Two new subergane-based sesquiterpenes from a Taiwanese Gorgonian coral Subergorgia suberosa.

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Suberosols A−D, Four New Sesquiterpenes with β-Caryophyllene Skeletons from a Taiwanese Gorgonian Coral *Subergorgia suberosa*

Guey-Horng Wang,[†] Atallah F. Ahmed,[†,‡] Jyh-Horng Sheu,*[,†] Chang-Yih Duh,[†] Ya-Ching Shen,[†] and Li-Tang Wang[†]

Department of Marine Resources, National Sun Yat-Sen University, Kaohsiung 804, Taiwan, Republic of China, and Department of Pharmacognosy, Faculty of Pharmacy, Mansoura University, Mansoura 35516, Egypt

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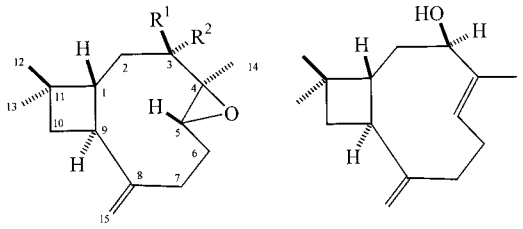
Four new β-caryophyllene-derived sesquiterpenes alcohols, suberosols A (**1**), B (**2**), C (**3**), and D (**4**), along with two known β-caryophyllene-derived sesqueterpene ketones, buddledins C (**5**) and D (**6**), were isolated from a Taiwanese gorgonian coral *Subergorgia suberosa*. The structures of **1**−**4** were determined on the basis of extensive spectroscopic analyses. Cytotoxicity of these compounds toward various cancer cell lines is also described.

In our screening for biologically active metabolites from the Taiwanese gorgonians, we have discovered a series of novel terpene metabolites from the gorgonian corals including a *Briareum* sp.,[1] *Briareum excavatum*,[2−7] *Junceella fragilis*,[8] and *Isis hippuris*.[9] In this paper, we wish to describe the isolation and structure characterization of four new caryophyllene-type sesquiterpenes, suberososls A−D (**1**−**4**), along with two known metabolites, buddledins C (**5**) and D (**6**),[10] from a Taiwanese gorgonian, *Subergorgia suberosa*. This coral occurs widely in the Indo-Pacific waters and has been found to contain a novel tricyclopentanoid cardiotoxin, subergorgic acid (**7**),[11] four analogues of subergorgic acid,[12] a cytotoxic sesquiterpene, suberosenone (**8**),[13] and new 9,11-secosterols.[14,15] The structures of these metabolites were determined by extensive NMR (^{1}H, ^{13}C NMR including DEPT, ^{1}H−^{1}H COSY, HMQC, HMBC, and NOESY) experiments. Cytotoxicity of metabolites **1**−**6** toward P-388 (mouse lymphocytic leukemia), A549 (human lung adenocarcinoma), and HT-29 (human colon adenocarcinoma) cancer cell lines is also reported.



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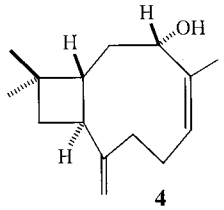

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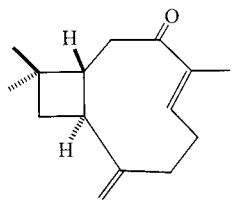

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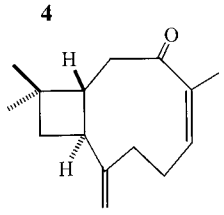

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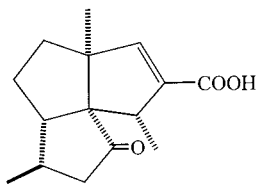

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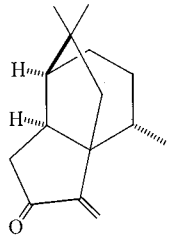

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Results and Discussion

The gorgonian *S. suberosa* was frozen immediately after collection and subsequently freeze-dried. The freeze-dried organism was extracted with ethyl acetate to afford a crude extract, which was subsequently separated by extensive column chromatography on silica gel and afforded sesquiterpenes **1**−**6** (Experimental Section).

