Cytotoxic Cyclic Norterpene Peroxides from a Red Sea Sponge *Diacarnus erythraenus*

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Investigation of the lipophilic extract of the Red Sea sponge *Diacarnus erythraenus* revealed one new norsesterterpene cyclic peroxide, aikupikoxide A (**1**), three new norditerpene cyclic peroxides, aikupikoxide B−D (**2**−**4**), and the known norterpene peroxides muqubilin and nuapapuin A methyl ester. In addition, a new sesquiterpene, *O*-methyl guaianediol, was isolated. Their structures were determined by means of spectroscopic methods. The cytotoxic activities for the isolated compounds have been reported.

The methanolic extract of the Red Sea sponge *Diacarnus erythraenus* yielded a new norsesterterpene cyclic peroxide, aikupikoxide A (**1**), three new norditerpene cyclic peroxides, aikupikoxide B−D (**2**−**4**), and the known norterpene peroxides muqubilin (**5**) and nuapapuin A methyl ester (**6**). Moreover, a new sesquiterpene *O*-methyl guaianediol (**7**) was also isolated and identified. Extensive NMR studies and high-resolution mass spectral measurements were used for determination of the structures of the isolated compounds. In addition, empirical rules for establishing the C-2, C-3, and C-6 relative stereochemistry of the peroxides were used in this study. Aikupikoxide A−D and *O*-methyl guaianediol show cytotoxicity to mouse lymphoma (P-388), human lung carcinoma (A-549), and human colon carcinoma (HT-29).

Sponges of the genus *Diacarnus* are known to produce terpene peroxides and related metabolites.[2,3] Terpene peroxides are a unique class of compounds of both terrestrial and animal origin. Interest has usually focused on such metabolites because of their biological activities. These activities have been associated with antimalarial,[3] antimicrobial,[4−7] sea urchin egg cell-division inhibitory,[8] antiviral,[9] ichtyotoxic,[10,11] and cytotoxic activities.[2,12−15] During our search for biologically active metabolites from marine sources we have examined the Red Sea sponge *Diacarnus erythraenus*.[16]

The hexane fraction of a methanolic extract of the sponge *Diacarnus erythraenus* was subjected successively to Sephadex LH-20 (MeOH/CH_2Cl_2, 1:1), silica, and reversed-phase flash chromatography as well as HPLC (both normal- and reversed-phase) to afford a new norsesterterpene cyclic peroxide, aikupikoxide A (**1**),[17] three new norditerpene cyclic peroxide methyl esters, aikupikoxide B−D (**2**−**4**), and the known peroxide norterpenes muqubilin (**5**)[8] and nuapapuin A methyl ester (**6**)[2] (formerly known as methyl nuapauanoate).[8] Moreover, a new sesquiterpene, *O*-methyl guaianediol (**7**), was isolated. Structures of the isolated compounds were secured by intensive 1D and 2D NMR studies and the exact mass determinations. The relative stereochemistry at C-2, C-3, and C-6 of the cyclic peroxides was determined by using established empirical rules[4] (see Experimental Section).

Results and Discussion

All isolated compounds are optically active and therefore are presumably the result of enzyme-mediated biosynthesis. The peroxide moiety for the isolated norterpene cyclic peroxides was determined by resonances at roughly chemical shifts at δ 81/4.2 (for C-3/H-3) and 80 (for the quaternary C-6).[8,12,13,18−21]

