Scalarane Sesterterpenoids: Semisynthesis and Biological Activity

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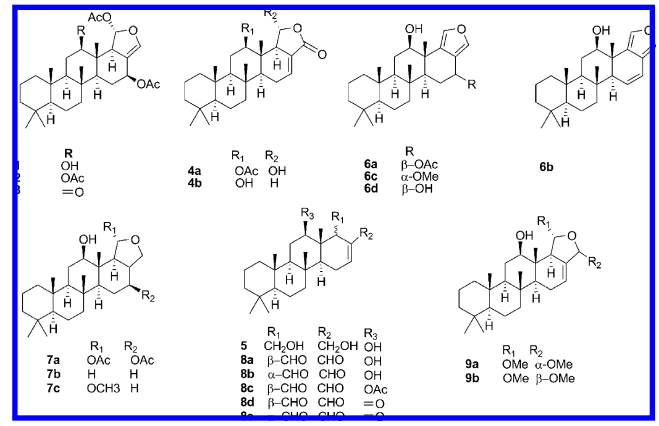
Received June 1, 2009

Aiming to improve the potency and selectivity of scalarane sesterterpenoids, a series of natural and semisynthetic analogues, derived from the cytotoxic naturally abundant sesterterpene heteronemin (**1**), were evaluated for their in vitro antimicrobial and cytotoxic properties. The new sesterterpenes 16-*O*-methylsesterstatin 4 (**6c**), 17, 24-dihydroheteronemin (**7a**), 16, 25-deacetoxy-17, 24-dihydroheteronemin (**7b**), and 16-deacetoxy-25-methoxy-17, 24-dihydroheteronemin (**7c**) were structurally defined via physical data analyses. Scalarane sesterterpenes possessing an unsaturated 1,4-dialdehyde moiety showed potent inhibitory activity against methicillin-resistant *Staphylococcus aureus* at concentrations that are not significantly cytotoxic to mammalian cells. The structural features for the cytotoxicity of scalarane sesterterpenoids are discussed.

Scalaranes constitute an important structural class of sesterterpenes prominent in Dictyoceratida sponges, e.g., *Hyrtios erecta*,[1] *Spongia* spp.,[2] *Hyatella intestinalis*,[3] *Phyllospongia madagascarensis*,[4] and *Cacospongia scalaris*.[5] These sesterterpenes have also been isolated from nudibranchs associated with the sponges, and hence, the sesterterpenoids in the nudibranch were suggested to have a dietary origin.[6] Several structural classes of the scalarane sesterterpenoids were isolated including C_{25} (scalarane), C_{26} (homoscalarane), and C_{27} (bishomoscalarane) compounds containing tetra- and pentacyclic skeletons.[14] The structural diversity in the scalarane family mainly arises from the different oxidation states at C-24 and C-25.[15] The structural types of natural scalarane sesterterpenes have been outlined by Crews.[14] All of them possess a conserved *trans*-configuration of the A/B/C/D ring junctions, and the majority are characterized by oxygenation at C-12. Natural scalaranes with structural diversity in the A, D, and/or E ring systems have been reported.[10d,14,16] Additionally, a radical relay hydrogenation synthetic method has recently been used to effect B ring functionalization.[15]

Owing to their diverse biological properties, interest in scalarane sesterterpenes and their synthesis has increased noticeably in recent years. This family of compounds have been reported to have anti-inflammatory,[7] antimicrobial,[8] platelet-aggregation inhibitory,[9] and cytotoxic[10] activities. In addition, the role of these compounds in the defensive mechanism of sponges has been demonstrated, e.g., their antifeedant,[11] antifouling,[12] and ichthyotoxic[13] activities. Despite the large number of scalarane sesterterpenes exhibiting moderate to significant cytotoxic activities, there are no systematic reports on their structure−cytotoxicity relationship. Additionally, further biological studies were prohibited due to the nonselective cytotoxicities associated with some analogues. Herein, a series of semisynthetic and natural scalarane sesterterpenes were evaluated for their in vitro antimicrobial and cytotoxic properties. Structural features for their cytotoxicity were defined and antimicrobial activities for a number of scalaranes at nontoxic concentrations were found.



Results and Discussion

In our semisynthetic approach, we used the sesterterpene heteronemin (**1**), available in large quantities from the sponge *Hyrtios* sp.,[17] as a starting material for the synthesis of a series of scalaranes including four new (**6c**, **7a**−**7c**) and 15 known analogues previously isolated in low yields from marine sponges. Heteronemin (**1**) is an abundant sesterterpene first reported from the sponge *Heteronema erecta* in 1976[17] and subsequently identified as an antitubercular[8b] and a farnesyl transferase inhibitor.[18] However, its strong but nonselective cytotoxicity prevented further biological studies.