Suberosol A (**1**) was isolated as a colorless oil, $[\alpha]^{29}_D$ −17.4° (*c* 0.13, $CHCl_3$). Its molecular formula, $C_{15}H_{24}O_2$, was established by HREIMS (*m*/*z* 236.1776, $[M]^+$) and ^{1}H and ^{13}C NMR spectral data. Thus, four degrees of unsaturation were determined for compound **1**. The mass spectrum of **1** exhibited a peak at *m*/*z* 218 $[M - H_2O]^+$, suggesting the presence of a hydroxy group in **1**. The ^{1}H NMR (Table 1) spectrum of **1** showed three tertiary methyl groups (δ 1.23, 1.03 and 1.00, 3H, s each), two methine protons (δ 1.69, 1H, t, J = 10.5 Hz, and 2.65, 1H, q, J = 9.5 Hz), two oxymethine protons (δ 3.16, 1H, q, J = 5.5 Hz, and 2.87, 1H, dd, J = 4.5, 10.5 Hz), and one olefinic exomethylene group (δ 4.99, 1H, s, and 4.88, each 1H, s). The ^{13}C NMR spectrum of **1** showed the presence of 15 carbons, as shown in Table 2. The DEPT spectrum of **1** exhibited three methyl, five methylene, and four methine signals. The remaining three signals in the broad-band spectrum were attributed to the quaternary carbon atoms. Two olefinic carbons (δ 113.2, t; 151.3, s) and three oxygen-bearing carbons (δ 61.4, d; 62.3, s; 79.2, d) were further identified. On the basis of the above observation, together with the molecular formula, suberosol A was suggested to

* To whom correspondence should be addressed. Tel: 886-7-5252000, ext. 5030. Fax: 886-7-5255020. E-mail: sheu@mail.nsysu.edu.tw.
[†] National Sun-Yat-Sen University.
[‡] Mansoura University.

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Table 1. ^{1}H NMR Chemical Shifts of Compounds **1**−**4**

	1[a]	**2**[b]	**3**[b]	**4**[b]
H-1	1.69 t (10.5)[c]	2.09 t (10.0)	1.60 m	1.67 t (10.4)
H-2	1.54 dd (8, 14.0)	1.69 m	1.57 d m	1.55 m
	1.81 dd (5.5, 13.5)	1.87 ddd (1, 3.3, 15.3)	1.66 m	1.55 m
H-3	3.16 q (5.5)	3.88 t (3)	4.09 dd (5.3, 9.9)	4.66 dd (4.6, 10.6)
H-5	2.87 dd (4.5, 10.5)	3.35 dd (3.9, 11.1)	5.44 br dd (5.0, 8.8)	5.40 t (8.0)
H-6	1.40 m	1.30 m	2.05 m	2.19 m
	2.26 m	2.26 m	2.46 m	2.29 m
H-7	2.15 m	2.15 m	2.05 m	2.09 m
	2.33 m	2.29 m	2.20 m	2.29 m
H-9	2.65 q (9.5)	2.64 q (8.5)	2.38 q (10.5)	2.56 q (8.9)
H-10	1.60 m	1.57−1.69 m	1.57 m	1.51 t (10.5)
	1.72 t (9.0)		1.66 m	1.74 dd (8.5, 10.6)
12-Me	1.00 3H, s	1.00 3H, s	0.97 3H, s	1.00 3H, s
13-Me	1.03 3H, s	1.02 3H, s	0.99 3H, s	1.04 3H, s
14-Me	1.23 3H, s	1.22 3H, s	1.64 3H, s	1.65 3H, s
H-15	4.88 s	4.84 s	4.84 s	4.74 s
	4.99 s	4.95 s	4.95 s	4.84 s

[a] Spectra recorded at 500 MHz in $CDCl_3$ at 25 °C. [b] 300 MHz in $CDCl_3$ at 25 °C. [c] The *J* values are in Hz in parentheses.