Aikupikoxide A (**1**) was isolated as a colorless oil with a molecular formula of $C_{24}H_{40}O_6$ as deduced from HRFABMS (*m*/*z* 425.2908, $[M + H]^+$). Two of five degrees of unsaturation required by this formula were attributed to ketone functionalities (δ 208.7 and 215.1), one due to carboxylic acid (δ 178.3), and one due to a monosubstituted double bond. The remaining two oxygens in the molecular formula are incorporated into an endoperoxide ring to complete the degrees of unsaturation. The ^1H and ^{13}C NMR data (Table 1) revealed resonances for five singlets and one doublet (δ 1.25/13.2) belonging to methyl groups, nine methylenes, an oxymethine (δ 4.14/81.1), an alkylmethine (δ 2.64/42.6), one proton for a monosubstituted sp^2 group (δ 5.08/124.3 and 134.5), a carboxylic acid (δ 178.3), and diketone moieties (δ 215.1 and 208.7). The $^1H-^1H$ COSY and HMQC experiments allowed the assembly of the C-1/C-5, C-7/C-9, C-11/C-12, and C-15/C-17 units. Assignment and connection between these units was supported by a HMBC experiment. HMBC correlations between H-2/C-1, H-2/C-3, and H-5/C-6, together with correlations between H_3-20 and C-1, C-2, and C-3, and between H_3-21 and C-5, C-6, and C-7, supported the assignments of these signals. Further correlations from H_2-11 to C-9, C-10, C-12, C-13, and C-22, and from H_2-15 to C-13, C-14, C-16, C-23, and C-24, confirmed the assignments of these signals and positioning the ketone moiety at C-13 (δ 215.1). The terminal methyl H_3-19 showed correlation to the resonating signal at δ 208.7 (C-18), which supports the assignment of the methyl ketone. Applying the established empirical rules by Capon and MacLeod,[4] the chemical shift of the C-6 methyl (δ 20.6, H_3-21) indicated an axial orientation, the chemical shift of the methyl signal at C-2 (δ 1.24 d, H_3-20) requires a C-2/C-3-*threo* configuration, and the coupling constant of H-3 (δ

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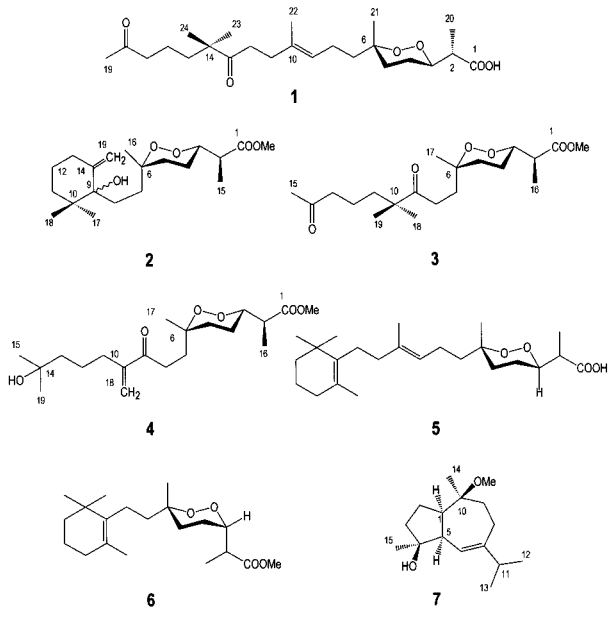
Table 1. ^{1}H and ^{13}C NMR Assignments for **1** and **2** ($CDCl_3$)

	1			**2**		
posit.	δ_C	δ_H (mult., J_{Hz})	HMBC (H→C)	δ_C	δ_H (mult., J_{Hz})	HMBC (H→C)
1	178.3			173.9		
2	42.6	2.64 (quin., 7.5)	3	42.9	2.56 (quin., 7.1)	1, 15
3	81.1	4.14 (ddd, 9.5,7.5,3.0)		82.1	4.25 (ddd, 9.6, 7.2, 2.5)	
4	23.3	1.75 (m)		23.1	1.74 (m), 1.63 (m)	3
5	31.9	1.65 (m)	6	34.1	1.74 (m)	3, 6, 7
6	80.1			80.0		
7	39.5	1.42 (m)		27.1	2.17 (m)	8
8	21.6	1.99 (m)		23.1	1.50 (m)	14
9	124.3	5.08 (t, 7.0)		89.2		
10	134.5			40.4		
11	33.4	2.18 (t, 7.7)	9, 10, 12, 13, 22	40.2	1.51 (m), 1.33 (m)	
12	35.5	2.53 (t, 7.7)	13	19.5	2.31 (dt, 11.4, 1.3)1.97 (m)	10
13	215.1			35.2	2.21 (m), 1.94 (m)	9
14	47.4			146.7		
15	39.1	1.46 (m)	13, 14, 16, 23, 24	12.9	1.14 (d, 7.0)	1, 3
16	18.9	1.42 (m)	15, 17	23.6	1.09 (s)	5, 6, 7
17	43.9	2.40 (t, 6.9)		26.8	1.18 (s)	14
18	208.7			22.7	1.01 (s)	14
19	29.9	2.12 (s)	17, 18	110.0	4.84 (br. s), 4.78 (br. s.)	11
20	13.2	1.24 (d, 7.1)	1, 2, 3			1
21	20.6	1.28 (s)	5, 6, 7			
22	16.0	1.59 (s)	9, 10			
23	24.3	1.10 (s)	13, 14			
24	24.3	1.10 (s)	13, 14			
OMe				52.0	3.70 (s)	1

