemistry as that of plakortolide.[12] In addition, comparison of the NMR data of **1** and plakortolide showed clearly that the 11(12)-double bond of plakortolide is replaced in **1** by a diene (Table 1). The location of the diene between C-8 and C-11 was elucidated from the HMBC CH correlations and especially the correlations between the vinyl methyl CH_3-23 (δ 1.68 (s), 3H) to C-7, which itself is

* To whom correspondence should be addressed. Tel: +972-3-6408419. Fax: +972-3-6409293. E-mail: kashman@post.tau.ac.il.
[†] Tel Aviv University.
[#] PharmaMar.
[‡] Université de la Réunion.
[§] Marseille III.
[⊥] Centre d'Oceanologie de Marseille.

10.1021/np020589a CCC: $25.00
Published on Web 04/12/2003

Table 1. ^{1}H and ^{13}C NMR Data of Compound **1** in $CDCl_3$

position	δ_C	δ_H[a] mult (*J*)	HMBC (H to C#)	COSY
1	173.0 (C)			
2	34.0 (CH_2)	A 2.53 d (18.6)	1, 3	2B
		B 2.80 dd (18.6, 6.0)	1	2A, 3
3	80.7 (CH)	4.38 d (5.7)	H_3-21, 4, 1	2B
4	82.5 (C)			
5	41.1 (CH_2)	A 1.62 d (15.0)	H_3-21, H_3-22, 7, 6	5B, 7A
		B 2.18 d (15.0)	7, 6, 4	5A
6	80.8 (C)			
7	46.9 (CH_2)	A 2.05 d (14.0)	H_3-23, 6	5A, 7B
		B 2.57 d (14.0)	H_3-23, H_3-22, 5, 6, 9, 8	7A
8	131.6 (C)			
9	129.9 (CH)	5.73 d (10.8)	H_3-23, 7, 10, 11	H_3-23, 10
10	126.8 (CH)	6.16 m	12	9, 11
11	133.0 (CH)	5.53 m	9, 12, 13	10, 12
12	32.5 (CH_2)	2.05 m	10, 11, 13, 14	11, 13
13	29.7 (CH_2)	1.50 m	11, 12, 14, 15	12, 14
14	32.4 (CH_2)	2.17 m	12, 13, 15	13, 15
15	130.7 (CH)	6.12 m	13, 16, 17	14, 16
16	130.1 (CH)	6.27 d (15.8)	14, 15, 17, 18	14, 15
17	137.9 (C)			
18	125.5 (CH)	7.26 d (7.8)	16, 19, 20	
19	128.5 (CH)	7.20 t (7.6)	16	
20	126.9 (CH)	7.07 t (7.5)	18	
21	25.8 (CH_3)	1.28 s	3, 4, 5	
22	24.0 (CH_3)	1.08 s	5, 6, 7	
23	18.3 (CH_3)	1.68 s	7, 8, 9	

[a] A and B denote a geminal pair.

Table 2. ^{1}H and ^{13}C NMR Data of Compound **3** in $CDCl_3$

position	δ_C	δ_H[a] mult (*J*)	HMBC (H to C#)	COSY
1	173.0 (C)			
2	43.6 (CH_2)	A 2.67 d (14.9)	1, 3, 4	2B
		B 2.71 d (14.9)		2A
3	83.8 (C)			
4	55.6 (CH_2)	A 2.20 d (12.4)	2, 3, 5, 6, 20, 21	4B
		B 2.37 d (12.4)		4A
5	86.6 (C)			
6	39.7 (CH_2)	1.70 m		7, 8
7	24.5 (CH_2)	1.50 m		8
8-13	29.3−29.9 (CH_2)	1.20 brs		
14	31.5 (CH_2)	1.54 m	13, 15	13, 15
15	35.9 (CH_2)	2.53 m	13, 14, 16, 17	14
16	142.9 (C)			
17	128.4 (CH)	7.10 m	19	
18	128.8 (CH)	7.10 m	19	
19	125.5 (CH)	7.20 m	17, 18, 16	
20	23.7 (CH_3)	1.40 s	2, 3, 4	
21	23.1 (CH_3)	1.22 s	4, 5, 6	

[a] A and B denote a geminal pair.

correlated to CH_3-22. Thus, plakortolide H (**1**) was determined to be the $\Delta^{8,11}$ diene analogue of plakortolide.

