New Metabolites from the Sponge *Spongia agaricina*

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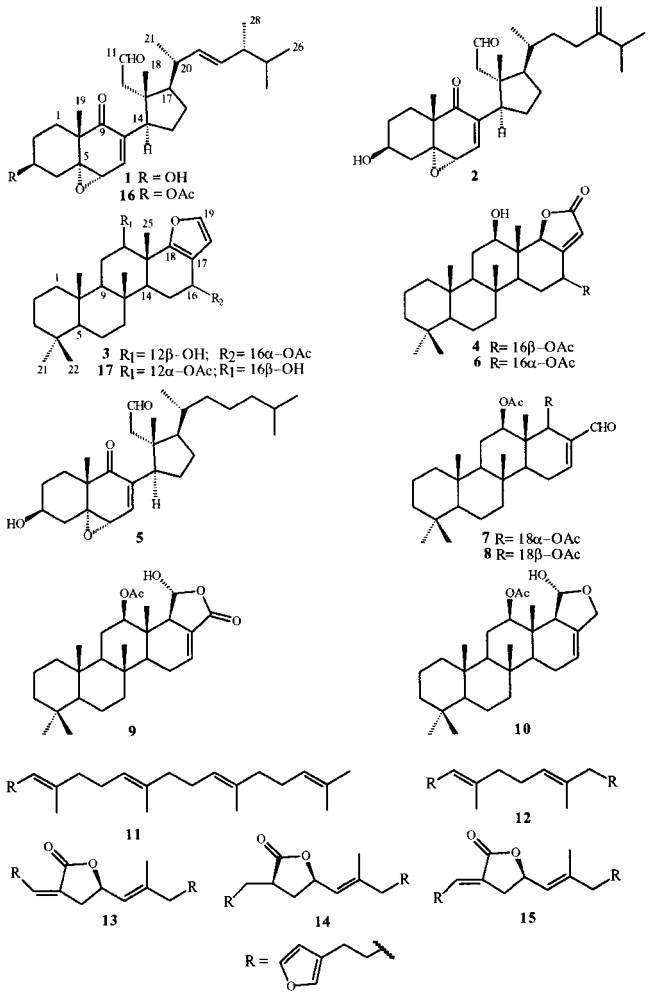
The sponge *Spongia agaricina* from Tarifa, Cádiz, Spain, contains two new 9,11-secosterols, [3-*O*-deacetylluffasterol B (**1**) and 3-*O*-deacetyl-22,23-dihydro-24,28-dehydroluffasterol B (**2**)] and two new sesterterpenoids [12,16-di-*epi*-12-*O*-deacetyl-16-*O*-acetylfuroscalarol (**3**) and 16-*epi*-scalarolbutenolide (**4**)], in addition to the known compounds **5**−**15**. The structures of all compounds were elucidated by interpretation of spectroscopic data. The metabolites **1**−**3** showed significant cytotoxicity against four tumor cell lines (IC_{50} 1 μg/mL).

Marine sponges of the genus *Spongia* have been extensively studied and have given rise to a great array of structurally diverse metabolites.[2] Most of the compounds isolated from this genus, however, are mevalonate-derived metabolites, suggesting a biosynthetic origin rather than a symbiotic source. Recent examples include the chemical study of *S. matamata*[3] and *S. officinalis*.[4]

Among the mevalonate-derived metabolites isolated from sponges of the genus *Spongia*, the most recently described are 9,11-secosterols.[2,3] This type of compounds has also been isolated from other Dictyoceratida and Dendroceratida sponges and from soft corals.[2,5] The 9,11-secosterols from *Spongia* sponges are structurally characterized by a *trans*-decalin system containing a 3-hydroxyl or acetoxyl and a double bond at C-7, C-8. Differences reside in the side chain, the functionalization at C-11 (either formyl or hydroxymethyl), and the oxygenated functions at C-5 and C-6 (either hydroxyl or an epoxide bridge). Other main structural types shown by marine 9,11-secosterols include 3,11-dihydroxysecosteroids possessing a C-5,C-6 double bond or additional hydroxyl groups at C-2, C-4, C-6, and C-19 and a minor group formed by those containing a *cis*-decalin system hydroxylated at C-2 and C-6.