4.14, ddd, $J_{3,4ax} = 9.5$ Hz) established an axial H-3 (see Experimental Section).



Although aikupikoxide A shares many previously reported norsesterterpenes in the structure and stereochemistry of the endoperoxide ring, it shows a unique structural feature by the presence of the diketone functionalities in the aliphatic nonbranched side chain at C-13 and C-18.

Aikupikoxide B (**2**) was obtained as colorless oil and analyzed for $C_{20}H_{34}O_5$ as determined from HRFABMS [*m*/*z* 353.2295, $(M - H)^+$]. Its ^{1}H NMR spectrum (Table 1) showed resonances for a methyl ester (δ 3.70, s), a secondary methyl (δ 1.14, d), three tertiary methyls (δ 1.09, 1.01 and 1.18), seven methylenes, an exomethylene (δ 4.48 and 4.78, each s), an oxymethine (δ 4.25), and an alkylmethine (δ 2.56). Interpretation of the ^{13}C NMR data, together with ^{1}H−^{1}H COSY and HMQC experiments, led to the assembly of the fragments C-1/C-5 and C-7/C-8 and the substituted cyclohexyl moiety. The molecular formula of **2** needs four degrees of unsaturation. Accounting for the carboxylic ester, the exomethylene and the endoperoxide left one remaining ring. Two methyl singlets (*CDCl*$_3$, δ 1.01, 1.18, each s), one additional hydroxylated quaternary carbon (δ 89.2), and terpene biogenetic considerations suggested one remaining ring was present as a 1-hydroxy-2,2-dimethyl-6-methylenecyclohexyl moiety tethered to C-6 by two methylenes (C-7/C-8). This substructure, with the exception of the OH substitution, has appeared in several other marine metabolites,[22−24] which in turn served as a supportive model for the NMR data of **2**. Connections between the fragments of **2** as well as the assignments of all signals were securely supported by HMBC correlations and are listed in Table 1. Applying the empirical rules,[4] the chemical shift of the C-6 methyl (δ 23.6, H_3-16) requires equatorial geometry; the chemical shift of the methyl signal at C-2 (δ 1.14, d, H_3-15) indicated a C-2/C-3-*erythro* configuration, and the coupling constant of the H-3 signal (δ 4.25, ddd, $J_{3,4ax} = 9.6$ Hz) establishes an equatorial side chain (see Experimental Section).

Aikupikoxide C (**3**) was obtained as colorless oil possessing a molecular formula of $C_{20}H_{34}O_6$ as deduced from HRFABMS [*m*/*z* 371.2418, $(M + H)^+$]. Its ^{1}H and ^{13}C NMR spectra (Table 2) displayed resonances for a methyl ester (δ 3.68/51.9 and 174.3), a secondary methyl (δ 1.12/12.8), three tertiary methyls, one methyl ketone (δ 2.12/29.9), seven methylenes, an oxymethine (δ 4.23/81.3), and an alkylmethine (δ 2.49/42.6), together with diketone functionalities (δ 215.7 and 208.6). Interpretation of the ^{1}H−^{1}H COSY and HMQC experiments revealed the assignments of the C-1/C-5 including the endoperoxide moiety and C-7/C-8 and C-11/C-13 fragments. Assignments and connections of these fragments were supported by HMBC correlations and are listed in Table 2. HMBC correlations from H-7, H-8, H-11, H_3-18, and H_3-19 to the resonating signal at δ 215.7, as well as from H-12, H-13, and H_3-15 to the resonating signal at δ 208.6 supported the assignments of these ketone signals and positioning these groups at C-9 and C-14, respectively. Similarly to **2**, and by applying the empirical rules,[4] compound **3** showed the same relative