A second peroxylactone, plakortolide I (**2**), whose planar structure was identified as 4,6-dimethyl-4-hydroxy-3,6-peroxy-16-phenylhexadecanoic acid 1,4-lactone by interpretation of the NMR data including 2D NMR (COSY, TOCSY, HMQC, HMBC, and NOESY), was isolated together with **1**. The enantiomer of compound **2**, the (3*S*,4*S*,6*R*)-isomer, was first reported by Faulkner's group from the Philippine sponge *Plakinastrella* sp.[4] Whereas the optical rotation of Faulkner's compound is −8°, that of compound **2** is +8°; therefore it has to be the (3*R*, 4*R*, 6*S*)-isomer.

The third isolated compound, andavadoic acid (**3**), was obtained as a colorless oil. The molecular formula of $C_{23}H_{36}O_4$ was established from HREIMS and ^{13}C NMR data. The IR spectrum showed a band at 1720 and a broad band at 2500 cm^{-1}, typical of a carboxylic acid.[13] The ^{13}C spectrum (Table 2) displayed 21 distinct signals, of which those at 173.0 (C), 142.9 (C), 128.4 (2CH), 128.8 (2CH), and 125.5 (CH) could easily be distinguished as a carboxylic carbonyl and a phenyl group, respectively. The signals at 83.8 (C) and 86.6 (C) were assigned to two oxygenated quaternary carbons. Together with the results of the ^{1}H NMR, DEPT, and HMQC experiments, the presence of three sp^2 methines, 12 methylene, and two methyl groups was established. The above data clearly pointed to a normal aliphatic chain with a phenyl group terminus, a moiety that was confirmed by MS data (*m*/*z* 91 ($PhCH_2^+$, 100%), 105 ($PhCH_2CH_2^+$, 70%) and 217 ($Ph(CH_2)_{10}^+$, 18%)). The need for an additional ring, the sixth degree of unsaturation, and the easy loss of an oxygen in the MS (*m*/*z* 361, $[MH - O]^+$, 70%) and the absence of OH absorptions in the IR spectrum of the methyl ester suggested a 1,2-dioxolane ring system. Indeed, the NMR data of **3** are in excellent agreement with models for a five-membered 1,2-dioxolane peroxide ring.[3,7] HMBC correlations of the latter dioxolane moiety (Table 2) confirmed, unequivocally, the substitution pattern of the heterocycle which is identical to that of epiplakinic acid C, isolated from a *Plakortis* sp. collected in the Fiji Islands.[3] The relative stereochemistry of the dioxolane ring of **3** was established using NOE experiments.

1D-NOE measurements showed correlations nearly identical to those reported for epiplakinic acid[3] and a recently reported new 1,2-dioxolane peroxide acid from the sponge *Plakinastrella onkodes*.[7] H-2 shows strong coupling to CH_3-20, CH_3-21, and H-4(B) (δ 2.37). H-4B, in turn, showed strong coupling to H-4A, CH_3-21, and H-2, and H-4A showed strong coupling to H-4B, C*H*$_3$-20, and H-6. All of these couplings are consistent with a *trans* relative stereochemistry.

The fourth isolated compound was obtained from the $CHCl_3$ partition after Sephadex LH-20 chromotography as a white powder and was identified by its NMR and MS data as N(3)-methyladenine. To the best of our knowledge, this is the first report of this well-known compound being isolated from a marine source.

Compound **3** has been found to be responsible for the cytotoxicity of the crude extract of the sponge. It demonstrated good activity (GI_{50} in the submicromolar range) against 13 human tumor cell lines; however, it did not show any selectivity.

Experimental Section

General Experimental Procedures. $[\alpha]_D$ spectra were obtained with a Jasco P-1010 polarimeter. IR spectra were obtained with a Bruker FTIR Vector 22 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on Bruker ARX-500 and Avance-400 spectrometers. All chemical shifts are reported with respect to TMS ($\delta_H = 0$) and $CDCl_3$ ($\delta_C = 77.0$). ^{1}H, ^{13}C, COSY, HMQC, HMBC, TOCSY, and NOESY were recorded using standard Bruker pulse sequences. ElMS, CIMS, and HREIMS were recorded on a Fisons, Autospec Q instrument.