In the course of our investigations directed toward the search for bioactive compounds from marine sources, we obtained specimens of the sponge *Spongia agaricina* Pallas (Spongiidae) collected near Tarifa Island (Cádiz, Spain). Our specimens afforded two new 9,11-secosterols (**1**, **2**) and two new sesterterpenes (**3**, **4**), together with a known 9,11-secosterol (**5**),[3] the five known tetracarbocyclic sesterterpenoids scalarolbutenolide (**6**),[6] 12,18-di-*epi*-scalaradial (**7**),[7] 12-*epi*-scalaradial (**8**),[7] 12-*epi*-scalarin (**9**),[8] and 12-*epi*-deoxoscalarin (**10**),[8] and the five known furanoterpenes furospinosulin-1 (**11**),[9] anhydrofurospongin-1 (**12**),[10] nitenin (**13**),[11,12] dihydronitenin (**14**),[11−13] and isonitenin (**15**).[13] Nitenin (**13**) and dihydronitenin (**14**) had been previously isolated from two different collections of *S. agaricina* one from the Bay of Naples (Italy)[14] and the other from off Blanes (northeastern Spain),[12] respectively.

Specimens of *S. agaricina* were collected by hand using scuba and were immediately frozen. The medium polar material of a Me_2CO extract was chromatographed on Si gel. Further purification using both normal and reversed-phase HPLC allowed isolation of the following compounds: 3-*O*-deacetylluffasterol B (**1**, 0.0006% dry wt); 3-*O*-deacetyl-22,23-dihydro-24,28-dehydroluffasterol B (**2**, 0.0003% dry wt); 12,16-di-*epi*-12-*O*-deacetyl-16-*O*-acetylfuroscalarol (**3**, 0.0008% dry wt); 16-*epi*-scalarolbutenolide (**4**, 0.0005% dry wt), together with the 11 known compounds mentioned above (**5**−**15**).



3-*O*-Deacetylluffasterol B (**1**) was isolated as an

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amorphous solid. The molecular formula, $C_{28}H_{42}O_4$, was obtained from the HREIMS measurement. The IR absorption at 3420 cm^{-1}, together with the ^{1}H-NMR signal at 3.98 (1H, m) and the ^{13}C-NMR signal at δ 68.3 (d), indicated that **1** was a secondary alcohol. The ^{1}H-NMR signal at δ 6.84 (1H, dd, J = 4.6, 1.0 Hz) and the ^{13}C-NMR signals at δ 200.6 (s), 140.5 (s), and 139.7 (d) were due to an α-substituted α,β-unsaturated ketone. The signal at δ 6.84 was coupled with a signal at δ 3.40 (1H, d, J = 4.6 Hz) that was assigned to an epoxide proton, indicating the presence of an epoxide ring in γ, δ to the unsaturated ketone. These structural features, together with the general analysis of the ^{1}H- and ^{13}C-NMR data, suggested that compound **1** was a 9,11-secosterol. In particular, comparison of ^{1}H-NMR spectroscopic data of the alcohol **1** with those of luffasterol B (**16**)[5] indicated that **1** was the corresponding deacetyl derivative of **16**. Furthermore, acetylation of **1** with Ac_2O in pyridine afforded a compound identical in all respects to luffasterol B (**16**).