Table 2. ^{13}C NMR Chemical Shifts of Compounds **1**−**4**

	1[a]	**2**[b]	**3**[b]	**4**[b]
C-1	47.3 (d)[c]	43.2 (d)	51.0 (d)	47.4 (d)
C-2	35.3 (t)	33.6 (t)	37.7 (t)	34.3 (t)
C-3	79.2 (d)	69.0 (d)	78.7 (d)	68.0 (d)
C-4	62.3 (s)	61.5 (s)	137.2 (s)	137.4 (s)
C-5	61.4 (d)	57.9 (d)	123.6 (d)	126.8 (d)
C-6	29.3 (t)	29.7 (t)	27.8 (t)	28.4 (t)
C-7	29.6 (t)	29.1 (t)	34.3 (t)	34.0 (t)
C-8	151.3 (s)	151.5 (s)	154.2 (s)	155.3 (s)
C-9	47.6 (d)	48.7 (d)	47.1 (d)	40.9 (d)
C-10	39.8 (t)	40.1 (t)	40.1 (t)	40.5 (t)
C-11	34.0 (s)	34.2 (s)	33.0 (s)	33.5 (s)
C-12	29.8 (q)	29.6 (q)	30.0 (q)	29.9 (q)
C-13	21.7 (q)	21.7 (q)	22.8 (q)	23.0 (q)
C-14	11.4 (q)	16.4 (q)	10.8 (q)	16.3 (q)
C-15	113.2 (t)	113.2 (t)	112.2 (t)	113.4 (t)

[a] Spectra recorded at 125 MHz in $CDCl_3$ at 25 °C. [b]75 MHz in $CDCl_3$ at 25 °C. [c]Multiplicity deduced by DEPT and indicated by usual symbols. The values are in ppm downfield from TMS.

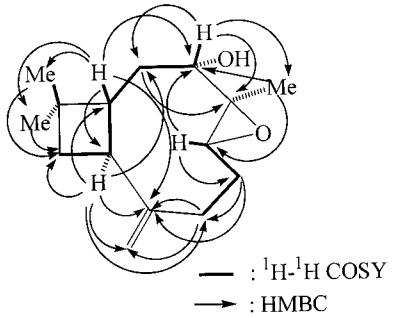

Figure 1. ^{1}H−^{1}H COSY and key HMBC correlations for **1**.

be a bicyclic sesquiterpene possessing an exomethylene-containing carbon−carbon double bond, a hydroxy-bearing methine, and a trisubstituted epoxide. By ^{1}H−^{1}H COSY, it was possible to establish two partial structures (Figure 1). Furthermore, the HMBC spectrum showed key correlations (Figure 1) of H-1 to C-3, C-8, C-9, C-11, C-12, and C-13; H_2-2 to C-3, C-4, and C-11; H-3 to C-2, C-4, and C-14; H-5 to C-3 and C-6; H_2-6 to C-7 and C-8; H_2-7 to C-8, C-9, and C-15; H-9 to C-1, C-2, C-7, C-8, C-10, and C-15; both H_3-12 and H_3-13 to C-1 and C-10, and H_3-14 to C-3 and C-5, successfully establishing the molecular framework of **1**. Thus, **1** was suggested to be a 4,5-epoxy-3-hydroxycaryophyllene.