Animal Material. *Plakortis* sp., a massive black anthracite colored sponge with blue-gray interior, was collected in Madagascar, in the area of Andavadoc (100 km north of Tulear) at Nosy Fasy 22 04 080 S, 43 10 827 E and two other localities, using scuba at a depth of 10−20 m in May 2000. A voucher sample (AM 631) is deposited in Dr. M. Aknin's laboratory in La Réunion. Re-collection and further studies are required for the species identification. The voucher specimen (Museum d'Histoire Naturelle de Marseille, No. MHNM2002Im3) includes two sponges: a massive haplosclerid (an unidentified *Haliclona* sp. with large strongyle spicules) covered by a thick encrusting homoscleromorph belonging to the genus *Plakortis*. The two sponges have the same black color in alcohol and are intimately mixed in the contact area. The homoscleromorph sponge has a spicule complement of numerous diods, 90−160 μm/1.5−6 μm, and very rare triods with actines 30−40 μm/2−4 μm, which is similar to that of *Plakortis simplex* Schulze, 1880. It differs from this species only by its black color in alcohol, which could be due to staining by the haplosclerid sponge. The identification, however, is tentative. *P. simplex* is a Mediterranean species with an apparent cosmopolitan distribution according to the literature records, but the conspecificity of biogeographically disjunct specimens is doubtful.[14] It is likely that the specimen belongs to a presently undescribed species of the *P. simplex* complex, as its spiculation differs slightly from that of other species described from the Indo-Pacific area.[15] A distinction from the Mediterranean *P. simplex* would need a detailed anatomical and biological study, which is not possible from the presently available morphological characteristics.

Extraction and Isolation. After collection, the sponge was immediately frozen and kept at −20 °C until processed. The sponge was freeze-dried and then homogenized and extracted (30 g, dry wt) with MeOH−EtOAc (1:1, ×3) to afford a brown gum (385 mg). Solvent partition between 10% aqueous MeOH and petrol ether gave the active compound in the petrol ether phase (130 mg). Si gel chromatography of the latter phase with a gradient of hexanes−EtOAc afforded the active compound **2** (2 mg) in the 1:1 EtOAc−petrol ether fraction, as well as compounds **1** and **3** (10 and 6 mg, respectively).

Compound 1: $[\alpha]_D$ −17.3° (*c* 0.012, $CHCl_3$); IR (neat) ν_{max} 2931, 2855, 1780 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; CIMS (%) *m*/*z* 397 (MH^+, 30%), 225 ($C_{12}H_{17}O_4$, 35), 173 ($C_8H_{11}O_4$+H, 61), 171 ($C_8H_{11}O_4$, 45), 155 ($C_7H_7O_4$, 100); HREIMS *m*/*z* 396.2278 (calcd for $C_{25}H_{32}O_4$, 396.2275 $[M]^+$).

Compound 2: $[\alpha]_D$ 8.0° (*c* 0.0173, $CHCl_3$); IR (neat) ν_{max} 2929, 2852, 1777 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.70 (H, d, J = 12.4, H-2), 2.91 (H, dd, J = 12.4, 6.0, H-2′), 4.44 (H, d, J = 6.0, H-3), 1.71 (H, d, J = 15.0, H-5), 2.17 (H, d, J = 15.0, H-5′), 1.50 (2H, m, H-7,7′), 1.25 (14H, m, H_2-8 to 14), 1.58 (2H, m, H-15,15′), 2.60 (2H, t, J = 7.0, H-16, 16′), 7.20−7.25 (5H, m, Ph), 1.37 (3H, s, CH_3-21) and 1.27 (3H, s, CH_3-22); ^{13}C NMR δ 174.2 (C-1), 34.2 (C-2), 80.1 (C-3), 82.0 (C-4), 41.0 (C-5), 80.2 (C-6), 36.0 (C-7), 22.4 (C-8), 29.5 (strong) (C9−C14), 31.4 (C-15), 39.5 (C-16), 142.2 (C-17), 128.3 (2C-18), 128.2 (2C-19), 125.5 (C-20), 25.8 (C-21), and 23.0 (C-22); CIMS (%) *m*/*z* 389 (MH^+, 10), 373 (M − O, 100), 355 (M − O − H_2O, 45), 155 ($C_7H_7O_4$, 45); HREIMS *m*/*z* 388.2611 (calcd for $C_{24}H_{36}O_4$, 388.2615 $[M]^+$).