3-*O*-Deacetyl-22,23-dihydro-24,28-dehydroluffasterol B (**2**) was obtained as a white solid. The molecular formula $C_{28}H_{42}O_4$, derived from HREIMS data, indicated that **2** was an isomer of 3-*O*-deacetylluffasterol B (**1**). Analysis of ^{1}H-NMR, ^{13}C-NMR, and COSY spectra revealed that the tetracyclic system of **2** was identical to that of compounds of the luffasterol series. The main difference observed in the ^{1}H-NMR spectrum of **2**, upon comparison with its isomer **1**, was the absence of the methyl proton signal at δ 0.91 (3H, d, J = 7.0 Hz) and the olefinic proton signals at 5.24 (1H, dd, J = 17.6, 7.4 Hz) and 5.20 (1H, dd, J = 17.6, 7.4 Hz); these signals were replaced by two exomethylene proton signals at 4.73 (1H, br s) and 4.65 (1H, d, J = 1.5 Hz), attributable to a C-24,C-28 double bond. Structure **2** was therefore proposed for 3-*O*-deacetyl-22,23-dihydro-24,28-dehydroluffasterol B.

It has been proposed that 9,11-secosterols isolated from *Spongia officinalis* possessing a C-7,C-8 double bond, hydroxyl functions at C-5 and/or C-6, and a formyl group at C-11 are biogenetically derived from a 5,7,9-(11)-triene sterol through oxidation at C-5 and/or C-6 and concomitant oxidative cleavage of the C-9,C-11 double bond.[15] The same biogenetic origin may be proposed for compounds **1**, **2**, and **5** from *S. agaricina*, which might arise from 5,7,9(11)-triene sterols by epoxidation of the C-5,C-6 double bond and oxidative cleavage.

12,16-Di-*epi*-12-*O*-deacetyl-16-*O*-acetylfuroscalarol (**3**) was isolated as a colorless oil. The molecular formula $C_{27}H_{40}O_4$ was obtained from the HREIMS. The IR absorption at 3450 cm^{-1} and the ^{13}C-NMR signal at 77.1 (d) were consistent with the presence of a secondary hydroxyl group. The ^{1}H-NMR signal at δ 2.05 (3H, s) and the ^{13}C-NMR signals at δ 170.8 (s) and 21.4 (q) clearly indicated the presence of an acetoxyl group in the molecule. The remaining 25 carbon atoms were assigned to a pentacyclic sesterterpene of the furoscalarol family upon observation of the ^{1}H-NMR singlets at 1.17 (3H, s), 0.91 (3H, s), 0.87 (3H, s), 0.86 (3H, s), and 0.82 (3H, s) and the furan proton signals at δ 7.24 (1H, d, J = 2.0 Hz) and 6.32 (1H, d, J = 2.0 Hz). A comparison of the spectroscopic data of compound **3** with those reported for furoscalarol (**17**)[16,17] revealed significant differences on the signals of the protons geminal to the oxygenated functionalities. Thus, the ^{1}H-NMR signal at δ 5.73 (1H, dd, J = 4.1, and 1.9 Hz) was assigned to an equatorial proton geminal to the acetoxyl group adjacent to the furan ring, and the signal at δ 3.82 (1H, dd, J = 11.2 and 4.1 Hz) was assigned to an axial proton geminal to the hydroxyl group at C-12. It was concluded that compound **3** was 12,16-di-*epi*-12-*O*-deacetyl-16-*O*-acetylfuroscalarol. Thus, compound **3** presents identical functionalization and stereochemistry at C-12 and C-16 as does the co-metabolite scalarolbutenolide (**6**).