The relative stereochemistry of **1** was disclosed by the key NOESY correlations as shown in Figure 2. It was found that H-1 showed NOE interactions with H-5 and H_3-12,

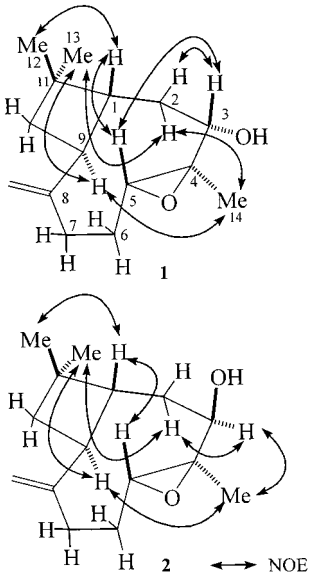

Figure 2. Key NOESY correlations of **1** and **2**.

but not with H-9, H_3-13, and H_3-14, and H-3 showed NOE responses with H-5, but not with H_3-14, indicating that H-1, H-3, H-5, and H_3-12 are situated on the same face and H-9, H_3-13, and H_3-14 should be positioned on the other face. This could be further confirmed, as H-9 exhibited NOE interactions with both H_3-13 and H_3-14. Based on the above analyses, the structure of suberosol A was established as (1*R**,3*S**,4*S**,5*R**,9*S**)-4,5-epoxy-3-hydroxy-β-caryophyllene, as described by formula **1**.

Suberosol B (**2**) was isolated as a colorless oil, $[\alpha]^{29}_D$ −10.7° (*c* 0.24, $CHCl_3$). On the basis of its HRFABMS (*m*/*z* 237.1855, $[M + H]^+$) and the ^{13}C NMR data, the molecular formula of **2** was established as $C_{15}H_{24}O_2$. Inspection of the ^{13}C NMR spectral data (Table 2) for compound **2**, including a DEPT spectrum, revealed the presence of three methyl carbons (δ 16.4, 21.7, and 29.6), four sp^3 methylene carbons (δ 33.6, 29.7, 29.1, and 40.1), one olefinic exomethylene carbon (δ 113.2), and two methine and two oxygenated methine carbons (δ 43.2, 48.7, 57.9, and 69.0, respectively). The remaining three carbon signals derived from one olefinic quaternary carbon (δ 151.5) and two sp^3 quaternary carbons (δ 34.2 and 61.5). It was found that ^{1}H and ^{13}C NMR spectral data of **2** were very similar to those of suberosol A (**1**), suggesting that **2** could be the stereoisomer of **1**. By the assistance of 2D NMR spectra, including COSY, HMQC, and HMBC, **2** was shown to possess the same

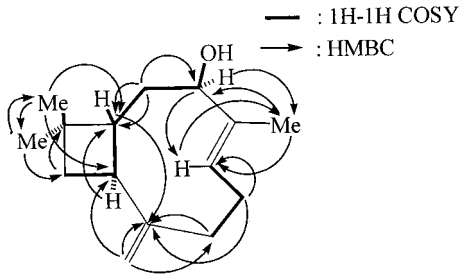

Figure 3. $^1H-^1H$ COSY and key HMBC correlations for **3**.

molecular framework as that of **1**. However, the significant downfield shifts for H-3 ($\Delta\delta$ +0.72 ppm), H-5 ($\Delta\delta$ +0.48 ppm), H-1 ($\Delta\delta$ +0.40 ppm), and C-14 ($\Delta\delta$ +5.0) and upfield shifts for C-3 ($\Delta\delta$ −10.2 ppm), C-1 ($\Delta\delta$ −4.1 ppm), and C-5 ($\Delta\delta$ −3.5 ppm) of **2**, in comparison with those of **1**, suggested that **2** could be the C-3 epimer of **1**. By careful study of the NOESY spectrum of **2** (Figure 3), it was found that H-1 showed NOE interactions with both H-5 and H_3-12 but not with H-3, H-9, and H_3-14, and H-3 exhibited interactions with H-2α (δ 1.82) and H_3-14 but not with H-1 and H-5. Thus, compound **2** was described as the C-3 epimer of **1**, and the structure of suberosol B (**2**) was identified as (1*R**,3*R**,4*S**,5*R**,9*S**)-4,5-epoxy-3-hydroxy-*β*-caryophyllene.

