Notes

Bioactive Scalaranes from the Thai Sponge *Hyrtios gumminae*

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Chemical investigation of the Thai sponge *Hyrtios gumminae* collected from Similan Island in the Andaman Sea, Thailand, yielded four new sesterterpenoids, similan A (**1**), 12β,20-dihydroxy-16β-acetoxy-17-scalaren-19,20-olide (**2**), 12β-acetoxy-20-hydroxy-17-scalaren-19,20-olide (**3**), and 12β,16α,20-trihydroxy-17-scalaren-19,20-olide (**4**), together with seven known compounds. The structures of these new compounds were elucidated on the basis of their spectroscopic data and chemical transformations. Some of the isolated compounds were tested for their cytotoxic activity.

Previous chemical investigations of different *Hyrtios* spp. and their associated microorganisms have revealed the presence of numerous structurally unique natural products including steroids,[1,2] acyclic triterpenoids,[3] indole alkaloids,[4,5] macrolides,[6,7] and scalarane sesterterpenoids.[4,8−15] Of particular interest, scalarane-type sesterterpenoids have been reported to display a variety of biological activities including cytotoxicity.[8−11,13] As part of our project directed toward the search for cytotoxic metabolites from sponges, an EtOAc extract of *Hyrtios gumminae* was found to exhibit cytotoxic activity against HuCCA-1, KB, HeLa, MDA-MB-231, T47D, and H69AR cancer cell lines. Chemical investigation of the EtOAc-soluble fraction of the methanolic extract of *H. gumminae* has now led to the isolation of four new sesterterpenes (**1**−**4**), together with seven known compounds, hyrtiosal (**5**),[8,15] scalarafuran (**6**),[16] scalarolide,[4,13,16] 16-acetoxyscalarolide or sesterstatin 7,[12] 12-epi-*O*-deacetyl-19-deoxyscalarin (**7**),[12,17] hyrtiolide (**8**),[13] and cholesterol.[2] Compounds **2**−**4** were characterized as their acetate derivatives **2a**−**4a** and **2b**−**4b**, respectively. Some of the isolated compounds were tested for cytotoxic activity.

Compound **1** was isolated as a solid with a molecular formula of $C_{26}H_{40}O_3$, as established from its HRAPCIMS. The ^{13}C NMR spectrum revealed signals for 26 carbons (Table 1) including six methyl, seven methylene, seven methine, and six quaternary carbons. Partial structures of C-1 to C-3, C-5 to C-7, and C-13 to C-15 with a hydroxy group at C-15 were deduced by the $^1H-^1H$ COSY and HSQC analysis. The proton signals at δ 7.30 (d, J = 1.8 Hz, H-24) and 6.53 (brd, J = 1.5 Hz, H-25) are representative of a disubstituted furan moiety in **1**. Furthermore, the appearance of the signal of H-18 as a sharp singlet at δ 3.85 suggested the quaternary nature of the carbons on both sides (C-12 and C-17) of C-18. Connectivities of the five-ring system of **1** were deduced by HMBC experiments (Table 1). For example, fusion of rings C and D was supported by significant HMBC cross-peaks from H-11 to C-9, C-10, C-12, C-18, and C-23, as well as H-18 to C-11, C-12, C-13, and C-23. Similarly, fusion of the furan moiety to the seven-membered ring through C-16 and C-17 was secured by HMBC correlations between H-18 and C-16 and C-17, H-24 and C-16 and C-17, and H-25 and C-17. The methoxy group at C-18 was secured by HMBC correlation of H-18 to the methoxy carbon. In the NOESY spectrum of **1** (Figure 1), Me-21 (δ 0.78) was correlated with Me-22 (δ 0.83) and Me-23 (δ 0.95), while the methine proton H-9 (δ 1.34) was correlated with the methine protons H-5 (δ 0.94) and H-13 (δ 1.89). These data were in agreement with the all-*trans* fusion of rings A−B−C−D of the tetracyclic skeleton, having Me-21, Me-22, and Me-23 axially oriented to the β-face, whereas

Table 1. ^{13}C (150 MHz) and ^1H NMR (600 MHz) Data of **1** ($CDCl_3$)[a]

position	δ_C, mult.	δ_H, mult (J in Hz)	HMBC[b]
1	40.0, CH_2	1.39, m	
		0.96, m	2
2	18.4, CH_2	1.62, m	
		1.41, m	10
3	42.6, CH_2	1.37, m	
		1.18, td (13.9, 4.0)	2, 20
4	33.2, qC		
5	57.7, CH	0.94, m	6
6	18.8, CH_2	1.57, m	8, 10
		1.38, m	
7	40.0, CH_2	1.67, m	5, 9
		1.11, td (12.5, 3.9)	6
8	44.9, qC		
9	60.8, CH	1.34, m	8
10	36.8, qC		
11	35.1, CH_2	1.81, dd (8.6, 3.3)	8, 9, 10, 12, 18, 23
		1.32, m	9, 12
12	43.7, qC		
13	49.7, CH	1.89, d (11.3)	9, 14, 15, 22, 23
14	33.0, CH_2	1.83, dd (12.5, 5.6)	13, 15
		1.64, m	13, 15, 16
15	69.9, CH	4.66, dd (9.9, 5.3)	
16	124.5, qC		
17	149.9, qC		
18	85.0, CH	3.85, s	11, 12, 13, 16, 17, 23, 18-OMe
19	33.6, CH_3	0.85, s	3, 5, 20
20	21.3, CH_3	0.83, s	5
21	16.8, CH_3	0.78, s	7, 8, 9, 13
22	15.3, CH_3	0.83, s	5, 9
23	25.3, CH_3	0.95, s	11, 12, 13, 18
24	140.6, CH	7.30, d (1.8)	16, 17, 24
25	111.4, CH	6.53, brd (1.5)	15, 16, 17, 25
18-OMe	57.9, CH_3	3.30, s	18

[a] Assignments are based on HSQC, HMBC, and DEPT experiments; δ in ppm. [b] HMBC correlations are from proton(s) stated to the indicated carbon.