Structurally, heteronemin (**1**) possesses a pentacyclic scalarane skeleton including a dihydrofuran moiety. It has nine stereogenic centers, a secondary hydroxy group at C-12, an acetal moiety at C-25, and a trisubstituted C-17−C-24 double bond. The arrangement of the various functionalities is unique and allows for numerous chemical transformations. Treatment of heteronemin (**1**) with acetic anhydride in pyridine afforded 16-*O*-acetylheteronemin (**2**)[19] (Scheme 1), while oxidation with pyridinium chlorochromate afforded 12-oxoheteronemin (**3**) as the major product (Scheme 2). 12-Episcalarin (**4a**)[20] was available from the sponge extract. The triol **5** was obtained from heteronemin (**1**) via $NaBH_4$ reduction. Oxidation of **5** with an excess of barium permanganate (10−20 equiv) at room temperature afforded 12-*O*-deacetyl-25-deoxy-12-episcalarin (**4b**) (Scheme 1). The ease of oxidation of the allylic C-24 alcohol into the carboxylic acid functionality resulted in rapid lactone ring formation. The product **4b** is identical to the reported naturally occurring material.[20]

Next, we investigated the conversion of heteronemin (**1**) into scalarane-based furanosesterterpenes. Treatment of heteronemin (**1**)

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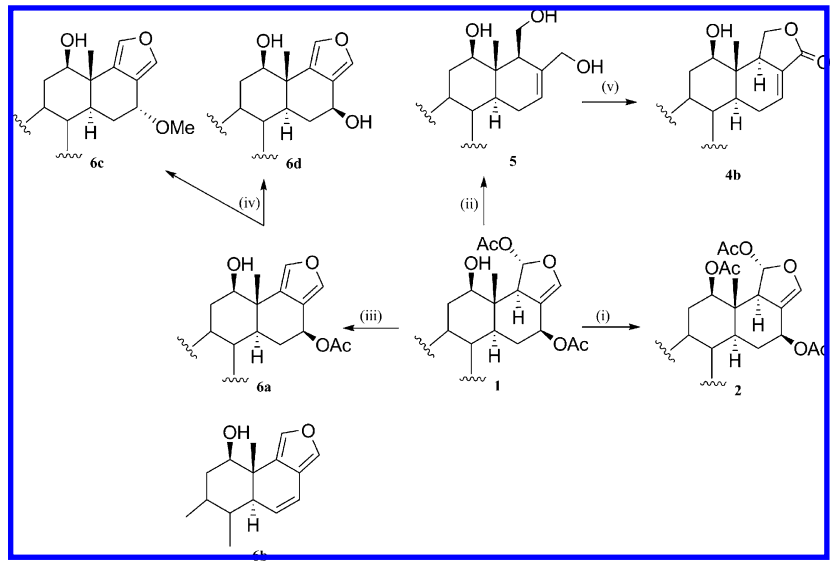
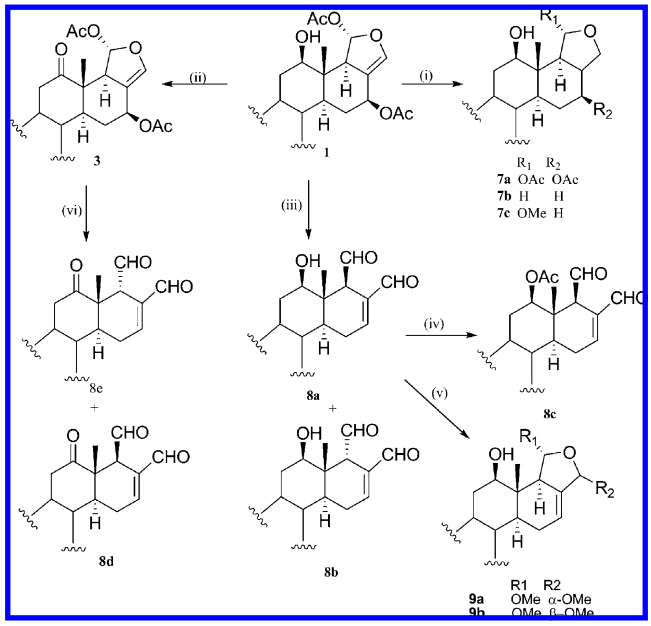
10.1021/np900326a CCC: $40.75
Published on Web 08/06/2009

Scheme 1[a]



[a] Reagents and conditions: (i) Ac_2O, pyr; (ii) $NaBH_4$, MeOH, rt, 1 h; (iii) *p*-TsOH, CH_2Cl_2, reflux, 3 h; (iv) KOH, MeOH, rt, 24 h; (v) $Ba(MnO_4)_2$, CH_2Cl_2, rt, 24 h.