Compound 3: $[\alpha]_D$ +34.7° (*c* 0.004, $CHCl_3$); IR (neat) ν_{max} 2500 and 1720 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 2; CIMS (%) *m*/*z* 377 (MH^+, 40%), 361 (MH−O, 70%), 343 (MH−O−H_2O, 71%), 315 ($C_{21}H_{31}O_2$, 31%), 259 ($C_7H_{11}O_4(CH_2)_7$+H, 75%), 242 ($C_7H_{11}O_4(CH_2)_4CH{=}CH_2$, 40%), 217 ($Ph(CH_2)_{10}$, 18%), 202 ($C_{10}H_{17}O_4$, 26%), 173 ($C_8H_{13}O_4$, 18%), 105 ($PhCH{=}CH_2$, 70%), 91 ($PhCH_2$, 100%); CIMS of the methyl ester *m*/*z* 391 (MH^+, 7%), 373 (MH − H_2O, 26%), 261 (MH − OCH_3, 30%), 357 (MH − O − H_2O, 100%); HREIMS *m*/*z* 376.2619 (calcd for $C_{23}H_{36}O_4$ 376.2615 $[M]^+$).

Compound 4: identical by its ^{1}H and ^{13}C NMR and MS spectrum to an authentic sample of N(3)-methyladenine (Aldrich 28,087-9);[16] ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.29 (H, s, H-2), 7.83 (2H, s, NH_2), 7.76 (H, s, H-8), 3.89 (3H, s, CH_3); ^{13}C NMR (125 MHz) δ 144.0 (C-2), 36.1 (CH_3), 150.6 (C-4), 120.5 (C-5), 155.2 (C-6), 152.6 (C-8).

Biological Results. A colorimetric type of assay, using sulforhodamine B (SRB) reaction,[17−19] has been employed for the cytotoxicity assays against 13 human tumor cell lines: leukemia, K-562 (ATCC-CCL-243); lung carcinoma A-549 (ATCC-CCL-185); melanoma, SK-MEL-28 (ATCC HTB-72); colon carcinoma, HT-29 (ATCC-HTB-38), LoVo (ATCC-CCL-229) & LoVo-Dox, MDR cell line; prostate carcinoma, DU-145 (ATCC-HTB-81) & LNCaP (ATCC-CRL-1740); breast carcinoma SK-BR3 (ATCC-HTB-30); ovary carcinoma SK-OV-3 (ATCC-HTB-77) JGROV & IGROV-ET resistant to ET-743 (both provided by Mario Negri Institute of Milan); and pancreas carcinoma, PANC1 (ATCC-CRL-1469).

Cell lines were maintained in RPMI 1640, supplemented with 10% FBS, 2 mM L-glutamine, 0.1 g/L penicillin, and 0.1 g/L streptomycin sulfate. Cells were incubated at 37 °C, 5% CO_2, and 98% humidity. Cells were seeded in 96-well microtiter plates, at 5000 cells per well in aliquots of 195 μL of medium, and they were allowed to attach to the plate surface by growing in drug-free medium for 24 h. Afterward, cells were treated separately with 5 μL of each compound dilution for 72 h.

The antitumor effect was measured by the SRB (sulforhodamine B) methodology.[17] Cells are seeded in 96-well microtiter plates, at 5 × 103 cells per well in aliquots of 195 μL of medium, and they are allowed to attach to the plate surface by growing in drug-free medium for 18 h. Afterward, samples are in alloquots of 5 μL ranging from 10 to 10−8 μL/mL dissolved in DMSO/EtOH (0.2% in PS buffer). Cells were then fixed by adding 50 μL of cold 50% (wt/vol) trichloroacetic acid (TCA) and incubated for 60 min at 4 °C. Plates were washed with deionized water, and 100 μL of SRB solution (0.4 wt %/vol in 1% acetic acid) was added to each microtiter well and incubated for 10 min at room temperature. Unbound SRB was removed by washing with 1% acetic acid. Plates were air-dried, and the bound stain was solubilized with Tris buffer. Optical densities were read on an automated spectrophotometer plate reader at a single wavelength of 490 nm. Data analysis was generated automatically, and some parameters for cellular responses have been calculated.