Suberosol C (**3**) was obtained as a colorless oil, $[\alpha]^{29}_D$ −67.9° (*c* 0.14, $CHCl_3$). According to the HREIMS (*m*/*z* 220.1821, $[M]^+$) and ^{13}C NMR data, its molecular formula was established as $C_{15}H_{24}O$. Thus, four degrees of unsaturation were determined for **3**. The EIMS of **3** exhibited a peak at *m*/*z* 202 $[M - H_2O]^+$, indicating the presence of a hydroxy group in **3**. The ^1H NMR spectrum of compound **3** (Table 1) showed signals of two methyls (δ 0.97, 3H, s; 0.99, 3H, s) and an olefinic exomethylene (δ 4.84, 1H, s; 4.95, 1H, s) group. In addition, a methyl-bearing trisubstituted carbon−carbon double bond could be further identified by the proton resonances at δ 1.64 (3H, s) and 5.44 (1H, dd, J = 5.0, 8.8 Hz). A signal appearing at δ 4.09 (1H, dd, J = 5.3, 9.9 Hz) was attributed to a hydroxy-bearing methine proton. The ^{13}C NMR spectral data (Table 2), assigned by the assistance of a DEPT spectrum, revealed the presence of three methyl (δ 10.8, 22.8, and 30.0), four sp^3 methylene (δ 27.8, 34.3, 37.7, and 40.1), one sp^2 methylene (δ 112.2), one sp^3 oxygenated methine (δ 78.7), one sp^2 methine (δ 123.6), and two normal sp^3 methine (δ 47.1 and 51.0) carbons. The remaining three carbon signals were a sp^3 quaternary (δ 33.0) carbon and two olefinic (δ 137.2 and 154.2) carbons. On the basis of the above results and by comparing the molecular formula and spectral data of **1**, it was suggested that **3** is the 4,5-deoxygenated product of **1**. These findings, together with the connectivities observed in the $^1H-^1H$ COSY and HMBC spectra (Figure 3), established the *β*-caryophyllene-based molecular skeleton of suberosol C (**3**).

The relative stereochemistry of **3** was tentatively assigned through the inspection of the available NOE correlations (Figure 5) and other steric considerations. The *E* geometry of the 4,5-endocyclic double bond in **3** was established by the lack of NOE correlation between the methyl protons attached at C-4 (δ 1.64) and H-5 (δ 5.44) and the chemical shift of C-14 (δ 10.8). It is worthwhile to mention that the abnormal upfield-shifted δ_C observed for that of C-14 in **3**, as in the case of **1**, can be explained by the strong γ-effect arising from the steric compression of a *gauche* interaction between the methyl group attached at C-4 and the hydroxy group attached at C-3. The above observation, together with the NOE correlations observed

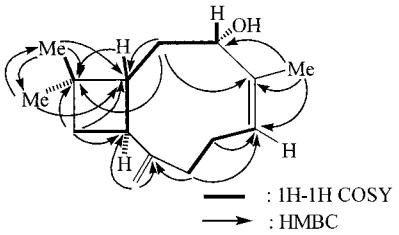

Figure 4. $^1H-^1H$ COSY and key HMBC correlations for **4**.

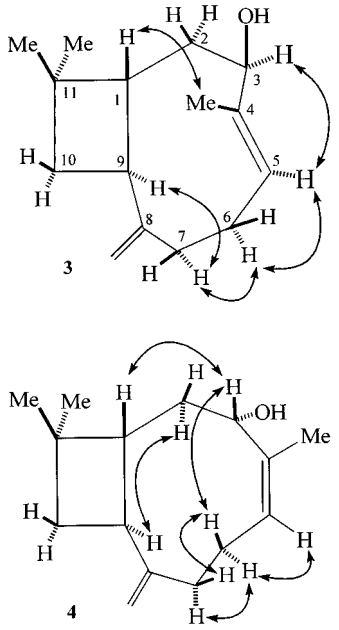

Figure 5. Distinguishing NOESY correlations of **3** and **4**.

between H-3 and H-5, H-5 and H-6α, H-6α and H-7α, and H-7 α and H-9 (see Figure 5), indicated that the structure of suberosol C should be established as (1*R**,3*R**,9*S**)-3-hydroxy-*β*-caryophyllene (**3**).

