Full Papers

Salmahyrtisol A, a Novel Cytotoxic Sesterterpene from the Red Sea Sponge *Hyrtios erecta*

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The lipophilic partition of a methanol extract of the Red Sea sponge *Hyrtios erecta* yielded a novel pentacyclic sesterterpene ester salmahyrtisol A (**1**), three new scalarane-type sesterterpenes, 3-acetyl sesterstatin 1 (**3**), 19-acetyl sesterstatin 3 (**4**), and salmahyrtisol B (**5**), together with the previously reported sesterterpenes hyrtiosal (**2**), scalarolide (**6**), and salmahyrtisol C (**7**). The structure determination was based on extensive NMR studies and high-resolution mass spectral measurements. In addition, salmahyrtisol A has a previously unknown pentacyclic carbon skeleton. The new compounds show significant cytotoxicity to murine leukemia (P-388), human lung carcinoma (A-549), and human colon carcinoma (HT-29). A biosynthetic relationship between **1** and **2** is briefly discussed.

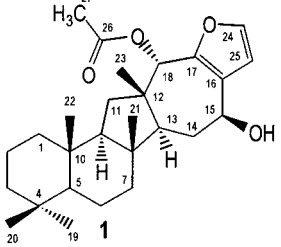
Sponges have been proven to be copious producers of novel terpenoids which display a variety of biological activities.[2] The order Dictyoceratida is a rich source of scalarane-based sesterterpenes.[3−7] They exhibit a variety of biological activities including cytotoxic,[5,7,8−13] antifeedant,[14,15] ichthyotoxic,[16] platelet-aggregation inhibitory,[17] and antiinflammatory[18] activities. The genus *Hyrtios* has yielded structurally diverse metabolites with useful biological activities. For example, it provides the remarkable anticancer spongistatin series,[19−21] 15-oxopuupehenol (cancer cell line and malarial inhibitory),[22] dipuupehedione (cancer cell line inhibitory),[23] dipuupehenone, and related metabolites with antibacterial, antiviral, antifungal, cytotoxic, and immunomodulatory activities.[24,25]

The hexane fraction of a methanolic extract of the sponge *Hyrtios erecta* was separated successively on Sephadex LH-20 (MeOH/CH_2Cl_2, 1:1) by silica flash column chromatography as well as reversed-phase HPLC to afford a new sesterterpene, salmahyrtisol A (**1**),[26] three new scalarane-type sesterterpenes, 3-acetylsesterstatin 1 (**3**), 19-acetylsesterstatin 3 (**4**), and salmahyrtisol B (**5**), together with the known sesterterpenes hyrtiosal (**2**),[27] scalarolide (**6**),[28] and the salmahyrtisol C (**7**).[29] Structures of the isolated sesterterpenes were assigned and secured by high-field 2D NMR techniques and exact mass spectral determinations.

Results and Discussion

Salmahyrtisol A (**1**) was isolated as colorless needles with a molecular formula of $C_{27}H_{40}O_4$, as established by HR-FABMS and ^{13}C NMR data. Its ^1H NMR spectrum revealed resonances for 39 protons including six singlets (δ 0.79, 0.82, 0.83, 0.86, 1.07, and 2.09) belonging to methyl groups, seven methylenes, five aliphatic methines, and two sp^2 methines. The ^{13}C NMR spectrum showed resonances for 27 carbons including six quartets, seven triplets, seven doublets, and seven singlets. Partial structures of C-1 to C-3, C-5 to C-7, and C-13 to C-15 with an OH group at C-15 were assigned from the interpretation of the $^1H-^1H$ COSY and HMQC experiments. In addition, significant COSY correlations from H-9 to the protons at C-11 supported the existence of the partial structure of C-9 to C-11 and the quaternary nature of all carbons (C-8, C-10, and C-12) adjacent to C-9 and C-11, which in turn support the five-membered ring as ring C of **1**. Furthermore, the appearance of the signal of H-18 as a sharp singlet at δ 5.86 suggests the quaternary nature of the carbons on both sides (C-12 and C-17) of C-18. The 2,3-disubstituted furan moiety (ring E) was assigned from doublets at δ 7.30 and 6.50 ($J_{24,25}$ = 1.9 Hz).