Table 2. ^1H and ^{13}C NMR Assignments for **3** and **4** ($CDCl_3$)

	3			**4**		
posit.	δ_C	δ_H (mult., J_{Hz})	HMBC (H→C)	δ_C	δ_H (mult., J_{Hz})	HMBC (H→C)
1	174.3			174.3		
2	42.6	2.49 (quin., 7.7)	1, 3, 15	42.7	2.50 (quin., 7.7)	1
3	81.3	4.23 (ddd, 8.6, 7.7, 3.0)		81.4	4.24 (ddd, 9.5, 7.7, 2.5)	
4	22.5	1.68 (m)	3, 5	22.6	1.69 (m)	
5	33.4	1.73 (m)	4, 6	33.4	1.74 (m)	
6	79.2			79.2		
7	28.3	2.24 (ddd, 14.5, 10.5, 5.0)	8, 9	28.8	2.33 (ddd, 16.0, 11.0, 4.8)	
		1.57 (ddd, 14.5, 10.5, 5.0)			1.61 (ddd, 16.0, 11.0, 5.0)	
8	30.9	2.66 (ddd, 15.8, 10.5, 5.0)	6, 7, 9	31.9	2.91 (ddd, 16.5, 11.0, 4.8)	6, 7, 9
		2.42 (ddd, 15.8, 10.5, 5.0)			2.63 (ddd, 16.5, 11.0, 5.0)	
9	215.7			202.0		
10	47.6			148.5		
11	39.2	1.47[a]	9,10,12, 18, 19	31.3	2.27 (t, 7.2)	10, 13
12	19.0	1.43[a]	11, 13, 14	23.3	1.48[a]	10, 14
13	43.9	2.40 (t, 6.1)	12, 14	43.5	1.47[a]	11, 12
14	208.6			70.9		
15	29.9	2.12 (s)	13, 14	29.5	1.20 (s)	13, 14
16	12.8	1.12[b] (d)	1, 2, 3	12.8	1.13 (d, 7.2)	1, 2, 3
17	23.7	1.06 (s)	5, 6, 7	23.7	1.08 (s)	5, 6, 7
18	24.3	1.12[b] (s)	9, 10, 11	124.2	6.08 (s), 5.75 (s)	11
19	24.3	1.12[b] (s)	9, 10, 11	29.5	1.20 (s)	13, 14
OMe	51.9	3.68 (s)	1	51.9	3.69 (s)	1

[a,b] In each column signals are partially overlapped.

stereochemistry at C-2, C-3, and C-6 (see Experimental Section).

With the exception of the similarity of the peroxide ring in aikupikoxide C (**3**) with previously reported norditerpenes, it possesses the diketone moieties in the aliphatic nonbranched side chain as in **1**.

Aikupikoxide D (**4**) was isolated as colorless oil and has a molecular formula of $C_{20}H_{34}O_6$ as determined by HRFABMS (*m*/*z* 371.2418, $[M + H]^+$). Its ^1H and ^{13}C NMR spectra (Table 2) displayed resonances for a methyl ester (δ 3.69/51.9), an exomethylene (δ 5.57 and 6.08/124.0 and 148.5), an oxymethine (δ 4.24/81.4), an alkylmethine (δ 2.50/42.7), seven methylenes, three tertiary methyl groups, and a ketone moiety (δ 202.0). Comparison of the NMR data of **4** with those of **3** showed the absence of two tertiary methyl groups at C-10 and the terminal methyl ketone at C-14 and the replacement of these functionalities with an exomethylene and a tertiary alcohol terminus at C-10 and C-14, respectively. Assignments of the proton and carbon chemical shifts were made possible from combination of the $^1H-^1H$ COSY, HMQC, and HMBC data (Table 2). As **2** and **3**, and by applying the empirical rules,[4] compound **4** showed the same relative stereochemistry at C-2, C-3, and C-6.