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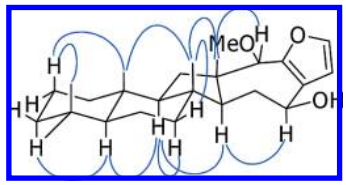
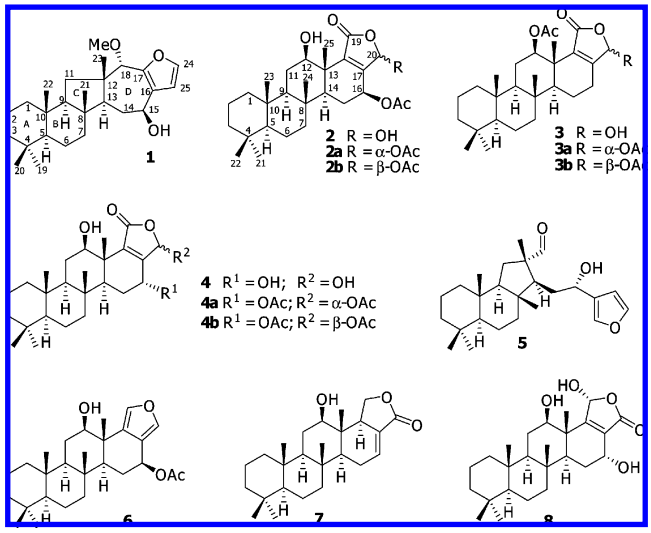


Figure 1. Key NOESY correlations of **1**.



H-5, H-9, and H-13 were axially oriented to the α-face. The equatorial nature of OH-15 was deduced from the large coupling constant of H-15 (9.9 Hz) and also from the NOESY cross-peak of H-15 (δ 4.66) with H-13 (δ 1.89). The NOESY correlation between H-18 (δ 3.85) and Me-23 (δ 0.95) was observed, revealing that H-18 had the β-configuration and the equatorial C-18 methoxy group occupied the α-face. All these data supported structure **1** for similan A. The structure of similan A is closely related to salmahyrtisol A[8] except for the replacement of an acetoxy group by a methoxy at C-18 in **1**. Considering that the extraction was made with MeOH, **1** may be an artifact.

The inseparable mixture of 12β,20α-dihydroxy-16β-acetoxy-17-scalaren-19,20-olide and 12β,20β-dihydroxy-16β-acetoxy-17-scalaren-19,20-olide (**2**) had the molecular formula $C_{27}H_{40}O_6$, from HRAPCIMS. Treatment of the mixture with Ac_2O and pyridine gave a mixture of the diacetate derivatives **2a** and **2b**. The diacetates **2a** and **2b** were easily separated by preparative TLC (30% EtOAc−hexane).

Compound **2a** was obtained as a solid with a molecular formula of $C_{29}H_{42}O_7$, as determined by HRAPCIMS and ^{13}C NMR data. The ^{13}C NMR spectrum displayed 29 signals; a combination of ^1H NMR, ^{13}C NMR, and HSQC data of **2a** revealed seven methyl, seven methylene, six methine, and nine quaternary carbons. The COSY and HSQC analysis led to assignment of the following spin systems: $CH_2-CH_2-CH_2$ (C-1−C-3), $CH-CH_2-CH_2$ (C-5−C-7), CH_2-CHOH (C-11−C-12), and $CH-CH_2-CH(O)$ (C-14−C-16). The attachment of the hydroxy group at C-12 was deduced from the proton signal at δ 3.70 (ddd, J = 10.8, 4.5, 1.3 Hz, H-12), which was correlated in the HMBC spectrum with C-9 (57.9), C-18 (142.6), and C-25 (16.4). Furthermore, the large $^1H-^1H$ coupling constants of H-12 (10.8 Hz) indicated the axial (α-face) orientation for this proton. The acetoxy group at C-16 was assigned from the proton signal (H-16) at δ 5.58 in **2a** (5.60/5.61 in **2**) coupling with H-15 by COSY, as well as from HMBC correlations to C-14 (54.1), C-18 (142.6), and C-20 (90.8). The significantly downfield shifted signal of H-20 at δ 6.90 (6.06/6.05 in **2**) was assigned to an acetylated hemiacetal methine proton. This methine proton together with two carbonyl signals [$\delta_{C\text{-}19}$ 171.5 and δ_C 168.6 (20-O*C*OCH$_3$)] and a tetrasubstituted double bond ($\delta_{C\text{-}17}$ 155.8 and $\delta_{C\text{-}18}$ 142.6) defined the presence of a γ-acetoxy-α,β-unsaturated-γ-lactone involving carbons C-17, C-18, C-19, and C-20 of scalarane framework. The location of the double bond at C-17 and C-18 was supported by the HMBC correlations of the olefinic carbons at δ 155.8 (C-17) and 142.6 (C-18) with H-15 and H-12 and H-25, respectively. Furthermore, the ^{13}C chemical shifts of C-17 and C-18 indicated the location of the carbonyl at C-19. The all-*trans*-fused A−B−C−D ring system in **2a** was confirmed by ROESY correlations between the axial H-12α and H-9α and H-14α and between the axial H-16α and H-14α. In the NOE spectrum of **2a**, H-20 did not show enhancement with the signal of H-16α, indicating the β-orientation of H-20. All these data were consistent with the structure **2a**, which was thus deduced as 12β-hydroxy-16β,20α-diacetoxy-17-scalaren-19,20-olide.