Connectivities of the five-ring system of **1** were deduced by HMBC and are listed in Table 1. For example, coexistence and fusion of rings C and D were supported by significant HMBC cross-peaks from H_2-11 to C-9, C-10, C-12, C-18, and C-23, as well as from H-18 to C-11, C-12, and C-13. Similarly, fusion of the furan moiety to the seven-membered ring through C-16 and C-17 was secured by HMBC correlations of H-18/C-16, H-18/C-17, H-24/C-16, H-24/C-17, and H-25/C-17. The position of the acetate moiety at C-18 was secured from the downfield chemical shift of H-18 (δ 5.86) and from HMBC correlation of H-18/C-26. The stereostructure of **1** was elucidated from a 2D ROESY experiment (Figure 1). The all trans-fused-A−B−C−D ring system within **1** was confirmed from ROESY



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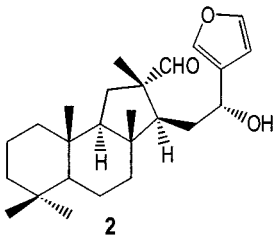

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Table 1. ^{1}H and ^{13}C NMR Data for Compounds **1** and **5** ($CDCl_3$)

	1			**5**		
position	δ_C	δ_H (mult., J_{Hz})	HMBC (H→C)	δ_C	δ_H (mult., J_{Hz})	HMBC (H→C)
1	40.5, t	1.38[a] (m) 0.74 (dt, 12.7, 3.4)	10	39.9,[b] t	1.69 (td, 11.2, 3.0) 0.81 (m)	
2	18.7, t	1.60 (m)		18.0, t	1.59 (m) 1.40 (m)	
3	42.6, t	1.40 (m) 1.14 (dd, 13.0, 4.1)		41.9, t	1.36 (m) 1.11 (ddd, 13.4, 13.4, 4.2)	
4	33.1, s			33.2,[c] s		
5	58.2, d	0.85 (m)		56.5, d	0.79 (m)	
6	18.3, t	1.60 (m) 1.40 (m)	8, 10	18.5, t	1.61 (m) 1.44 (m)	
7	40.1, t	1.72 (dd, 12.3, 3.2) 1.06 (m)	5, 8, 9	41.2, t	1.75 (ddd, 12.4, 3.4, 3.1) 0.95 (m)	5, 8, 9, 21
8	44.6, s			37.5, s		
9	61.3, d	1.18 (dd, 12.0, 7.0)	5, 10, 21, 22	58.5, d	0.98 (dd, 12.4, 2.0)	
10	36.7, s			37.4, s		
11	34.4, t	1.58 (dd, 12.0, 7.0) 1.39[a] (m)	9, 10, 12, 18, 23	27.8, t	1.82 (ddd, 12.6, 4.3, 2.0) 1.57 (m)	8, 9, 10, 12
12	43.0, s			79.2, d	3.76 (dd, 11.4, 4.4)	23
13	50.7, d	1.82 (d, 11.3)	8, 12, 14, 15, 18, 21, 23	39.9,[b] s		
14	32.7, t	1.92 (dd, 12.7, 6.2) 1.73 (ddd, 12.7, 11.3, 10.9)	8, 12, 13, 15, 16	54.7, d	1.64 (dd, 12.0, 4.4)	8, 15, 21, 23
15	69.6, d	4.68 (dd, 10.9, 6.2)	16	35.5, t	2.55 (m) 2.52 (m)	13, 14, 16, 17
16	126.4, s			195.7, s		
17	146.4, s			122.8, s		
18	75.9, d	5.86 (s)	11, 12, 13, 16, 17, 23, 26	135.7, s		
19	33.5, q	0.86 (s)	3, 4, 5, 20	33.2,[c] q	0.83 (s)	3, 4, 5, 20
20	21.2, q	0.83 (s)	3, 4, 5, 19	21.6, q	0.80 (s)	3, 4, 5
21	16.5, q	0.79 (s)	7, 9, 13	17.0,[d] q	0.94 (s)	7, 8, 9, 14
22	15.1, q	0.82 (s)	1, 5, 9, 10	16.3	0.86 (s)	1, 5, 9
23	24.9, q	1.07 (s)	11, 12, 13, 18	17.0,[d] q	1.23 (s)	12, 13, 14, 18
24	141.6, d	7.30 (d, 1.9)	16, 17	138.2, d	7.60 (d, 1.5)	
25	111.1, d	6.50 (d, 1.9)	17, 24	143.4, d	7.89 (d, 1.5)	
26	170.5, s					
27	21.3, q	2.09 (s)	26			

[a] Signals are partially overlapped. [b] Exact δ values are 39.90 (C-1) and 39.91 (C-13). [c] Exact δ values are 33.26 (C-4) and 33.22 (C-19). [d] Exact δ values are 17.03 (C-21) and 17.07 (C-23).