16-*epi*-Scalarolbutenolide (**4**) was isolated as an amorphous powder. The molecular formula $C_{27}H_{40}O_5$ was derived from HREIMS data. The ^{1}H NMR methyl singlets at δ 0.87, 0.85, 0.84, 0.80, and 0.73, together with the ^{13}C-NMR quartets at 33.2, 21.3, 17.3, 16.4, and 7.0, indicated that **4** was a scalarane sesterterpene. The IR absorption at 1755 and 1656 cm^{-1}, along with the ^{13}C-NMR signals at δ 169.9 (s), 166.5 (s), 112.3 (d), and 89.8 (d), were diagnostic of an α,β-unsaturated γ-lactone of the scalarolbutenolide type. A comparison with the data reported[6] for its isomer scalarolbutenolide (**6**) revealed that compound **4** had the same functionality and stereochemistry at C-12 upon observation of the H-12α signal at δ 3.70 (1H, dd, J = 11.1, 4.5 Hz); however, the acetoxyl geminal proton signal at δ 5.55 (1H, ddd, J = 9.4, 7.3, 2.1 Hz) indicated an axial orientation for the H-16 proton and therefore a configuration at C-16 opposite to that of scalarolbutenolide (**6**). It was concluded that compound **4** was 16-*epi*-scalarolbutenolide.

The new compounds **1**−**4** isolated from *S. agaricina* showed cytotoxicity against P-388 mouse lymphoma, A-549 human lung carcinoma, HT-29 human colon carcinoma, and MEL-28 human melanoma tumor cell lines with IC_{50} values of 1μg/mL in all cases, with exception of butenolide **4**, which showed a weaker activity (IC_{50} 5 μg/mL).

Experimental Section

General Experimental Procedures. IR spectra were recorded on a Perkin−Elmer 881 spectrophotometer. Optical rotations were measured on a Perkin−Elmer 241 polarimeter. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Varian 400 at 399.952 and 100.577 MHz, respectively, using $CDCl_3$ as solvent. The resonances of residual $CHCl_3$ at δ_H 7.26 and of $CDCl_3$ at δ_C 77.0 were used as internal reference for ^{1}H-NMR and ^{13}C-NMR spectra, respectively. Values with the same superscript in the same compound may be interchanged. Mass spectra were measured on a VG 12250 or on a Kratos MS 80RFA spectrometer. In HPLC separations, LiChrosorb Si-60 was used in normal-phase mode and LiChrosorb RP-18 was used in reversed-phase mode, using a differential refractometer in both cases. All solvents were distilled from glass prior to use.

Collection, Extraction, and Isolation Procedures. Specimens of *Spongia agaricina* (528 g dry wt) were collected by hand using scuba near Tarifa Island and immediately frozen. The frozen tissue was extracted exhaustively with Me_2CO at room temperature. The filtered Me_2CO solution was evaporated under reduced pressure, and the aqueous residue was ex-

Table 1. Selected ^{1}H-NMR Data for Compounds 1−4,[a,b] 6,[b,c] and 16[d]