Compound **2b** has the same molecular formula as **2a**, and the ^1H,^{13}C NMR data for the two compounds were almost identical. The configuration of rings A, B, C, and D of **2b** was also the same as that of **2a**, which was confirmed by NOESY. Therefore, the differences between **2a** and **2b** must be due to the configurations of the hemiacetal chiral centers. Irradiation of H-16α of **2b** caused an NOE enhancement of H-20 (3%). Compound **2b** was thus deduced as 12β-hydroxy-16β,20β-diacetoxy-17-scalaren-19,20-olide.

The mixture of 12β-acetoxy-20α-hydroxy-17-scalaren-19,20-olide and 12β-acetoxy,20β-hydroxy-17-scalaren-19,20-olide (**3**) was obtained as a solid. Treatment of the mixture of **3** with Ac_2O and pyridine gave a mixture of the diacetates **3a** and **3b**, which were separated by HPLC.

Compound **3a** was obtained as a solid with a molecular formula of $C_{29}H_{42}O_6$, which indicated that **3a** had one oxygen less than **2a** and **2b**. The NMR spectra of **3a** were similar to those of **2a** and **2b**, except **3a**, which contained only one methine carbon bearing an acetoxy group ($\delta_{H\text{-}12}$ 4.93, $\delta_{C\text{-}12}$ 75.8). The HMBC cross-peaks of C-12 (δ 75.8) with the protons at δ 1.25 (H-25) and 0.98 (H-9) confirmed the location of the acetoxy group at C-12. The β-orientation of H-20 was proposed from the NOESY correlation between H-20 (δ 6.60) and H-16β (δ 2.32). Thus the structure of **3a** was determined to be 12β,20α-diacetoxy-17-scalaren-19,20-olide.

The NMR spectra of **3b** were almost identical to those of **3a** except in regard to the signal of H-20 appearing at δ 6.63 (d, J = 1.0 Hz), suggesting that **3b** differed from **3a** only in the configuration at C-20. The NOESY spectrum of **3b** showed the cross-peak correlation between H-20 and H-16α (δ 2.12). All these data suggested the α-orientation of H-20, and therefore the structure of **3b** was assigned as 12β,20β-diacetoxy-17-scalaren-19,20-olide.

The mixture of 12β,16α,20α-trihydroxy-17-scalaren-19,20-olide and 12β,16α,20β-trihydroxy-17-scalaren-19,20-olide (**4**) had the molecular formula $C_{25}H_{38}O_5$ by HRAPCIMS. Treatment of the mixture with Ac_2O and pyridine gave a mixture of the diacetate derivatives **4a** and **4b**, which were separated by preparative TLC (EtOAc−hexane−CH_2Cl_2, 1:3:3).

Compound **4a** had the same molecular formula as **2a**, and the NMR data of the two compounds were almost identical except for the signal of H-16. In particular, H-16 of **4a** appeared as a broad doublet with the coupling constant 4.1 Hz and the high-field chemical shift of C-16 at δ 63.1 ($\delta_{C\text{-}16}$ 66.5 in **2a**), suggesting that the configuration at C-16 of **4a** and **2a** was different. In the NOESY spectrum of **4a**, a cross-peak was observed between H-16 and H-20. Irradiation of H-16 caused an NOE enhancement of the H-20 (5.0%). These data suggested the β-orientation of both hydrogen atoms H-16 and H-20. Compound **4a** was thus assigned as 12β-hydroxy-16α,20α-diacetoxy-17-scalaren-19,20-olide.

The NMR spectra of **4b** were almost identical to those of **4a** except in regard to the resonances of 20-OCO*C*H$_3$ (δ_H 2.19 in **4b**; 2.10 in **4a**) and C-20 ($\delta_{C\text{-}20}$ 91.8 in **4b**; 93.0 in **4a**), suggesting that **4b** differed from **4a** only in the configuration at C-20. In the NOE spectrum of **4b**, H-20 showed an enhancement of only 1.7% (5%

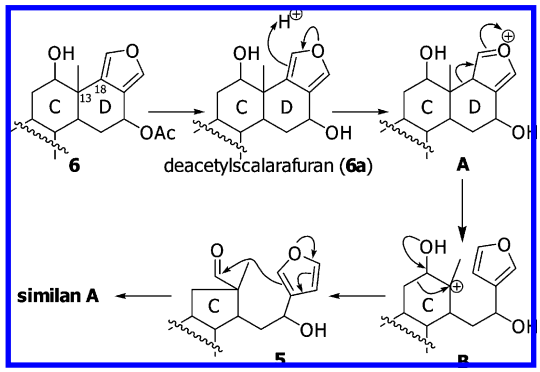


Figure 2. Proposed relationships of **1**, **5**, and **6**.

Table 2. Cytotoxic Activity of **1** and **4**−**8**

	IC$_{50}$ (μM)					
compound	HuCCA-1	KB	HeLa	MDA-MB-231	T47D	H69AR
1	90	75	125	58	70	>125
4	65	14	26	29	48	—[a]
5	9.1	7.8	18	5.4	9.1	31
6	49	58	63	14	28	51
7	42	7.0	23	5.9	5.2	57
8	57	12	22	26	34	—[a]
etoposide	5.1	0.5	0.4	0.3	0.1	46

[a] Not determined.

in **4a**) with the signal of H-16, indicating the α-orientation of H-20. Compound **4b** was thus deduced as 12β-hydroxy-16α,20β-diacetoxy-17-scalaren-19,20-olide.