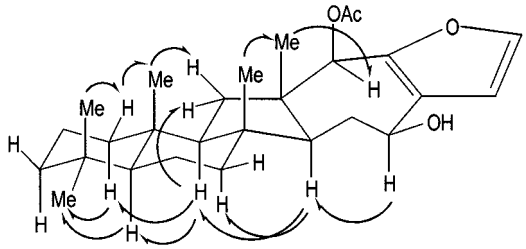
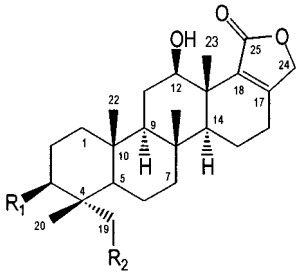


Figure 1. Important ROESY NMR correlations observed for **1**.

cross-peaks of H-5$_\alpha$/H-9$_\alpha$, H-9$_\alpha$/H-13$_\alpha$/H-9$_\alpha$/H-11$_\alpha$, H_3-20/H-1$_\beta$, H-1$_\beta$/H_3-22, H_3-22/H-11$_\beta$, and H_3-21/H_3-23. The equatorial nature of OH-15 was deduced from the large ^{1}H−^{1}H coupling constant of H-15 (10.9 Hz)[10,12] and also from the ROESY cross-peak of H-15/H-13, while ROESY correlation between H-18 and H_3-23 revealed a equatorial geometry of H-18.

Hyrtiosal (**2**) was isolated as colorless needles and had a molecular formula of $C_{25}H_{38}O_3$ based on the results of HRFABMS and ^{13}C NMR data. The ^{1}H and ^{13}C NMR spectral data of **2** were consistent with those of hyrtiosal.[27] Since **1** and **2** represent novel skeletons of marine sesterterpenes, we believe that **2** might be the logical biosynthetic intermediate for **1**. The coexistence of **1** together with **2** is noteworthy from the viewpoint of the biosynthesis of such unusual sesterterpenes.

3-Acetylsesterstatin 1 (**3**) was isolated as an amorphous solid with a molecular formula of $C_{27}H_{40}O_5$, as determined by HREIMS and ^{13}C NMR data. The ^{1}H NMR and ^{13}C NMR spectra (Table 2) showed four methyl singlets (δ 0.83, 0.88 (× 2), and 1.12), nine methylene groups, five methine groups, five quaternary carbons, and two fully substituted (δ 162.1 and 135.8) sp^2 carbon atoms incorporated into an α,β-unsaturated lactone ring (C=O at δ 175.9). Signals for OH (δ 5.93, s) and acetate (δ 2.05 (s)/21.0 and 171.3) moieties were also recognized. Analysis of the ^{1}H−^{1}H COSY and HMQC spectra allowed the assembly of the partial structures of C-1 to C-3, C-5 to C-7, and C-9 to C-12 with an OH group attached to C-12, and C-14 to C-16. The connection of the five-ring system of **3** was secured from HMBC correlations, which are listed in Table 2. The position of the acetate moiety at C-19 was established from the HMBC cross-peak of H_2-19/C-26. The equatorial geometry of OH at C-12 was established from the large coupling constant of H-12 (J = 10.9 Hz).[10,12]