Similarly to **3**, aikupikoxide D (**4**) shows oxygenation at the C-9 and C-14 in the aliphatic side chain. The oxygenation at C-9 and C-14 in the norditerpene peroxides (aikupikoxide C and D) as well as at C-13 and C-18 in the norsesterterpene peroxides (aikupikoxide A) make this group of compounds unique. This feature has never been seen before in the reported norterpenes and is noteworthy in the view of biosynthesis of such metabolites.

Muqubilin (**5**) was isolated as a colorless oil and has a molecular formula of $C_{24}H_{40}O_4$ as established by the HRFABMS [*m*/*z* 415.2829, $(M + Na)^+$]. The structure of **5** was established from intensive 1D and 2D (COSY, HMQC, and HMBC) NMR spectra. The ^1H and ^{13}C NMR data of **5** were comparable with those reported data for muqubilin.[8]

Nuapapuin A methyl ester (**6**) was obtained as a colorless oil. Its molecular formula of $C_{20}H_{34}O_4$ was determined by HRDCIMS [*m*/*z* 339.2534, $(M + H)^+$]. The structure of **6** was assigned and secured from intensive 1D and 2D (COSY, HMQC, and HMBC) NMR spectra. The ^1H and ^{13}C NMR data of **6** are comparable with those reported data for nuapapuin A methyl ester.[2,8]

O-Methyl guaianediol (**7**) was obtained as a colorless oil with a molecular formula of $C_{16}H_{28}O_2$ as established by HREIMS [*m*/*z* 220.1816, $(M - H - OMe)^+$]. Its ^1H and ^{13}C NMR spectra (see Experimental Section) revealed resonances for a guaiane sesquiterpene skeleton, including resonances for three methine, one olefinic proton for a monosubstituted double bond, two tertiary methyls, and one methoxy group. $^1H-^1H$ COSY, HMQC, and HMBC experiments secured the assignments of all signals. The relative stereochemistry at C-1, C-2, C-5, and C-6 was determined from 2D NOESY experiments through NOE cross-peaks between H-1 and H-5, H-1 and H_3-14, and H-5 and H_3-15, respectively. With the exception of a difference in the stereochemistry at C-1 and C-4, compound **7** is the *O*-methyl derivative of the previously reported compound guaianediol.[25]

Experimental Section

General Experimental Procedures. NMR spectra were determined on either a General Electric GN Omega 500 spectrometer or Varian Unity INOVA 400 WB instrument (^1H at 500 or 400 MHz; ^{13}C at 125 or 100 MHz, respectively). Homonuclear ^1H connectivities were determined by using the 2D double-quantum-filtered COSY. One-bond heteronuclear $^1H-^{13}C$ connectivities were determined by a 2D proton-detected HMQC experiment; two- and three-bond $^1H-^{13}C$ connectivities were determined by a 2D proton-detected HMBC experiment. High-resolution mass spectra were determined in the EI and FAB modes. Optical rotations were measured on a Jasco-DIP-700 using CH_2Cl_2 at 20 °C at the sodium D line (589 nm).

Animal Material. The sponge is ramose-digitate and forms an anastomosing mat and was collected using scuba at a depth of 15−20 m from the Red Sea at El Qusier, 120 km south of Hurghada, Egypt, on January 21, 1999. The surface is covered in dull spines, but is microscopically smooth and fleshy, the color mottled dark reddish maroon and oak brown. The sponge is fleshy and compressible but difficult to cut. This sponge is characterized by huge primary fibers, which appear as "sinews" in the sponge, and large yolk-like larvae 1−2 mm in diameter. The sponge is *Diacarnus eythraenus* Kelly-Borges and Vacelet (1995) (*insertae sedis* within Latrunculiidae, order

Hadromerida). A voucher specimen has been deposited at the Natural History Museum, London, United Kingdom (BMNH 1999.12.20.4).