It is of interest to consider the biogenetic relationship between sesterterpenes **1**, **5**, and **6**, and our proposed route is illustrated in Figure 2. Similan A may arise from deacetyl scalarafuran (**6a**) by means of an acid-catalyzed rearrangement. Protonation of the furan ring of **6a** can generate transient oxonium ion A, which induces further fragmentation of the C_{13}−C_{18} bond, giving rise to the stable tertiary carbocation intermediate B. Subsequent ring contraction of the resulting intermediate B via the Wagner−Meerwein rearrangement provides the five-membered C ring present in hyrtiosal (**5**). The aldehyde formed in **5** can then undergo an electrophilic addition with the adjacent furan, followed by methoxy incorporation and rearomatization to deliver the final similan A (**1**). Furthermore, γ-hydroxybutenolide compounds **2**, **3**, **4**, and **8** can arise by singlet oxygen oxidation of the corresponding furans via the [4+2] cycloaddition.[18]

Some of the isolated compounds were evaluated for their cytotoxic activity against cancer cell lines, including HuCCA-1 (human cholangiocarcinoma), KB (human epidermoid carcinoma of the mouth), HeLa (human cervical carcinoma), MDA-MB-231 (hormone-independent breast cancer), T47D (hormone-dependent breast cancer), and H69AR (multidrug-resistant small-cell lung cancer). Compounds **5** and **7** exhibited moderate cytotoxic activity (IC$_{50}$ values of 5.2−57 μM), while scalarolide and cholesterol were inactive at >100 μM. Compounds **1**, **6**, and **8** and the mixture of **4** showed weakly cytotoxic activities (IC$_{50}$ values of 15−65 μM) (Table 2).

Experimental Section

General Experimental Procedures. Melting points were determined on a Buchi 535 apparatus and are uncorrected. Optical rotations were recorded on a JASCO DIP 1020 polarimeter using a cylindrical glass cell (10 mm i.d. × 10 mm). UV spectra were measured with a UV-1700 Pharma Spec (Shimadzu) spectrophotometer. FTIR spectra were obtained using a universal attenuated total reflectance attached to a Perkin-Elmer Spectrum One spectrometer. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ solution containing Me_4Si as internal standard on a Bruker AM400 or AVANCE600 spectrometer. MS spectra were determined using a Finnigan Polaris mass spectrometer, and HRMS were performed on a Bruker microTOF mass spectrometer. HPLC was performed on Thermo Separation Products (San Jose, CA) instruments (pump, P4000; detector, UV6000LP for analysis, UV2000 for preparative; columns, Exsil 100-5ODS (150 × 4.60 mm) for analytical and Exsil 100-10ODS (250 × 21.20 mm) for preparative applications). All commercial grade solvents were distilled prior to use, and spectral grade solvents were used for spectroscopic measurements.

Sponge Material. The sponge, *Hyrtios gumminae*, was collected from Similan Island in the Andaman Sea (Thailand) in April 2005 at a depth of 30−40 feet by hand via scuba diving. This sponge was identified by Mr. Saharath Dheerakomporn, Faculty of Marine Technology, Burapha University, Chanthaburi Campus, Thailand. A voucher specimen (CRI210) is presently deposited at the Laboratory of Natural Products, Chulabhorn Research Institute, Bangkok, Thailand.

Extraction and Isolation. A frozen sample (wet wt 2.3 kg) of *H. gumminae* was cut into small pieces and extracted exhaustively with MeOH. The extract was filtered through cotton and then evaporated under reduced pressure to give an aqueous residue, which was partitioned with EtOAc. The organic layer was concentrated to give a dark brown solid (8 g). The EtOAc-soluble fraction was subjected to vacuum liquid chromatography on silica gel and eluted with an EtOAc−hexane gradient (0 → 80%). Seven fractions (F1−F7) were obtained. F3 (400 mg) was further chromatographed on a C_{18} reversed-phase HPLC and eluted with MeOH−H_2O (85:15, flow rate 12 mL/min) to yield compounds **5** (50 mg), **6** (24.5 mg), and **1** (9.4 mg). F4 (790 mg) was subjected to reversed-phase HPLC [C_{18}, CH_3CN−H_2O (95:5), flow rate 10 mL/min] to give cholesterol. F5 (480 mg) was subjected to column chromatography on Sephadex LH-20, using CH_2Cl_2−MeOH (1:1), to give subfractions f_1−f_3. Subfraction f_1 (297 mg) was subjected to silica gel column chromatography using hexane−CH_2Cl_2−acetone (25:25:1) to give scalarolide (6.8 mg). Subfraction f_2 (32 mg) was subjected to reversed-phase HPLC [C_{18}, MeOH−H_2O (93:7), flow rate 2.3 mL/min] to give 16-acetoxyscalarolide (5 mg). Subfraction f_3 (90 mg) was subjected to reversed-phase HPLC [C_{18}, MeOH−H_2O (77:23), flow rate 8 mL/min] to give **8** (13 mg) and a mixture of **4** (10 mg). F6 (200 mg) was subjected to reversed-phase HPLC [C_{18}, MeOH−H_2O (88:12), flow rate 8 mL/min] to give **2** (12 mg), **3** (10 mg), **7** (30 mg), and **8** (12 mg).

Similan A (1): colorless solid; mp 164−165 °C; $[\alpha]^{22}_D$ −4.5 (*c* 0.16, $CHCl_3$); UV λ_{max} (MeOH) (log ε) 221 (3.7) nm; IR (UATR) ν_{max} 3371, 2922, 2851, 1766, 1720, 1462, 1386, 1376, 1090, 742 cm^{-1}; ^{1}H and ^{13}C NMR (Table 1); EIMS *m*/*z* (%) 399 [M − H]$^+$(1), 351(30), 245(100); HRAPCIMS *m*/*z* 399.2888 [M − H]$^+$ (calcd for $C_{26}H_{39}O_3$, 399.2894).