C no.	1	2	3	4	6	16
	δ_H, mult, J(Hz)	δ_H, mult, J(Hz)	δ_H, mult, J (Hz)	δ_H, mult, J(Hz)	δ_H, mult, J(Hz)	δ_H, mult, J(Hz)
2	2.09 *m*, 1.68 *m*	2.13 *m*, 1.68 *m*				
3	3.98 *m*	3.98 *m*				4.96 *m*
4	2.18 *m*, 1.56 *m*	2.18 *m*, 1.54 *m*				2.22 *t* (12), 1.62 *m*
6	3.40 *d* (4.6)	3.40 *d* (4.5)				3.38 *d* (4.5)
7	6.84 *dd* (4.6, 1.0)	5.86 *dd* (4.5, 1.1)				6.82 br *d* (4.5)
11	9.88 *dd* (3.8, 1.7)	9.88 *dd* (3.8, 1.5)				9.86 *dd* (4, 1.5)
12	2.27 *dd* (15.9, 3.8)	2.30 *dd* (16.2, 3.8)	3.82 *dd* (11.2,4.1)	3.70 *dd* (11.1, 4.5)	3.80 *dd* (10, 4)	2.23 *dd* (16, 4)
	2.00 *dd* (15.9, 1.7)	1.98 *dd* (16.2, 1.5)				1.97 *dd* (16,1.5)
14	3.51 *dd* (10.3, 9.2)	3.53 *dd* (10.3, 9.8)				3.48 br *t* (9.5)
15	1.78 *m*, 1.71 *m*	2.08 *m*, 1.73 *m*				
16			5.73 *dd* (4.1, 1.9)	5.55 *ddd* (9.4, 7.3, 2.1)	5.8 *m* (w/2 = 5)	
18	0.76 *s*	0.76 *s*		4.54 *d* (1.6)	4.76 br *s* (w/2 = 3)	0.73 *s*
19	1.21 *s*	1.21 *s*	7.24 *d* (2.0)			1.19 *s*
20	2.18 *m*	1.94 *m*	6.32 *d* (2.0)	5.84 *dd* (2.1, 1.7)	6.0 br *s* (w/2 = 3)	2.15 *m*
21	1.00 *d* (6.8)	0.97 *d* (6.8)	0.87* s	0.84† *s*	0.90‡ *s*	0.98 *d* (7)
22	5.20 *dd* (17.6,7.4)		0.82 *s*	0.80 *s*	0.90‡ *s*	5.19 *dd* (15, 5)
23	5.24 *dd* (17.6,7.4)		0.86* *s*	0.85† *s*	0.90‡ *s*	5.22 *dd* (15, 6)
24	1.87 *m*		0.91 *s*	0.87 *s*	0.84‡ *s*	1.82 *m*
25	1.47 *m*	2.21 *m*	1.17 *s*	0.73 *s*	0.68‡ *s*	
26	0.83 *d* (6.8)					0.81 *d* (7)
27	0.82 *d* (6.8)					0.79 *d* (7)
28	0.91 *d* (7.0)	4.73 br *s*, 4.65 *d* (1.5)				0.88 *d* (7)
OAc			2.05 *s*		2.1 *s*	2.02 *s*

[a] Assignments aided by COSY experiments. [b] Values with the same superscript in the same column may be interchanged. [c] Values reported by Cimino et al.[6] [d] Values reported by Reddy et al.[5]

tracted with Et_2O. The solvent was evaporated to give an oil residue (14.5 g) that was chromatographed on a Si gel column using solvents of increasing polarity from hexane to Et_2O and, subsequently, EtOAc. Fractions eluted with 10% ether in hexane were further chromatographed on Si gel, and selected fractions were subjected to reversed-phase HPLC separation eluting with MeOH−H_2O (96:4) to afford furospinosulin-1 (**11**, 16.5 mg, 0.0031% dry wt) and with MeOH−H_2O (9:1) to afford anhydrofurospongin-1 (**12**, 5.4 mg, 0,0010% dry wt). Fractions of the general chromatography eluted with 20% ether in hexane yielded nitenin (**13**, 2.96 g, 0.5606% dry wt) and dihydronitenin (**14**, 947 mg, 0.1794% dry wt). Fractions eluted with 30% ether in hexane were further separated by normal-phase HPLC eluting with He−EtOAc (88:12) to yield 12,16-di-*epi*-12-*O*-deacetyl-16-*O*-acetylfuroscalarol (**3**, 4.4 mg, 0.0008% dry wt) and isonitenin (**15**, 17 mg, 0.0032% dry wt). Fractions eluted with 50% ether in hexane afforded 12,-18-di-*epi*-scalaradial (**7**, 10.1 mg, 0.0019% dry wt) and 12-*epi*-scalaradial (**8**, 528.6 mg, 0.1001% dry wt). Fractions eluted with 70% ether in hexane were crystallized from hexane−EtOAc, yielding a mixture that was further separated by reversed-phase HPLC (MeOH−H_2O, 87:13) to obtain 12-*epi*-deoxoscalarin (**10**, 50 mg, 0.0095% dry wt) and scalarolbutenolide (**6**, 45 mg, 0.0085% dry wt). The mother liquors were purified by reversed-phase HPLC eluting with MeOH−H_2O (9:1) to obtain 16-*epi*-scalarolbutenolide (**4**, 2.9 mg, 0.0005% dry wt). Polar fractions eluted with EtOAc were exhaustively subjected to separation by reversed-phase HPLC eluting with 20% to 18% H_2O in MeOH to afford 12-*epi*-scalarin (**9**, 103.7 mg, 0.0196% dry wt), 3-*O*-deacetylluffasterol B (**1**, 3 mg, 0.0006% dry wt), 3β-hydroxy-5α,6α-epoxy-9-oxo-9,11-seco-5α-cholest-7-en-11-al (**5**, 6.2 mg, 0.0012% dry wt), and 3-*O*-deacetyl-22,23-dihydro-24,28-dehydroluffasterol B (**2**, 1.8 mg, 0.0003% dry wt).