Extraction and Isolation. Freshly collected specimens (250 g, wet wt) of the sponge were immersed quickly in MeOH on site. The sponge was extracted with MeOH (3 × 500 mL) at room temperature. The combined methanolic extracts were concentrated under reduced pressure and dissolved in 500 mL of MeOH/H_2O (9:1) and extracted with hexane (4 × 250 mL) to give 1.72 g of hexane residue. The remaining methanolic layer was diluted with H_2O to (3:2) MeOH/H_2O and then extracted with CH_2Cl_2 (4 × 250 mL) to afford 558 mg of CH_2Cl_2 extract. The hexane residue was loaded on a Sephadex LH-20 column equilibrated with CH_2Cl_2/MeOH (1:1). Six fractions were collected. Fraction 4 (583 mg) was subjected to a SiO_2 flash column eluted with hexane/CH_2Cl_2/acetone gradients to afford 14 subfractions. Subfraction 4-5 was concentrated under vacuum to give pure **6** (30 mg). Subfraction 4-11 (27 mg) was then purified by reversed-phase HPLC with 65% MeCN (1.8 mL/min at 220 nm) to give **3** (4.6 mg). Subfraction 4-13 (16 mg) was subjected to purification on reversed-phase HPLC eluted with 95% MeCN (2 mL/min at 220 nm) to afford **4** (0.9 mg). Fraction 5 (423 mg) was subjected to a reversed-phase flash column chromatography (YMC Gel ODS-A, 60 Å 230/70 mesh) eluting with 70%→0% H_2O/MeCN followed by CH_2Cl_2 to obtain 12 subfractions. Subfraction 5-6 (37 mg) was purified on a reversed-phase column eluting with 50% MeCN (2 mL/min at 220 nm) to afford **7** (2 mg). Subfraction 5-7 (19 mg) was purified on a reversed-phase HPLC column (starting with 80% MeCN to 50% MeCN in 50 min, 2 mL/min at 220 nm), affording **1** (1.9 mg). Fraction 5-8 (21 mg) was further subjected to separation on normal-phase HPLC (Microsorb Si, 300 × 7.0 mm) eluting with hexane/2-propanol (95:5) (2 mL/min and monitoring at 220 nm) to afford **2** (1.0 mg). Subfraction 5-11 (45 mg) was purified by reversed-phase HPLC (starting with 90% MeCN to MeCN in 35 min, 2 mL/min at 220 nm) to give **5** (9.1 mg).

Stereochemical Analysis. The relative stereochemistry in all of the norterpene peroxides reported is based on the empirical rules for C-2, C-3, and C-6 relative stereochemistry introduced by Capon and MacLeod.[4] The empirical rule we adopted from those originally proposed is as follows. First, the ^{13}C NMR chemical shift of the C-6 tertiary methyl reveals axial (δ 20.5−20.9) or equatorial (δ 23.5−24.0) geometry. Second, the ^1H NMR chemical shift of the C-2 secondary methyl reveals whether C-2 and C-3 are in an *erythro* (*R,R* or *S,S*; δ 1.13−1.14) or a *threo* configuration (*R,S* or *S,R*; δ 1.22−1.24). Last, the $^3J_{H,H}$ value of the H-3 establishes the axial or equatorial nature of this proton.

Aikupikoxide A (**1**): colorless oil (1.9 mg, 0.00076%, based on wet wt); $[\alpha]_D$ +81° (*c* 0.8, CH_2Cl_2); NMR data, see Table 1; HRFABMS *m/z* 425.2908 (calcd for $C_{24}H_{41}O_6$, $[M + H]^+$, 425.29031).

Aikupikoxide B (**2**): colorless oil (1 mg, 0.0004%, based on wet wt); $[\alpha]_D$ +76° (*c* 0.5, CH_2Cl_2); NMR data, see Table 1; HRFABMS *m/z* 353.2295 (calcd for $C_{20}H_{33}O_5$, $[M - H]^+$, 353.2328).