Mixture of 12β,20α-Dihydroxy-16β-acetoxy-17-scalaren-19,20-olide and 12β,20β-Dihydroxy-16β-acetoxy-17-scalaren-19,20-olide (2) (ratio ~2:1): ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.06/6.05 (s, H-20), 5.60/5.61 (dd, J = 9.8, 7.0 Hz, H-16), 3.63/3.67 (dd, J = 10.8, 4.3 Hz, H-12), 2.12/2.10 (s, 16-OCOC*H*$_3$), 1.23/1.19 (m, H-14); ^{13}C NMR (150 MHz, $CDCl_3$) δ 172.0 (C, C-19), 170.0/171.2 (C, 16-O*C*OCH$_3$), 157.4/155.2 (C, C-17), 141.5/142.3 (C, C-18), 96.4/97.7 (CH, C-20), 74.9/75.0 (CH, C-12), 67.5/68.2 (CH, C-16), 57.9/57.8 (CH, C-9), 56.6 (CH, C-5), 53.9/54.2 (CH, C-14), 42.7/43.1 (C, C-13), 42.1/42.0 (CH_2, C-3), 41.5/41.6 (CH_2, C-7), 39.6/39.7 (CH_2, C-1), 37.5/37.4 (C, C-10), 37.2/37.18 (C, C-8), 33.8/33.4 (C, C-4), 33.2/33.3 (CH_3, C-21), 25.4/25.6 (CH_2, C-11), 23.8 (CH_2, C-15), 21.3 (CH_3, C-22), 20.9/20.93 (CH_3, 16-OCO*C*H$_3$), 18.5 (CH_2, C-2), 18.2 (CH_2, C-6), 17.2/17.19 (CH_3, C-24), 16.3/16.9 (CH_3, C-25), 15.94/15.9 (CH_3, C-23); HRAPCIMS *m*/*z* 461.2911 [M + H]$^+$ (calcd for $C_{27}H_{41}O_6$, 461.2898). Treatment of the mixture of **2** (8 mg) with Ac_2O−pyridine and subsequent separation by preparative TLC (30% EtOAc−hexane) yielded compounds **2a** (4.9 mg) and **2b** (1.5 mg).

Compound 2a: colorless solid; mp 206−207 °C; $[\alpha]^{28}_D$ −7.9 (*c* 0.49, $CHCl_3$); UV λ_{max} (MeOH) (log ε) 220 (3.4) nm; IR (UATR) ν_{max} 3459, 2925, 2853, 1743, 1370, 1227, 1027, 983 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 6.90 (1H, brd, J = 0.4 Hz, H-20), 5.58 (1H, dd, J = 9.9, 7.2 Hz, H-16), 5.27 (1H, d, J = 1.3 Hz, 12−OH), 3.70 (1H, ddd, J = 10.8, 4.5, 1.3 Hz, H-12), 2.30 (1H, ddd, J = 12.8, 7.2, 1.3 Hz, H-15a), 2.15 (3H, s, 20-OCOC*H*$_3$), 2.09 (3H, s, 16-OCOC*H*$_3$), 1.89 (1H, ddd, J = 13.5, 4.5, 2.0 Hz, H-11a), 1.78 (1H, dt, J = 12.5, 3.2 Hz, H-7a), 1.72 (1H, m, H-1a), 1.71 (1H, m, H-15b), 1.58 (2H, m, H-2a, H-6a), 1.50 (1H, td, J = 13.2, 11.0 Hz, H-11b), 1.41 (2H, m, H-2b, H-6b), 1.38 (1H, m, H-3a), 1.26 (1H, m, H-14), 1.21 (3H, s, H-25), 1.11 (1H, td, J = 13.5, 4.1 Hz, H-3b), 0.94 (1H, m, H-7b), 0.91 (1H, m, H-9),

0.89 (3H, s, H-24), 0.84 (6H, s, H-21, H-23), 0.81 (3H, s, H-22), 0.78 (1H, m, H-5), 0.77 (1H, m, H-1b); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 171.5 (C, C-19), 169.9 (C, 16-O*C*OCH$_3$), 168.6 (C, 20-O*C*OCH$_3$), 155.8 (C, C-17), 142.6 (C, C-18), 90.8 (CH, C-20), 74.5 (CH, C-12), 66.5 (CH, C-16), 57.9 (CH, C-9), 56.7 (CH, C-5), 54.1 (CH, C-14), 43.0 (C, C-13), 42.1 (CH_2, C-3), 41.6 (CH_2, C-7), 39.7 (CH_2, C-1), 37.4 (C, C-10), 37.2 (C, C-8), 33.3 (C, C-4), 33.3 (CH_3, C-21), 25.5 (CH_2, C-11), 23.8 (CH_2, C-15), 21.2 (CH_3, C-22), 20.7 (CH_3, 16-OCO*C*H$_3$), 20.5 (CH_3, 20-OCO*C*H$_3$), 18.5 (CH_2, C-2), 18.1 (CH_2, C-6), 17.2 (CH_3, C-24), 16.4 (CH_3, C-25), 15.9 (CH_3, C-23); HRAPCIMS *m/z* 503.3010 $[M + H]^+$ (calcd for $C_{29}H_{43}O_7$, 503.3003).