	R_1	R_2
3	H	$OCOCH_3$
4	$OCOCH_3$	H
6	H	H

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

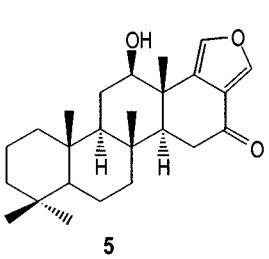
	3			**4**		
position	δ_C	δ_H (mult., J_{Hz})	HMBC (H→C)	δ_C	δ_H (mult., J_{Hz})	HMBC (H→C)
1	39.2, t	1.74 (m)		37.8, t	1.77 (m)	
		0.78 (m)			1.43 (m)	
2	17.7, t	1.64 (m)	4	23.6, t	1.66 (m)	10
		1.50 (m)				
3	35.7, t	1.33 (m)	4	80.6, d	4.46 (dd, 11.2, 5.4)	1, 4, 19
4	36.5, s			37.7, s		
5	50.6, d	1.06 (m)	4, 10	55.5, d	0.85 (m)	
6	18.2, t	1.44 (m)		17.8, t	1.56 (m)	
7	41.4, t	1.82 (m)		41.6, t	1.83 (m)	8
		0.85 (m)			0.92 (m)	
8	37.1, s			37.0, s		
9	58.0, d	0.88 (m)		57.7, d	0.84 (m)	
10	37.3, s			37.1, s		
11	25.8, t	1.85 (m)	18, 23	25.9, t	1.82 (m)	9, 10, 12
		1.69 (m)			1.50 (m)	
12	75.5, d	3.67 (dd, 10.9, 3.8)	11, 12	75.4, d	3.66 (dd, 10.5, 4.6)	11, 23
OH		5.93 (s)	11, 12		5.94 (s)	11, 12
13	42.2, s			42.2, s		
14	55.1, d	1.09 (m)	8, 13, 21	55.0, d	1.05 (d, 12.2)	8, 13, 15
15	16.4, t	1.90 (m)	8, 13, 14, 16, 17	16.5, t	1.91 (dd, 13.6, 7.3)	8, 13, 17
		1.69 (m)			1.66 (m)	
16	25.2, t	2.28 (ddd, 19.0, 10.9, 7.0)	5, 14, 15, 17, 18	25.2, t	2.43 (dd, 19.4, 5.9)	14, 15, 17, 18
		2.43 (dd, 19.0, 5.6)			2.27 (ddd, 19.4, 11.0, 7.0)	
17	162.1, s			162.0, s		
18	135.8, s			135.8, s		
19	72.9, t	3.86 (d, 10.9)	3, 4, 5, 26	27.9, q	0.84 (s)	3, 5
		3.61 (d, 10.9)				
20	17.1,[a] q	0.83 (s)	4, 5, 19	16.3, q	0.85 (s)	3, 5
21	16.3, q	0.88 (s)	9, 14	17.1, q	0.89 (s)	7, 9, 14
22	17.1,[a] q	0.88 (s)	1, 5, 9, 10	16.0, q	0.87 (s)	9
23	16.7	1.12 (s)	12, 13, 14, 18	16.7, q	1.13 (s)	12, 13, 14, 18
24	72.1, t	4.70 (d, 17.6)	17, 18, 25	72.1, t	4.72 (d, 17.5)	17, 18, 25
		4.65 (d, 17.6)			4.65 (d, 17.5)	
25	175.9, s			175.8, s		
26	171.3, s			171.0, s		
27	21.0, q	2.05 (s)	26	21.7, q	2.04 (s)	26

[a] Exact δ values are 17.11 (C-20) and 17.15 (C-22).

19-Acetylsesterstatin 3 (**4**) was isolated as an amorphous solid and had a molecular formula of $C_{27}H_{40}O_5$, as determined by HRFABMS and ^{13}C NMR data. Compound **4** possesses the same molecular formula as **3**. Its ^{1}H and ^{13}C NMR spectra (Table 2) revealed resonances very close to those of **3**. The only difference is the position of the acetate moiety at C-3 instead of C-19 in compound **3**. The structure of **4** was secured from a study of both 1D and 2D NMR (COSY, HMQC, and HMBC) spectra. The large coupling constants observed for H-3 (J = 11.2 Hz) and H-12 (J = 10.5 Hz) indicated an equatorial geometry for the acetate and OH moieties at C-3 and C-12, respectively.[10,12]

The parent compounds sesterstatin 1 and 3 were reported from the marine sponge *Hyrtios erecta* collected from the Republic of Maldives.[12]

Salmahyrtisol B (**5**) was purified as an amorphous solid and had a molecular formula of $C_{25}H_{36}O_3$, as determined by HRFABMS and ^{13}C NMR data. Its ^{1}H NMR spectrum (Table 1) displayed signals for five methyl singlets (δ 0.80, 0.83, 0.86, 0.94, and 1.2), seven methylenes, and six methines. The ^{13}C NMR spectrum showed resonances for 25 carbons, including signals for an α,β-conjugated carbonyl group (δ 195.7, 135.7, and 122.8), five quaternary carbons, six methines including three oxygenated ones (δ 79.2, 143.4, and 138.2), seven methylenes, and five methyls, which accounted for 35 nonexchangeable protons. Interpretation of the ^{1}H−^{1}H COSY and HMQC experiments led to the assembly of the partial structures of C-1 to C-3, C-5 to C-7, C-9 to C-12 with OH attached to C-12, and C-14 to C-15. The remaining oxygenated methines of the furan ring showed long-range coupling ($J_{24,25}$ = 1.5 Hz) in the COSY spectrum. The structural units of **5** were connected through quaternary carbons on the basis of accurate study of the HMBC data (Table 1), which included cross-peaks of H_3-21/C-8, C-9, C-14; H_3-22/C-1, C-5, C-9; H_3-2/C-14, C-18; and H-14/C-8. HMBC correlations of H-15/C-17 and H-12/C-18 implied that the furan moiety was fused to ring D at C-17 and C-18. The large ^{1}H−^{1}H coupling constant of 11.4 Hz indicated an axial H-12.[10,12,13]