Aikupikoxide C (**3**): colorless oil (4.6 mg, 0.00184%, based on wet wt); $[\alpha]_D$ +88° (*c* 2.0, CH_2Cl_2); NMR data, see Table 2; HRFABMS *m/z* 371.2418 (calcd for $C_{20}H_{35}O_6$, $[M + H]^+$, 371.2433).

Aikupikoxide D (**4**): colorless oil (0.9 mg, 0.00036%, based on wet wt); $[\alpha]_D$ +69° (*c* 0.45, CH_2Cl_2); NMR data, see Table 2; HRFABMS *m/z* 371.2418 (calcd for $C_{20}H_{35}O_6$, $[M + H]^+$, 371.2433).

Muqubilin (**5**): colorless oil (9.1 mg, 0.00364%, based on wet wt); $[\alpha]_D$ +30.2° (*c* 1.82, CH_2Cl_2) (lit.[8] +31.6°); HRFABMS *m/z* 415.2829 (calcd for $C_{24}H_{40}O_4Na$, $[M + Na]^+$, 415.2824).

Nuapapuin A methyl ester (**6**): colorless oil (30 mg, 0.012%, based on wet wt); $[\alpha]_D$ +53.3° (*c* 2.25, CH_2Cl_2) (lit.[8] +53.7°); HRDCIMS *m/z* 339.2534 (calcd for $C_{20}H_{35}O_4$, $[M + H]^+$, 339.2535).

***O*-methyl guaianediol** (**7**): colorless oil (2 mg, 0.0008%, based on wet wt); $[\alpha]_D$ +22° (*c* 0.15, CH_2Cl_2); ^1H NMR ($CDCl_3$, 500 MHz) δ 2.03 (1H, m, H-1), 1.73 and 1.62 (each 1H, m, H_2-2), 1.7−1.59 (2H, m, 1.73, H_2-3), 2.22 (1H, m, H-5), 5.46 (1H, d, J = 2.1 Hz, H-6), 2.22 (1H, m, H-8a), 1.86 (1H, dd, J = 16 and 9.6 Hz, H-8b), 1.4 and 1.60 (each 1H, m, H_2-9), 2.20 (1H, m, H-11), 0.98 (3H, d, J = 6.9 Hz, H_3-12), 0.97 (3H, d, J = 6.9 Hz, H_3-13), 1.19 (3H, s, H_3-14), 1.18 (3H, s, H_3-15), 3.17 (3H, s, OMe); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 47.9 (d, C-1), 21.5 (t, C-2), 40.5 (t, C-3), 80.2 (s, C-4), 50.1 (d, C-5), 121.7 (d, C-6), 149.6 (s, C-7), 24.5 (t, C-8), 35.4 (t, C-9), 79.1 (s, C-10), 21.6 (d, C-11), 21.6 (q, C-12), 21.2 (q, C-13), 17.9 (q, C-14), 22.4 (q, C-15), 48.7 (q, OMe); HREIMS *m/z* 220.1816 (calcd for $C_{15}H_{24}O$, $[M - H - OMe]^+$, 220.1827).

Cytotoxicity Testing. Cytotoxicity assays (IC_{50}, μg/mL) were carried out against three types of cancer cells including murine leukemia (P-388; ATCC: CCL 46), human lung carcinoma (A-549; ATCC: CCL 8), and human colon carcinoma (HT-29; ATCC: HTB 38). A dilution assay limit corresponding to 1 μg/mL has been set as a cutoff value for further in vivo screening. The new compounds **1**, **2**, **3**, **4**, and **7** showed activity of $IC_{50} > 1$ μg/mL against the three types of cells. Such activity is not of sufficient interest to pursue such compounds with in vivo studies.