Compound 2b: colorless solid; $[\alpha]^{28}_D$ −39.1 (*c* 0.15, $CHCl_3$); UV λ_{max} (MeOH) (log ε) 220 (3.5) nm; IR (UATR) ν_{max} 3440, 2925, 2854, 1743, 1372, 1235, 1027, 985 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 6.87 (1H, d, *J* = 1.2 Hz, H-20), 5.55 (1H, ddd, *J* = 10.4, 7.1, 1.2 Hz, H-16), 5.41 (1H, brs, 12-OH), 3.69 (1H, dd, *J* = 11.0, 4.5 Hz, H-12), 2.22 (1H, m, H-15a), 2.14 (3H, s, 20-OCOC*H*$_3$), 2.08 (3H, s, 16-OCOC*H*$_3$), 1.88 (1H, m, H-11a), 1.87 (1H, m, H-15b), 1.78 (1H, m, H-7a), 1.72 (1H, m, H-1a), 1.60 (2H, m, H-2a, H-6a), 1.51 (1H, m, H-11b), 1.40 (2H, m, H-2b, H-6b), 1.38 (1H, m, H-3a), 1.25 (3H, s, H-25), 1.21 (1H, m, H-14), 1.13 (1H, m, H-3b), 0.91 (1H, m, H-7b), 0.91 (3H, s, H-24), 0.88 (1H, m, H-9), 0.84 (6H, s, H-22, H-23), 0.83 (3H, s, H-22), 0.79 (2H, m, H-1b, H-5); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 171.5 (C, C-19), 170.4 (C, 16-O*C*OCH$_3$), 168.1 (C, 20-O*C*OCH$_3$), 153.7 (C, C-17), 143.7 (C, C-18), 93.0 (CH, C-20), 74.6 (CH, C-12), 68.4 (CH, C-16), 57.8 (CH, C-9), 56.7 (CH, C-5), 54.2 (CH, C-14), 43.3 (C, C-13), 42.0 (CH_2, C-3), 41.6 (CH_2, C-7), 39.7 (CH_2, C-1), 37.4 (C, C-10), 37.3 (C, C-8), 33.3 (C, C-4), 33.2 (CH_3, C-21), 25.8 (CH_2, C-11), 24.0 (CH_2, C-15), 21.3 (CH_3, C-22), 20.6 (CH_3, 16-OCO*C*H$_3$), 20.4 (CH_3, 20-OCO*C*H$_3$), 18.5 (CH_2, C-2), 18.2 (CH_2, C-6), 17.1 (CH_3, C-24), 16.8 (CH_3, C-25), 15.9 (CH_3, C-23); HRAPCIMS *m/z* 503.3001 $[M + H]^+$ (calcd for $C_{29}H_{43}O_7$, 503.3003).

Mixture of 12*β*-Acetoxy-20α-hydroxy-17-scalaren-19,20-olide and 12*β*-Acetoxy-20*β*-hydroxy-17-scalaren-19,20-olide (3) (ratio ~ 1:1.5): ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.68/5.80 (s, H-20), 4.91 (m, H-12), 2.13/2.12 (s, 12-OCOC*H*$_3$), 1.21/1.24 (s, H-25); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.3 (C, 12-O*C*OCH$_3$), 161.0/160.8 (C, C-17), 136.7/137.2 (C, C-18), 96.3/95.6 (CH, C-20), 76.4/76.2 (CH, C-12), 57.5/57.9 (CH, C-9), 56.4/56.5 (CH, C-5), 55.7/56.1 (CH, C-14), 42.0/41.9 (CH_2, C-3), 41.5/41.6 (CH_2, C-7), 40.8 (C, C-13), 39.5/39.6 (CH_2, C-1), 33.3/33.2 (CH_3, C-21), 24.5/24.6 (CH_2, C-11), 24.5 (CH_2, C-16), 21.9/21.8 (CH_3, 12-OCO*C*H$_3$), 21.2 (CH_3, C-22), 17.2/17.4 (CH_3, C-25), 16.0/16.3 (CH_2, C-15). Treatment of the mixture of **3** (5 mg) with Ac_2O−pyridine and subsequent separation by HPLC (C_8, 70 → 100% of MeOH in H_2O for 120 min, flow rate 2.3 mL/min) yielded the acetates **3a** (1.1 mg) and **3b** (2.4 mg).

Compound 3a: colorless solid; $[\alpha]^{23}_D$ −20.2 (*c* 0.11, $CHCl_3$); UV λ_{max} (MeOH) (log ε) 220 (3.1) nm; IR (UATR) ν_{max} 2926, 2852, 1764, 1737, 1371, 1243, 1209, 1035, 974 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 6.60 (1H, s, H-20), 4.93 (1H, dd, *J* = 11.1, 4.7 Hz, H-12), 2.32 (1H, m, H-16a), 2.22 (1H, m, H-16b), 2.15 (3H, s, 12-OCOC*H*$_3$), 2.13 (3H, s, 20-OCOC*H*$_3$), 1.94 (1H, dd, *J* = 13.5, 7.1 Hz, H-15a), 1.83 (1H, m, H-7a), 1.80 (1H, m, H-11a), 1.63 (1H, m, H-1a), 1.59 (1H, m, H-15b), 1.58 (2H, m, H-2a, H-6a), 1.53 (1H, m, H-11b), 1.43 (2H, m, H-2b, H-6b), 1.36 (1H, m, H-3a), 1.25 (3H, s, H-25), 1.17 (1H, brd, *J* = 11.5 Hz, H-14), 1.11 (1H, td, *J* = 13.2, 3.6 Hz, H-3b), 0.98 (1H, m, H-9), 0.96 (1H, m, H-7b), 0.91 (3H, s, H-24), 0.84 (3H, s, H-19), 0.83 (1H, m, H-1b), 0.83 (3H, s, H-23), 0.81 (3H, s, H-22), 0.79 (1H, m, H-5); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 171.3 (C, 12-O*C*OCH$_3$), 169.3 (C, 20-O*C*OCH$_3$), 167.1 (C, C-19), 158.1 (C, C-17), 138.2 (C, C-18), 91.6 (CH, C-20), 75.8 (CH, C-12), 57.8 (CH, C-9), 56.7 (CH, C-5), 56.0 (CH, C-14), 42.1 (CH_2, C-3), 41.7 (CH_2, C-7), 41.2 (C, C-13), 39.7 (CH_2, C-1), 37.4 (C, C-10), 37.3 (C, C-8), 33.3 (CH_3, C-21), 33.2 (C, C-4), 24.7 (CH_2, C-16), 24.6 (CH_2, C-11), 21.7 (CH_3, 12-OCO*C*H$_3$), 21.3 (CH_3, C-22), 20.6 (CH_3, 20-OCO*C*H$_3$), 18.4 (CH_2, C-2), 18.3 (CH_2, C-6), 17.3 (CH_3, C-24), 17.0 (CH_3, C-25), 16.4 (CH_2, C-15), 16.2 (CH_3, C-23); HRESIMS *m/z* 509.2873 $[M + Na]^+$ (calcd for $C_{29}H_{42}NaO_6$, 509.2874).