Using control OD values, test OD values, and time zero OD values, calculations have been made for the following parameters: GI_{50} = concentration that causes 50% growth inhibition, which may predict the potencial of the compound in terms of tumor reduction; TGI = total growth inhibition, signifies the cytostatic effect; and LC_{50} = concentration that causes 50% cell killing, meaning the cytotoxic effect.

Acknowledgment. We thank PharmaMar Madrid for financial support.

Supporting Information Available: Cytotoxicity and antiproliferative activities of compound **3** (GI_{50}'s TGI's, LC_{50}'s [M]). This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Faulkner, D. J. *Nat. Prod. Rep.* **2001**, *18*, 1−49, and previous reports in this series.
(2) Casteel, D. A. *Nat. Prod. Rep.* **1999**, *16*, 55−73.
(3) Davidson, B. S. *J. Org. Chem.* **1991**, *56*, 6722−6724.
(4) Gureshi, A.; Salvá, J.; Harper, M. K.; Faulkner, D. J. *J. Nat. Prod.* **1998**, *61*, 1539−1542.
(5) Horton, P. A.; Longley, R. E.; Kelly-Borges, M. K.; McConnell, O. J.; Ballas, L. M. *J. Nat. Prod.* **1994**, *57*, 1374−1381.
(6) Chen, Y.; Killday, K. B.; McCarthy, P. J.; Schimoler, R.; Chilson, K.; Selitrennikoff, C.; Pomponi, S. A.; Wright, A. E. *J. Nat. Prod.* **2001**, *64*, 262−264.
(7) Chen, Y.; McCarthy, P. J.; Harmody, D. K.; Schimoler-O'Rourke, R.; Chilson, K.; Selitrennikoff, C.; Pomponi, S. A.; Wright, A. E. *J. Nat. Prod.* **2002**, *65*, 1509−1512.
(8) Kashman, Y.; Rotem, M. *Tetrahedron Lett.* **1978**, 1707−1708.
(9) Rudi, A.; Kashman, Y. *J. Nat. Prod.* **1993**, *56*, 1827−1830.
(10) Rudi, A.; Talpir, R.; Kashman, Y.; Benyahu, Y.; Schleyer, M. *J. Nat. Prod.* **1993**, *56*, 2178−2182.
(11) Yosief, T.; Rudi, A.; Wolde-ab, Y.; Kashman, Y. *J. Nat. Prod.* **1998**, *61*, 491−493.
(12) Davidson, B. S. *Tetrahedron Lett.* **1991**, *32*, 7167−7170.
(13) The CO_2H group was confirmed by its methylation with CH_2N_2 to yield the methyl ester: CIMS *m*/*z* (%) 391 (M, 7), 373 (M − H_2O, 26), 361 (M − OCH_3, 30), 357 (M − H_2O − O, 100), δ_H 3.67 (s) (3H).
(14) Diaz, M. C.; van Soest, R. W. M. In *Sponges in time and space; Biology, Chemistry, Paleontology*; van Soest, R. W. M., van Kempen, Th. M. G., Brackman, J. C., Eds.; A.A.Balkema: Rotterdam, 1994; pp 93−110.
(15) Muricy, G.; Diaz, M. C. In *Systema Porifera: A guide to the supraspecific classification of the phylum Porifera*; Hooper, J. N. A., van Soest, R. W. M., Eds.; Kluver Academic/Plenum Publishers: New York, 2002; pp 71−82.
(16) Guella, G.; Amade, P.; Pietra, F. *Helv. Chem. Acta* **1986**, *69*, 726−729.
(17) Faircloth, G. T.; Stewart, D.; Clement, J. J. *J. Tissue Cult. Methods* **1988**, *11*, 201−205.
(18) Monks, A.; Scudiero, D.; Skehan, P.; Shoemaker, R.; Paull, K.; Vistica, D.; Hose, C.; Langley, L.; Cronise, P.; Varigro-Wolf, A.; Gray-Goodrich, M.; Campbell, H.; Mayo, J.; Boyd, M. *J. Natl. Cancer Inst.* **1991**, *83*, 757−766.
(19) Skehan, P. A.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenny, S.; Boyd, M. *J. Natl. Cancer Inst.* **1990**, *82*, 1107−1112.

NP020589A