Suberosol D (**4**) was isolated as a colorless oil, $[\alpha]^{29}_D$ +2.5° (*c* 0.045, $CHCl_3$). The EIMS established a molecular formula of $C_{15}H_{24}O$ (*m*/*z* 220) for this metabolite; thus **4** was an isomer of **3**. Similar to those of **3**, the ^1H and ^{13}C NMR data of **4** (Tables 1 and 2) revealed the presence of three methyl, four sp^3 methylene, and three sp^3 methine (including one oxygenated) groups and four olefinic carbons attributed to a 1,1-disubstitued and a trisubstituted double bond. These findings, together with the $^1H-^1H$ COSY and HMBC correlations of **4** (Figure 4), revealed that **4** had a molecular framework similar to that of **3**. Despite the above findings, the NMR data (Tables 1 and 2) of **4** showed significant differences in comparison with those of **3**. For example, a significant downfield shift for H-3 ($\Delta\delta$ +0.57 ppm) and an upfield shift for C-3 ($\Delta\delta$ −10.7 ppm), a marked downfield shift for H-9 ($\Delta\delta$ +0.18 ppm) and an upfield shift for C-9 ($\Delta\delta$ −6.2 ppm), and a downfield shift for C-14 ($\Delta\delta$ +5.5 ppm) of **4** were shown when these data were compared with the corresponding chemical shifts of **3**.

By NOESY, it was found that **4** showed an NOE correlation between H-1 and H-3, proving the α-configuration of the 3-hydroxy group. No NOE response between H-1 and H-9 could be found, indicating the probable α-orientation of H-9. H_3-14 showed weak NOE interaction with H-5, suggesting the *cis*-geometry of a 4,5-double bond, which could be further supported by the downfield shift of C-14 of **4** in comparison with that of **3**. On the basis of the above findings and other key NOE interactions (Figure 5), the structure of suberosol D (**4**) was established as

Table 3. Cytotoxicity of Sesquiterpenes **1**−**6**[a]

compound	cell lines ED_{50} (μg/mL)		
	P-388	A549	HT-29
1	3.8	>50	>50
2	7.4	>50	>50
3	2.1	5.6	2.3
4	3.3	4.2	3.8
5	4.6	3.8	3.6
6	6.3	8.9	6.6

[a] For significant activity of pure compounds, an ED_{50} value of ≤4.0 μg/mL is required. See Geran et al.[23]

(1*R**,3*S**,9*S**)-3-hydroxy-β-caryophyllene as demonstrated by formula **4**.

The isolated less polar compounds **5** and **6** were found to be identical with the known buddledins C and D, respectively, according to the previously published MS and ^{1}H and ^{13}C NMR data.[10,16]

The cytotoxicity of metabolites **1**−**6** against the growth of P-388, A549, and HT-29 cancer cells was studied, and the results are shown in Table 3. These data revealed that metabolites **1**, **3**, and **4** exhibited significant cytotoxicity against P-388 cancer cells. Compound **5** exhibited significant cytotoxicity toward A549 cancer cells. Compounds **3**−**5** were found to exhibit significant activity against the growth of HT-29 cells.