Compound 3b: colorless solid; $[\alpha]^{23}_D$ −14.5 (*c* 0.24, $CHCl_3$); UV λ_{max} (MeOH) (log ε) 220 (3.9) nm; IR (UATR) ν_{max} 2926, 2852, 1769, 1737, 1370, 1243, 1210, 1030, 975 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 6.63 (1H, d, *J* = 1.0 Hz, H-20), 4.92 (1H, dd, *J* = 11.2, 4.6 Hz, H-12), 2.38 (1H, dd, *J* = 9.5, 5.3 Hz, H-16a), 2.14 (3H, s, 20-OCOC*H*$_3$), 2.13 (3H, s, 12-OCOC*H*$_3$), 2.12 (1H, m, H-16b), 1.93 (1H, m, H-15a), 1.84 (1H, dt, *J* = 12.4, 3.1 Hz, H-7a), 1.77 (1H, ddd, *J* = 13.0, 4.6, 2.1 Hz, H-11a), 1.62 (1H, m, H-1a), 1.57 (2H, m, H-2a, H-6a), 1.56 (1H, m, H-15b), 1.55 (1H, m, H-11b), 1.43 (2H, m, H-2b, H-6b), 1.37 (1H, m, H-3a), 1.27 (3H, s, H-25), 1.14 (1H, dd, *J* = 9.2, 1.2 Hz, H-14), 1.12 (1H, td, *J* = 13.5, 3.9 Hz, H-3b), 0.97 (1H, m, H-9), 0.94 (1H, m, H-7b), 0.91 (3H, s, H-24), 0.84 (3H, s, H-21), 0.83 (3H, s, H-23), 0.82 (1H, m, H-1b), 0.82 (3H, s, H-22), 0.81 (1H, m, H-5); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 171.3 (C, 12-O*C*OCH$_3$), 169.2 (C, 20-O*C*OCH$_3$), 167.7 (C, C-19), 158.6 (C, C-17), 138.3 (C, C-18), 91.7 (CH, C-20), 76.0 (CH, C-12), 57.9 (CH, C-9), 56.6 (CH, C-5), 56.0 (CH, C-14), 42.0 (CH_2, C-3), 41.8 (CH_2, C-7), 41.3 (C, C-13), 39.7 (CH_2, C-1), 37.4 (C, C-10), 37.3 (C, C-8), 33.3 (CH_3, C-21), 33.2 (C, C-4), 24.7 (CH_2, C-11), 24.3 (CH_2, C-16), 21.7 (CH_3, 12-OCO*C*H$_3$), 21.3 (CH_3, C-22), 20.7 (CH_3, 20-OCO*C*H$_3$), 18.5 (CH_2, C-2), 18.3 (CH_2, C-6), 17.4 (CH_3, C-24), 17.1 (CH_3, C-25), 16.3 (CH_2, C-15), 16.0 (CH_3, C-23); HRESIMS *m/z* 509.2875 $[M + Na]^+$ (calcd for $C_{29}H_{42}NaO_6$, 509.2874).

Mixture of 12*β*,16α,20α-Trihydroxy-17-scalaren-19,20-olide and 12*β*,16α,20*β*-Trihydroxy-17-scalaren-19,20-olide (4): UV λ_{max} (MeOH) (log ε) 208 (3.4), 222 (3.6) nm; IR (UATR) ν_{max} 3336, 2925, 2852, 1726, 1570, 1457, 1388, 1310, 1094, 777 cm^{-1}; ^{1}H NMR (600 MHz, pyridine-d_5) δ 6.93 (s, H-20), 5.20 (overlap, H-16), 3.85 (brd, *J* = 6.6 Hz, H-12), 2.13 (m, H-15a), 1.96 (m, H-11a), 1.95 (m, H-11b), 1.87 (m, H-15b), 1.74 (m, H-7a), 1.72 (m, H-14), 1.58 (m, H-1a), 1.51 (m, H-2a), 0.67 (m, H-1b), 1.31 (m, H-2b), 1.29 (m, H-3a), 1.07 (m, H-3b), 0.64 (m, H-5), 1.45 (m, H-6a), 1.29 (m, H-6b), 1.02 (m, H-7b), 1.12 (s, H-25), 0.81 (s, H-24), 0.80 (s, H-19), 0.79 (m, H-9), 0.75 (s, H-22), 0.74 (s, H-23); ^{13}C NMR (150 MHz, pyridine-d_5) δ 162.3 (C, C-17), 139.4 (C, C-18), 99.1 (C-20), 75.4 (CH, C-12), 60.3 (CH, C-16), 57.8 (CH, C-9), 56.7 (CH, C-5), 49.5 (CH, C-14), 43.1 (C, C-13), 42.0 (CH_2, C-3), 41.3 (CH_2, C-7), 39.6 (CH_2, C-1), 37.2 (C, C-10), 36.8 (C, C-8), 33.1 (C, C-4), 33.1 (CH_3, C-21), 27.6 (CH_2, C-15), 26.2 (CH_2, C-11), 21.2 (CH_3, C-22), 18.6 (CH_2, C-2), 18.1 (CH_2, C-6), 17.1 (CH_3, C-24), 15.8 (CH_3, C-23), 14.9 (CH_3, C-25); HRAPCIMS *m/z* 419.2804 $[M + H]^+$ (calcd for $C_{25}H_{39}O_5$, 419.2792). Treatment of the mixture of **4** (4 mg) with Ac_2O−pyridine and subsequent separation by preparative TLC (EtOAc−hexane−CH_2Cl_2, 1:3:3) yielded compounds **4a** (0.8 mg) and **4b** (2 mg).