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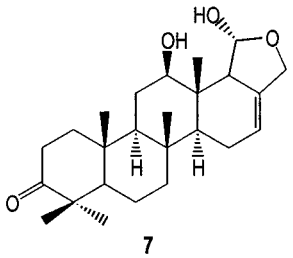

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Scalarolide (**6**) was isolated as a colorless solid with a molecular formula of $C_{25}H_{38}O_3$, as deduced from HR-FABMS and ^{13}C NMR data. The structural assignments of **6** were based on an accurate study of the 1D and 2D (COSY, HMQC, HMBC) NMR spectra. The ^{1}H and ^{13}C NMR (in $CDCl_3$) spectral data of **6** were consistent with the data for scalarolide, which was previously reported from *Spongia idia*.[28]

Salmahyrtisol C (**7**) was isolated as a colorless solid with a molecular formula of $C_{25}H_{38}O_4$, as determined from

HRFABMS and ^{13}C NMR data. The structure of **7** was established from an accurate study of the 1D and 2D (COSY, HMQC, HMBC) NMR spectra. The spectral data of **7** were consistent with the known but unnamed sesterterpene isolated previously from the sponge *Hyrtios erecta* collected in Kagoshima, Japan.[29]

The all trans-fused-A−B−C−D ring system for compounds **3**−**7** was confirmed from chemical shift values of H-5, H-9, H-14, H_3-21, H_3-22, and H_3-23 (Tables 1 and 2)[10,12,13] as well as from NOE correlations of H-5$_\alpha$/H-9$_\alpha$, H-9$_\alpha$/H-11$_\alpha$, H_3-20/H-1$_\beta$, H-1$_\beta$/H_3-22, H_3-22/H-11$_\beta$, and H_3-21/H_3-23.

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 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 with a Jasco-DIP-700 using CH_2Cl_2 at 20 °C at the sodium D line (589 nm). Reversed-phase HPLC was conducted on semipreparative Ultracarb 5μ ODS 30, 250 × 10 mm (Phenomenex).

Animal Material. The sponge forms large erect rounded digits, occasionally branched, with a rounded apex, and was collected from a depth of 15−20 m from El Quseir, 120 km south of Hurghada, Egypt, in the Red Sea, on January 22, 1999. The color in life is deep greenish black, the interior slightly lighter. The texture is incompressible, and the sponge easily snapped in half as the sponge fibers are packed solidly with sand grains. The surface is regularly conulose and quite spiky to the touch. The sponge is *Hyrtios erecta* (Keller) (family Thorectidae, order Dictyoceratida). A voucher specimen has been deposited in the Natural History Museum, London (BMNH 1999.12.20.5).

Extraction and Isolation. Freshly collected specimens (2.4 kg, wet wt) of the sponge were quickly immersed in MeOH on site. The sponge was extracted with MeOH (3 × 2 L) 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 × 400 mL) to give 635.0 mg 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 × 400 mL) to afford 553.8 mg of CH_2Cl_2 extract. All partition fractions as well as the column fractions were subjected to antitumor testing. Guided by the in vitro antitumor assay, the hexane residue (635 mg, IC_{50} = 2.5 μg/mL) was loaded on a Sephadex LH-20 column equilibrated with CH_2Cl_2/MeOH (1:1). Eight fractions were collected. Fraction 4 (130 mg, IC_{50} = 2.0 μg/mL) was subjected to a SiO_2 flash column eluted with hexane/CH_2Cl_2/acetone gradients to afford 13 subfractions. Subfractions 4−8 (29.1 mg, IC_{50} = 2.0 μg/mL) were then purified by reversed-phase HPLC with 85% MeCN (2.0 mL/min at 220 nm) to give **3** (3.7 mg). Compound **4** (4 mg) was purified by crystallization of the methanolic solution of subfractions 4−9 (16 mg). Fraction 5 (235.4 mg, IC_{50} = 2.0 μg/mL) was then subjected to a SiO_2 flash column eluted with hexanes/CH_2Cl_2/acetone gradients to yield 16 subfractions. Subfractions 5−8 (37.0 mg, IC_{50} = 2.0 μg/mL) were further purified on reversed-phase HPLC with 95% MeCN (2.0 mL/min at 220 nm) to afford **1** (1.9 mg), **2** (7 mg), and **6** (1.1 mg). Subfractions 5−13 (6.2 mg, IC_{50} = 2.0 μg/mL) were subjected to a reversed-phase HPLC with 70% MeCN (2.0 mL/min at 220 nm) to afford **7** (1.3 mg). Finally fraction 6 (81.4 mg, IC_{50} = 2.0 μg/mL) was flash chromatographed (YMC Gel ODS-A, 60 Å 230/70 mesh), eluting with 70%→0% H_2O/MeCN, to yield six subfractions. Subfractions 6−3 (15.4 mg, IC_{50} = 2.0 μg/mL) were purified on a reversed-phase HPLC with 85% MeCN (2.0 mL/min at 220 nm) to afford **5** (0.8 mg).