Scheme 2[a]



[a] Reagents and conditions: (i) H_2/Pd−C, anhydrous MeOH, rt, 24 h; (ii) PCC/silica gel, CH_2Cl_2, rt, 2 h; (iii) $NaHCO_3$, MeOH, rt, 5 min; (iv) Ac_2O, pyr; (v) MeOH, ion exchange resin, rt; (vi) K_2CO_3, MeOH, rt, 2 h.

with 1 equiv of *p*-toluenesulfonic acid at room temperature in CH_2Cl_2 for 30 min yielded the furan analogue scalarafuran (**6a**). However, if an excess of *p*-TsOH is used (10−20 equiv) or if the reaction is extended for longer periods of time (3 h), the C-15−C-16 vinylic furan, 16-deacetoxy-15,16-dehydroscalarafuran (**6b**), is obtained as the major product. It is notable that under these time-extended conditions the C-12 equatorial hydroxy group is not involved in a similar dehydration reaction. The conformational rigidity of the C ring would not permit a conformation in which the C-12 hydroxy group and a proton at C-11 would attain the requisite *trans*-diaxial relationship for a dehydration reaction. Scalarafuran (**6a**) was also obtained from heteronemin (**1**) via pyrolysis following reported procedures,[11] albeit in a low yield. Hydrolysis of scalarafuran (**6a**) in base/methanol afforded 16-*O*-methylsesterstatin 4 (**6c**) and sesterstatin 5[21] (**6d**). Compound **6c**

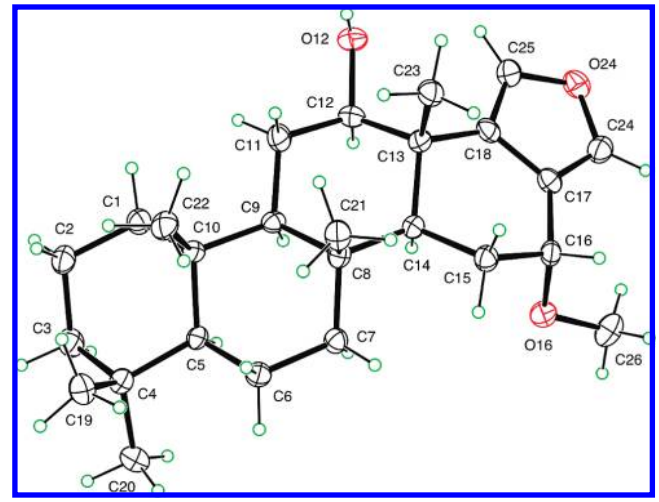


Figure 1. ORTEP drawing of compound **6c**.

is a new analogue, formed presumably via solvolysis of the allylic *O*-acetyl group in a predominantly S_N2 fashion.

Compound **6b** has a molecular formula of $C_{25}H_{36}O_2$ ([M + H]$^+$ 369.2782). Its ^{13}C NMR spectrum lacked the signals pertaining to the acetoxy functionality as well as the methine signal at δ 68.1 (C-16) in **6a** and, instead, displayed two olefinic methine signals at δ 119.2 and 127.6. Its ^1H NMR spectrum displayed two olefinic proton signals at δ 5.79 and 6.50. Analysis of the HMBC correlations confirmed the structure as 16-deacetoxy-15,16-dehydroscalarafuran. This compound was claimed to be formed via pyrolysis of heteronemin.[17] However, no NMR data to support the structure were presented. Compound **6c** has a molecular formula of $C_{26}H_{40}O_3$ [(M)$^+$ 400.2924]. The structure was elucidated by comparison of ^1H and ^{13}C NMR data with those of sesterstatin 5[21] (**6d**). It displayed an *O*-methyl signal in its ^{13}C NMR spectrum at δ 56.5, which correlated in the HMQC spectrum with a singlet at δ 3.35 (3H), suggesting the presence of an *O*-methyl group at C-16. The structure and configuration were confirmed by X-ray crystallography (Figure 1).