3-*O*-Deacetylluffasterol B (1): amorphous solid; $[\alpha]^{25}_D$ −21.4° (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (ϵ) 258 (4920) nm; IR (dry film) ν_{max} 3420 (O−H), 2872 (aldehyde C−H), 1721 (C=O), 1690 and 1684 (α,β-unsaturated ketone); ^{1}H NMR ($CDCl_3$, 400 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 2; HREIMS *m*/*z* 442.3115 (calcd for $C_{28}H_{42}O_4$, 442.3083).

Table 2. ^{13}C-NMR Data for Compounds 1−4[a,b] and 6[b,c]

C no.	1	2	3	4	6
1	27.8	27.8	39.9	40.0	40.0
2	30.5	30.5	18.2‡	18.2‡	18.2*
3	68.3	68.4	41.4*	42.0*	42.0
4	37.5	37.5	33.4	33.3	33.3
5	63.5	63.5	58.9	56.6	56.6
6	53.5	53.5	18.7‡	18.6‡	18.6*
7	139.7	139.9	42.2*	42.1*	42.0
8	140.5	140.5	36.9†	37.5†	37.3†
9	200.6	200.6	56.8	58.1	58.1
10	45.4	45.4	37.6†	37.8†	37.6†
11	203.4	203.3	25.3	25.3	25.3
12	50.8	50.7	77.1	80.6	80.7
13	46.3	46.5	42.7	46.6	47.5
14	45.0	45.0	50.1	50.0	47.8
15	26.7	26.7	24.9	27.6	26.9
16	25.8	25.9	65.7	69.1	65.9
17	51.9	51.7	114.3	166.5	162.8
18	17.1	16.7	162.6	89.8	89.5
19	20.0	21.1	141.2	169.9	169.2
20	43.0	34.7	110.1	112.3	116.8
21	19.7	19.4	33.3	33.2	33.3
22	133.4	33.9	21.3	21.3	21.3‡
23	134.0	31.5	16.2⊥	16.4	16.4
24	38.8	156.4	17.6⊥	17.3	17.1
25	33.2	33.8	15.4⊥	7.0	6.5
26	21.1	22.0*			
27	21.9	21.8*			
28	17.8	106.3			
$OCOCH_3$			170.8	171.3	170.4
$OCOCH_3$			21.4	20.8	21.1‡

[a] Assigments aided by APT experiments. [b] Values with the same superscript in the same column may be interchanged. [c] Values reported by Reddy et al.[5]

3-*O*-Deacetyl-22,23-dihydro-24,28-dehydroluffasterol B (2): amorphous solid; $[\alpha]^{25}_D$ −29.0° (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (ϵ) 259 (4756) nm; IR (dry film) ν_{max} 3420 (O−H), 2872 (aldehyde C−H), 1720 (C=O),

1690 and 1682 (α,β-unsaturated ketone), 916 (C=CH_2); ^{1}H NMR ($CDCl_3$, 400 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 2; HREIMS *m/z* 442.3065 (calcd for $C_{28}H_{42}O_4$, 442.3083).