Salmahyrtisol A (**1**): colorless needles (1.9 mg, 0.00008%, based on wet wt); $[\alpha]_D$ −59.15° (*c* 0.64, CH_2Cl_2); UV λ_{max} (MeOH) (log ϵ) 206 (3.19), 262 (2.57) nm; IR (film) ν_{max} 3380, 2920, 2849, 1756, 1604, 1485 cm^{-1}; NMR data, see Table 1; HRFABMS *m*/*z* 451.2803 (calcd for $C_{27}H_{40}O_4Na$ [M + Na]$^+$, 451.2824).

Hyrtiosal (**2**): colorless needles (7 mg, 0.0003%, based on wet wt); $[\alpha]_D$ −49.4° (*c* 1.6, CH_2Cl_2) (−73.8°);[27] HRFABMS *m*/*z* 409.2737 (calcd for $C_{25}H_{38}O_3Na$ [M + Na]$^+$, 409.2718).

3-Acetylsesterstatin 1 (**3**): amorphous solid (3.7 mg, 0.00016%, based on wet wt); $[\alpha]_D$ +82.7° (*c* 1.85, CH_2Cl_2); UV λ_{max} (MeOH) (log ϵ) 217 (2.71) nm; IR (film) ν_{max} 3380, 2924, 2850, 1752, 1714, 1655 cm^{-1}; NMR data, see Table 2; HREIMS *m*/*z* 444.2872 (calcd for $C_{27}H_{40}O_5$, [M]$^+$, 444.2875).

19-Acetylsesterstatin 3 (**4**): amorphous solid (4.0 mg, 0.00016%, based on wet wt); $[\alpha]_D$ +39° (*c* 1.05, CH_2Cl_2); UV λ_{max} (MeOH) (log ϵ) 219 (2.46) nm; IR (film) ν_{max} 3378, 2920, 2853, 1755, 1712, 1652 cm^{-1}; NMR data, see Table 2; HRFABMS *m*/*z* 445.2950 (calcd for $C_{27}H_{41}O_5$, [M + H]$^+$, 445.2954).

Salmahyrtisol B (**5**): amorphous solid (0.8 mg, 0.000033%, based on wet wt); $[\alpha]_D$ +68° (*c* 0.4, CH_2Cl_2); UV λ_{max} (MeOH) (log ϵ) 226 (2.13), 275 (2.41) nm; IR (film) ν_{max} 3390, 2926, 1695, 1610, 1480 cm^{-1}; NMR data, see Table 1; HRFABMS *m*/*z* 385.2755 (calcd for $C_{25}H_{37}O_3$, [M + H]$^+$, 385.2742).

Scalarolide (**6**): colorless needles (1.1 mg, 0.000045%, based on wet wt); $[\alpha]_D$ +26.3° (*c* 0.15, CH_2Cl_2) (+24.9°);[28] HRFABMS *m*/*z* 409.2730 (calcd for $C_{25}H_{38}O_3Na$, [M + Na]$^+$, 409.2718).

Salmahyrtisol C (**7**): colorless solid (1.3 mg, 0.000054%, based on wet wt); $[\alpha]_D$ +39.8° (*c* 0.18, CH_2Cl_2) (+40.3°);[29] HRFABMS *m*/*z* 425.2679 (calcd for $C_{25}H_{38}O_4Na$, [M + Na]$^+$, 425.2667).

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**, **3**, **4**, and **5** showed activity of $IC_{50} \geq 1$ 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 for **1**−**7**. This material is available free of charge via the Internet at http://pubs.acs.org.

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