Although the caryophyllene-based sesquiterpenes are known to be widespread in terrestrial plants[10,16−20] and less frequently in higher fungi,[21,22] it is worthwhile to mention that this is the first report of the isolation of new sesquiterpenes of this type from marine organisms.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Jasco DIP-1000 digital polarimeter. IR spectra were recorded on a Hitachi I-2001 infrared spectrophotometer. EIMS and FABMS were obtained with a VG Quattro GC/MS spectrometer. The NMR spectra were recorded on a Bruker AMX-300/5 FT-NMR at 300 MHz for ^{1}H and 75 MHz for ^{13}C or on a Varian Unity INOVA 500 FT-NMR at 500 MHz for ^{1}H and 125 MHz for ^{13}C, respectively, in $CDCl_3$ using TMS as internal standard. Si gel (Merck, 230−400 mesh) was used for column chromatography. Precoated Si gel plates (Merck, Kieselgel 60 F-254, 0.2 mm) were used for analytical TLC.

Organism. *Subergorgia suberosa* was collected by hand via scuba on the coast of Green Island, Taiwan, in July 1998, at a depth of 10−15 m, and stored in a freezer until extraction. A voucher sample was deposited at the Department of Marine Resources, Sun Yat-Sen University (specimen no. GISC-103).

Extraction and Isolation. The organism *S. suberosa* (1.4 kg, wet wt) was freeze-dried and then exhaustively extracted with EtOAc. The EtOAc extract was filtered and concentrated under vacuum to provide a brownish semisolid crude extract (24.8 g). The extract was subjected to column chromatography on Si gel 60. Elution was performed with EtOAc−*n*-hexane (stepwise, 0−100% EtOAc) to yield 18 fractions. Fraction 5 eluted with 5% EtOAc and was further chromatographed on Si gel 60 using a EtOAc−*n*-hexane gradient to yield **5** (6.0 mg) and **6** (25.5 mg). Fraction 6 eluted with 10% EtOAc was further chromatographed on Si gel 60 using a EtOAc−*n*-hexane (1:10 to 1:2) gradient to yield **1** (2.6 mg) and **2** (2.8 mg). Fraction 7 eluted with 20% EtOAc and was further chromatographed on Si gel 60 by HPLC using EtOAc−CH_2Cl_2 (1:20) to yield **3** (3.1 mg) and **4** (2.8 mg).

Suberosol A (1): colorless oil; $[\alpha]^{29}_D$ −17.4° (*c* 0.13, $CHCl_3$); IR (neat) ν_{max} 3435, 1643, 1454, 1385, 1370, 1044 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Tables 1 and 2, respectively; EIMS (70 eV) *m*/*z* 236 [1.0, $(M)^+$], 221 [2.5, $(M - Me)^+$], 218 [0.7, $(M - H_2O)^+$], 203 [1.6, $(M - Me - H_2O)^+$], 185 [2.2, $(M - Me - 2H_2O)^+$]; HREIMS *m*/*z* 236.1776 (calcd for $C_{15}H_{24}O_2$, 236.1777).

Suberosol B (2): colorless oil; $[\alpha]^{29}_D$ −10.7° (*c* 0.24, $CHCl_3$); IR (neat) ν_{max} 3445, 1630, 1456, 1383, 1368, 1117, 1074, 1055 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz), see Tables 1 and 2, respectively; FABMS *m*/*z* 237 [0.6, $(M + H)^+$], 219 [1.0, $(M + H - H_2O)^+$], 204 [0.6, $(M + H - Me - H_2O)^+$]; HRFABMS *m*/*z* 237.1855 (calcd for $C_{15}H_{24}O_2$ + H, 237.1856).

Suberosol C (3): colorless oil; $[\alpha]^{29}_D$ −67.9° (*c* 0.14, $CHCl_3$); IR (neat) ν_{max} 3433, 1633, 1456, 1385, 1370, 1044 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz), see Tables 1 and 2, respectively; EIMS (70 eV) *m*/*z* 220 [1.2, $(M)^+$], 205 [4.1, $(M - Me)^+$], 202 [3.1, $(M - H_2O)^+$], 187 [6.6, $(M - Me - H_2O)^+$]; HREIMS *m*/*z* 220.1821 (calcd for $C_{15}H_{24}O$, 220.1828).