12,16-Di-*epi*-12-*O*-deacetyl-16-*O*-acetylfuroscalarol (3): colorless oil; $[\alpha]^{25}_D$ −44.2° (*c* 0.33, $CHCl_3$); UV (MeOH) λ_{max} (ϵ) 209 (18840) nm; IR (dry film) ν_{max} 3450 (O−H), 1755 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz)[18] δ 7.24 (1H, d, J = 2.0 Hz, H-19), 6.32 (1H, d, J = 2.0 Hz, H-20), 5.73 (1H, dd, J = 4.1, 1.9 Hz, H-16), 3.82 (1 H, dd, J = 11.2, 4.1 Hz, H-12), 2.80 (1H, br s, O*H*), 2.05 (3 H, s, OCO*CH*$_3$), 2.00 (1 H, m, H-14), 1.93 (1 H, m, H.15), 1.87 (1 H, m, H-15'), 1.87 (1 H, m, H-11), 1.75 (1 H, m, H-7), 1.74 (1 H, m, H-1), 1.62 (1 H, m, H-2), 1.56 (1 H, m, H-6), 1.55 (1 H, m, H-11'), 1.45 (1 H, m, H-2'), 1.43 (1 H, m, H-6'), 1.38 (1 H, m, H-9), 1.32 (1 H, m, H-3), 1.17 (3 H, s, H-25), 1.13 (1 H, m, H-3'), 1.00 (1 H, m, H-5), 0.91 (3 H, s, H-24), 0.89 (1 H, m, H-7'), 0.87 (3 H, s, H-21),[a] 0.86 (3 H, s, H-23),[a] 0.84 (3H, s, H-21), 0.83 (1H, m, H-1'), 0.80 (3H, s, H-22), 0.79 (1H, m, H-5), 0.73 (3H, s, H-25); ^{13}C NMR ($CDCl_3$, 100 MHz), seeTable 2; HREIMS *m/z* 444.2895 (calcd for $C_{27}H_{40}O_5$, 444.2876).

16-*epi*-scalarolbutenolide (4): amorphous solid; $[\alpha]^{25}_D$ −7.3° (*c* 0.15, $CHCl_3$); UV (MeOH) λ_{max} (ϵ) 206 (7510) nm; IR (dry film) ν_{max} 3480 (O−H), 1722 (C=O), 1755 and 1656 (α,β-butenolide), 1236 (O=C−O) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz)[18] δ 5.84 (1H, dd, J = 2.1, 1.7 Hz, H-20), 5.55 (1H, ddd, J = 9.4, 7.3, 2.1 Hz, H-16), 4.54 (1H, d, J = 1.6 Hz, H-18), 3.70 (1H, dd, J = 11.1, 4.5 Hz, H-12), 2.46 (1H, br s, O*H*), 2.25 (1H, ddd, J = 12.8, 7.4, 2.7 Hz, H-15), 2.17 (3H, s, OCO*CH*$_3$), 1.84 (1H, m, H-11), 1.77 (1H, dt, J = 12.7, 3.2 Hz, H-7), 1.72 (1H, m, H-1), 1.62 (1H, m, H-15′), 1.59 (1H, m, H-6), 1.57 (1H, m, H-2), 1.44 (1H, m, H-11′), 1.41 (2H, m, H-2′ and H-6′), 1.37 (1H, m, H-3), 1.14 (1H, dd, J = 13.1, 3.7 Hz, H-3′), 1.08 (1H, dd, J = 12.8, 2.3 Hz, H-14), 1.00 (1H, m, H-7′), 0.88 (1H, m, H-9), 0.87 (3H, s, H-24), 0.85 (3H, s, H-23),[b] 0.84 (3H, s, H-21),[b] 0.83 (1H, m, H-1'), 0.80 (3H, s, H-22), 0.79 (1H, m, H-5), 0.73 (3H, s, H-25); ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 2; HREIMS *m/z* 444.2895 (calcd for $C_{27}H_{40}O_5$, 444.2876).