Compound 4a: colorless solid; $[\alpha]^{25}_D$ +27.8 (*c* 0.08, $CHCl_3$); UV λ_{max} (MeOH) (log ε) 220 (3.2) nm; IR (UATR) ν_{max} 3460, 2924, 2854, 1740, 1462, 1374, 1240, 1080, 970 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 6.86 (1H, s, H-20), 5.63 (1H, brd, *J* = 4.1 Hz, H-16), 5.37 (1H, brs, 12-OH), 3.75 (1H, brdd, *J* = 10.6, 4.1 Hz, H-12), 2.10 (3H, s, 20-OCOC*H*$_3$), 2.098 (3H, s, 16-OCOC*H*$_3$), 1.96 (2H, m, H-15), 1.92 (1H, m, H-11a), 1.74 (2H, m, H-1a, H-7a), 1.60 (2H, m, H-2a, H-6a), 1.52 (1H, m, H-11b), 1.45 (1H, m, H-14), 1.44 (2H, m, H-2b, H-6b), 1.38 (1H, m, H-3a), 1.12 (1H, td, *J* = 12.8, 3.6 Hz, H-3b), 1.11 (3H, s, H-25), 0.97 (1H, m, H-9), 0.90 (1H, m, H-7b), 0.88 (3H, s, H-24), 0.86 (3H, s, H-21), 0.85 (3H, s, H-23), 0.82 (1H, m, H-5), 0.82 (3H, s, H-22), 0.74 (1H, m, H-1b); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 171.3 (C, C-19), 169.5 (C, 16-O*C*OCH$_3$), 168.2 (C, 20-O*C*OCH$_3$), 152.0 (C, C-17), 145.3 (C, C-18), 93.0 (CH, C-20), 74.2 (CH, C-12), 63.1 (CH, C-16), 57.8 (CH, C-9), 56.7 (CH, C-5), 50.2 (CH, C-14), 43.3 (C, C-13), 42.2 (CH_2, C-3), 41.4 (CH_2, C-7), 39.8 (CH_2, C-1), 37.5 (C, C-10), 36.8 (C, C-8), 33.3 (C, C-4), 33.28 (CH_3, C-21), 25.8 (CH_2, C-11), 24.4 (CH_2, C-15), 21.3 (CH_3, C-22), 20.8 (CH_3, 16-OCO*C*H$_3$), 20.3 (CH_3, 20-OCO*C*H$_3$), 18.6 (CH_2, C-2), 18.2 (CH_2, C-6), 17.0 (CH_3, C-24), 15.9 (CH_3, C-23), 14.6 (CH_3, C-25); HRESIMS *m/z* 525.2826 $[M + Na]^+$ (calcd for $C_{29}H_{42}O_7Na$, 525.2823).

Compound 4b: colorless solid; $[\alpha]^{23}_D$ −4.7 (*c* 0.20, $CHCl_3$); UV λ_{max} (MeOH) (log ε) 220 (3.3) nm; IR (UATR) ν_{max} 3450, 2926, 2852, 1743, 1371, 1225, 1205, 1055, 984 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 6.82 (1H, s, H-20), 5.47 (1H, brd, *J* = 4.1 Hz, H-16), 5.33 (1H, brs, 12-OH), 3.73 (1H, brdd, *J* = 10.9, 4.5 Hz, H-12), 2.19 (3H, s, 20-OCOC*H*$_3$), 2.10 (3H, s, 16-OCOC*H*$_3$), 2.01 (1H, m, H-15a), 1.94 (1H, m, H-15b), 1.90 (1H, m, H-11a), 1.75 (1H, m, H-7a), 1.73 (1H, m, H-1a), 1.62 (1H, m, H-2a), 1.58 (1H, m, H-6a), 1.52 (1H, m, H-11b), 1.43 (1H, m, H-2b), 1.42 (1H, m, H-6b), 1.38 (1H, m, H-3a), 1.35 (1H, m, H-14), 1.14 (3H, s, H-25), 1.12 (1H, td, *J* = 13.6, 4.1 Hz, H-3b), 0.93 (1H, brd, *J* = 11.4 Hz, H-9), 0.89 (3H, s, H-24), 0.86 (1H, m, H-7b), 0.86 (3H, s, H-21), 0.85 (3H, s, H-23), 0.82 (3H, s, H-22), 0.81 (1H, m, H-1b); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 171.9 (C, C-19), 170.1 (C, 16-O*C*OCH$_3$), 168.7 (C, 20-O*C*OCH$_3$), 153.7 (C, C-17), 144.3 (C, C-18), 91.8 (CH, C-20), 74.4 (CH, C-12), 62.5 (CH, C-16), 57.8

(CH, C-9), 56.5 (CH, C-5), 50.1 (CH, C-14), 43.1 (C, C-13), 42.1 (CH_2, C-3), 41.4 (CH_2, C-7), 39.7 (CH_2, C-1), 37.4 (C, C-10), 36.8 (C, C-8), 33.3 (C, C-4), 33.26 (CH_3, C-21), 25.8 (CH_2, C-11), 24.1 (CH_2, C-15), 21.3 (CH_3, C-22), 20.9 (CH_3, 16-OCO*C*H_3), 20.5 (CH_3, 20-OCO*C*H_3), 18.6 (CH_2, C-2), 18.2 (CH_2, C-6), 17.1 (CH_3, C-24), 16.0 (CH_3, C-23), 14.9 (CH_3, C-25); HRAPCIMS *m/z* 503.3006 $[M + H]^+$ (calcd for $C_{29}H_{43}O_7$, 503.3003).

Cytotoxicity Assay. The cytotoxicity assay was performed using the method as previously described.[19]

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Supporting Information Available: ^1H and ^{13}C NMR spectra of **1**−**4**, **2a**−**4a**, and **2b**−**4b** are available free of charge via the Internet at http://pubs.acs.org.

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