Suberosol D (4): colorless oil; $[\alpha]^{29}_D$ +2.5° (*c* 0.045, $CHCl_3$); IR (neat) ν_{max} 3420, 1630, 1456, 1375, 1028 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz), see Tables 1 and 2, respectively; EIMS (70 eV) *m*/*z* 220 (0.8, $[M]^+$), 205 [3.2, $(M - Me)^+$], 202 [3.3, $(M - H_2O)^+$], 187 [4.5, $(M - Me - H_2O)^+$].

Buddledin C (5): colorless oil; $[\alpha]^{29}_D$ −300° (*c* 1.28, $CHCl_3$) (lit.,[10] −316°); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.32 (1H, ddd, *J* = 9.9, 8.0, 1.5 Hz, H-5), 4.95, 4.90 (2H, s each, H-15), 2.95 (1H, dd, *J* = 14.5, 12.1 Hz, H-2), 2.64 (1H, dt, *J* = 12.0, 4.2 Hz, H-7), 2.44 (2H, m, H-6), 2.43 (1H, ddd, *J* = 10.4, 9.8, 8.3 Hz, H-9), 2.29 (1H, dd, *J* = 14.5, 1.6 Hz, H-2), 2.24 (1H, m, H-7), 1.83 (1H, dd, *J* = 10.4, 8.3 Hz, H-10), 1.65 (1H, m, H-1); 1.65 (3H, s, H-14), 1.57 (1H, t, *J* = 10.4 Hz, H-10), 1.01 (6H, s, H-12, 13); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 206.8 (C-3), 153.2 (C-8), 143.7 (C-5), 136.4 (C-4), 111.7 (C-15), 55.6 (C-1), 47.5 (C-9), 45.0 (C-2), 41.0 (C-7), 40.5 (C-10), 33.3 (C-11), 30.9 (C-6), 29.3 (C-12), 21.9 (C-13), 13.1 (C-14); EIMS (70 eV) *m*/*z* 218 [0.3, $(M)^+$], 203 [0.4, $(M - Me)^+$]. The above data were found to be in full agreement with those reported previously.[10]

Buddledin D (6): colorless oil; $[\alpha]^{29}_D$ −152° (*c* 1.28, $CHCl_3$) (lit.,[10] −164°); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.53 (1H, ddd, *J* = 12.1, 5.3, 1.4 Hz, H-5), 4.89, 4.83 (2H, s each, H-15), 2.76 (1H, dd, *J* = 18.4, 10.9 Hz, H-2), 2.56 (1H, dd, *J* = 18.4, 1.7 Hz, H-2), 2.47 (1H, m, H-9), 2.46 (1H, m, H-6), 2.13 (1H, m, H-6), 2.32 (2H, m, H-7), 1.87 (1H, dd, *J* = 10.4, 3.2 Hz, H-10), 1.79 (1H, m, H-1), 1.79 (3H, m, H-14), 1.66 (1H, dd, *J* = 10.4, 8.0 Hz, H-10), 1.03 (3H, s, H-13), 1.01 (3H, s, H-12); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 209.3 (C-3), 151.0 (C-8), 137.4 (C-4), 132.4 (C-5), 110.1 (C-15), 50.2 (C-1), 46.0 (C-2), 42.6 (C-9), 38.0 (C-7), 36.7 (C-10), 34.0 (C-11), 29.8 (C-12), 27.0 (C-6), 22.5 (C-13), 20.8 (C-14); EIMS (70 eV) *m*/*z* 218 [1.0, $(M)^+$], 203 [1.0, $(M - Me)^+$]. The above data were found to be in full agreement with those reported previously.[10]

Cytoxicity Testing. P-388 cells were kindly provided by Prof. J. M. Pezzuto, University of Illinois at Chicago. A549 and HT-29 cells were purchased from the American Type Culture collection. The cytotoxic activities of tested compounds against the above three cancer cells were assayed with a modification of the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric methods.[24] Cytotoxicity assays were carried out according to the procedure described previously.[25]

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