Acetylation of 3-deacetylluffasterol B (1). An excess of Ac_2O was added to a solution of **1** (1 mg) in dry pyridine. The mixture was kept for 2 h at room temperature, and the residual pyridine and Ac_2O were removed by distillation under reduced presure. The residue was purified using reversed-phase HPLC eluting with MeOH−H_2O (75:15) to obtain luffasterol B (**16**) (0.4 mg).

Cytotoxicity Assays. The new compounds were tested against four tumor cell lines. The individual cell-lines identifiers are given along with the corresponding IC_{50} (μg/mL) values for each compound tested—3-*O*-Deacetylluffasterol B (**1**): P-388 (1), A-549 (1), HT-29 (1), MEL-28 (1); 3-*O*-deacetyl-22,23-dihydro-24,28-dehidroluffasterol B (**2**): P-388 (1), A-549 (1), HT-29 (1), MEL-28 (1); 12,16-di *epi*-12-*O*-deacetyl-16-*O*-acetylfuroscalarol (**3**): P-388 (1), A-549 (1), HT-29 (1), MEL-28 (1); 16-*epi*-scalarolbutenolide (**4**): P-388 (5), A-549 (5), HT-39 (5), MEL-28 (5).

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References and Notes

(1) Present address: Facultad de Ciencias del Mar de Mazatlán, Apdo. 610, 82000 Mazatlán-Sinaloa, Mexico.
(2) Faulkner, D. J. *Nat. Prod. Rep.* **1997**, *14*, 259−302, and previous reviews of this series.
(3) Lu, Q.; Faulkner, D. J. *J. Nat. Prod.* **1997**, *60*, 195−198.
(4) Garrido, L.; Zubía, E.; Ortega, M. J.; Salvá, J. *J. Nat. Prod.* **1997**, *60*, 794−797.
(5) Reddy, M. V. R.; Harper, M. K.; Faulkner, D. J. *J. Nat. Prod.* **1997**, *60*, 41−43.
(6) Cimino, G.; De Rosa, S.; De Stefano, S. *Experientia* **1981**, *37*, 214−215.
(7) Cimino, G.; De Stefano, S.; Di Luccia, A. *Experientia* **1979**, *35*, 1277−1278.
(8) Cimino, G.; De Stefano, S.; Minale, L.; Trivellone, E. *J. Chem. Soc., Perkin Trans. 1* **1977**, 1587−1593.
(9) Cimino, G.; De Stefano, S.; Minale, L. *Tetrahedron* **1972**, *28*, 1315−1324.
(10) Cimino, G.; De Stefano, S., Minale, L.; Fattorusso, E. *Tetrahedron* **1972**, *28*, 267−273.
(11) Fattorusso, E.; Minale. L.; Sodano, G.; Trivellone, E. *Tetrahedron* **1971**, *27*, 3909−3917.
(12) Fontana, A.; Albarella, L.; Scognamiglio, G.; Uriz, M.; Cimino, G. *J. Nat. Prod.* **1996**, *59*, 869−872.
(13) Lenis, L. A.; Núñez, L.; Jiménez, C.; Riguera, R. *Nat. Prod. Lett.* **1996**, *8*, 15−23.
(14) Cimino, G.; De Stefano, S.; Minale, L.; Sodano, G. *Comput. Biochem. Physiol.* **1975**, *50B*, 279−285.
(15) Adinolfi, R.; Migliuolo, A.; Piccialli, V.; Sica, D. *J. Nat. Prod.* **1994**, *57*, 1200−1226.
(16) Cafieri, F.; De Napoli L.; Fattorusso E.; Santacroce C.; Sica D. *Gazz. Chim. Ital.* **1977**, *107*, 71−74.
(17) Cimino, G.; Cafieri F.; De Napoli L.; Fattorusso E. *Tetrahedron Lett.* **1978**, 2041−2044.
(18) Assignments for signals carrying the same superscript may be interchanged.

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