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Supporting Information Available: ^1H and ^{13}C NMR spectra of **1**−**7**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Fulbright scholar at the University of Hawaii (1999−2000); on sabatical leave from Suez Canal University.
(2) Sperry, S.; Valeriote, F. A.; Corbett, T. H.; Crews, P. *J. Nat. Prod.* **1998**, *61*, 241−247.
(3) D'Ambrosio, M.; Guerriero, A.; Deharo, E.; Debitus, C.; Munoz, V.; Pietra, F. *Helv. Chim. Acta* **1998**, *81*, 1285−1292.
(4) Capon, R. J.; MacLeod, J. K. *Tetrahedron* **1985**, *41*, 3391−3404.
(5) Faulkner, D. J.; He, H.; Lu, H. S. M.; Clardy, J. *J. Org. Chem.* **1991**, *56*, 2112−2115.
(6) Capon, R. J. *J. Nat. Prod.* **1991**, *54*, 190−195.
(7) Sokoloff, S.; Halevy, S.; Usieli, V.; Colorni, A.; Sarel, S. *Experientia* **1982**, *38*, 337−338.
(8) Manes, L. V.; Bakus, G. J.; Crews, P. *Tetrahedron Lett.* **1984**, *25*, 931−934.
(9) Tanaka, J.; Higa, T.; Suwanborirux, K.; Kokpol, U.; Bernardinelli, G.; Jefford, C. W. *J. Org. Chem.* **1993**, *58*, 2999−3002.
(10) Albericci, M.; Collart-Lempereur, M.; Braekman, J. C.; Daloze, D.; Tursch, B. Declercq, J.-P.; Germain, G.; Van Meerssche, M. *Tetrahedron Lett.* **1979**, 2687−2690.
(11) Albericci, M.; Braekman, J. C.; Daloze, D.; Tursch, B. *Tetrahedron* **1982**, *38*, 1881−1890.
(12) Harrison, B.; Crews, P. *J. Nat. Prod.* **1998**, *61*, 1033−1037.
(13) Rudi, A.; Kashman, Y. *J. Nat. Prod.* **1993**, *56*, 1827−1830.
(14) Rudi, A.; Talpir, R.; Kashman, Y.; Benayahu, Y.; Schleyer, M. *J. Nat. Prod.* **1993**, *56*, 2178−2182.
(15) Gunasekera, S. P.; Gunasekera, M.; Gunawadana, G. P.; McCarthy, P.; Burres, N. *J. Nat. Prod.* **1990**, *53*, 669−674.
(16) Kelly-Borges, M.; Vacelet, J. A revision of *Diacarnus burton* and *Negombata* de Laubenfels (Demospongiae: Latrunculiidae) with descriptions of new species from the West Central Pacific and the Red Sea. *Mem. Queensland Mus.* **1995**, *38*, 477−503.
(17) *Aikupika* is the Hawaiian transliteration of Egypt, the country and the origin of the first author and of the sponge.
(18) Cambie, R. C.; Craw, P. A.; Bergquist, P. R.; Karuso, P. *J. Nat. Prod.* **1987**, *50*, 948−949.
(19) Ovenden, S. P. B.; Capon, R. J. *Aust. J. Chem.* **1998**, *51*, 573−579.
(20) Braekman, J. C.; Daloze, D.; De Groote, S.; Fernandes, J. B.; Van Soest, R. W. M. *J. Nat. Prod.* **1998**, *61*, 1038−1042.
(21) Qureshi, A.; Salva, J.; Harper, M. K.; Faulkner, D. J. *J. Nat. Prod.* **1998**, *61*, 1539−1542.
(22) Rudi, A.; Kashman, Y. *J. Nat. Prod.* **1992**, *55*, 1408−1414.
(23) Boyd, K. J.; Harper, M. K.; Faulkner, D. J. *J. Nat. Prod.* **1995**, *58*, 302−305.
(24) Wu, H.; Nakamura, H.; Kobayashi, J.; Ohizumi, Y.; Hirata, Y. *Tetrahedron Lett.* **1984**, *25*, 3719−3722.
(25) El Sayed, K. A.; Hamann, M. T. *J. Nat. Prod.* **1996**, *59*, 687−689.

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