Heteronemin (**1**) undergoes reduction with H_2 in the presence of Pd/C in MeOH to give a mixture of the sesterterpenes **7a**−**7c**. These 17,24-dihydroheteronemin derivatives are reported for the first time. Compound **7a** exhibited a molecular ion peak at *m*/*z*

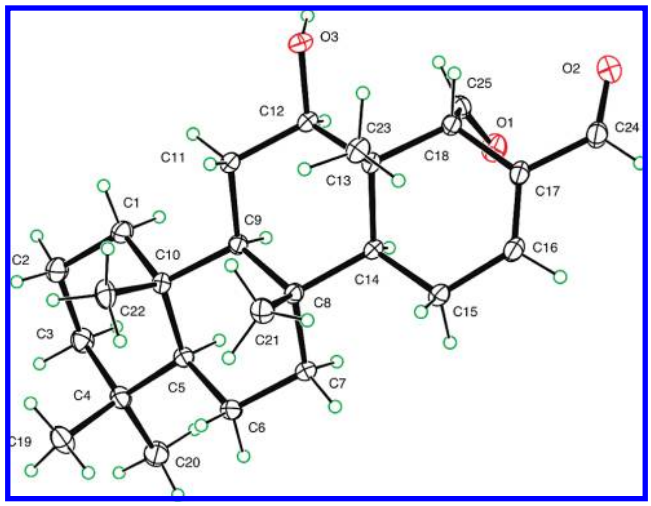


Figure 2. ORTEP drawing of compound **8b**.

Table 1. Cytotoxicity and Antimicrobial Data[a]

compound	SKOV3	SKMEL	BT549	Vero	MRSA	M. int.
1	3.4	15.3	11.2	8.2	NA	2.04
2	18.8	16.7	18.8	9.0	NA	18.8
3	>37.6	NA	>37.6	>37.6	NA	5.1
4a−4b	NA	NA	NA	NA	NA	NA
5	NA	NA	NA	NA	NA	NA
6a−6d	NA	NA	NA	NA	NA	NA
7a	18.3	>37.3	15.9	36.7	NA	NA
7b−7c	NA	NA	NA	NA	NA	NA
8a	31	26.4	19.4	19.4	22	38.8
8b	19.4	18.6	12.9	20.7	3.8	NA
8c	23.3	>42.7	21.0	22.2	6.3	NA
8d	41.6	14.6	15.6	18.2	27.8	NA
8e	31.2	14.3	15.6	15.6	30.1	NA
9a−9b	NA	NA	NA	NA	NA	NA
Ciprofloxacin					0.14	0.47
Doxorubicin	0.8	1.2	1.2	6.3		

[a] MRSA = methicillin-resistant *Staphylococcus aureus*, M. int. = *Mycobacterium intracellulare*, NA = not active. Data shown are the IC_{50} values in μM.

431.3236 ([M − OAc]$^+$), suggesting a molecular formula of $C_{29}H_{46}O_6$ with seven degrees of unsaturation. The ^{13}C NMR spectrum lacked the olefinic carbons at δ 114.0 and 134.9 in heteronemin (**1**) and displayed an oxymethylene carbon at δ 70.5 (C-24) and a methine carbon at δ 37.4 (C-17). On the basis of these results, **7a** was defined as 17,24-dihydroheteronemin. Compound **7b** had a molecular formula of $C_{25}H_{42}O_2$ (*m*/*z* 375.3257 [M + H]$^+$). The ^{13}C NMR spectrum lacked the olefinic (δ 114.0, 134.9) and ester carbonyl carbons (δ 170.3, 172.1) but displayed two oxymethylene carbons at δ 71.1 and 72.0 and an additional methylene carbon at δ 16.4, indicating hydrogenolysis of the allylic C-16 *O*-acetyl group. Detailed analysis of the 2D NMR data indicated the structure deduced for **7b** (16,25-deacetoxy-17,24-dihydroheteronemin) as shown. The molecular formula of **7c** was defined as $C_{26}H_{44}O_3$ from a molecular ion peak at *m*/*z* 427.3067 ([M + Na]$^+$) in the HRESIMS spectrum. The ^{1}H NMR spectrum exhibited an *O*-methyl resonance at δ 3.37, which correlated with the carbon at δ 55.2 in the HMQC spectrum and the carbon at δ 108.5 in the HMBC spectrum, indicating the attachment of the *O*-methyl group at C-25 of the scalarane skeleton. The structure was then confirmed by 2D NMR analysis. Compound **7c** originated via *trans*-acetalization at C-25 and hydrogenolysis of the allylic 16-*O*-acetyl group of heteronemin (**1**) in methanolic solution.

We then turned our attention to methods of synthesizing scalarane sesterterpenes possessing α,β-unsaturated dialdehyde groups to provide additional insights into structure−cytotoxicity relationships. Hydrolysis of heteronemin (**1**) under basic conditions gave 12-*O*-deacetyl-12-episcalaradial (**8a**) (Scheme 2) via acetal hydrolysis and subsequent dehydration involving the C-16 hydroxy group. Compound **8a** undergoes facile epimerization at C-18 to give 12-*O*-deacetyl-12,18-diepiscalaradial[23] (**8b**), whose structure was confirmed via X-ray crystallography (Figure 2). The vicinal dialdehyde functionality in **8a** is analogous to that of the antifeedant drimane sesquiterpene polygodial.[24] 12-Episcalaradial[23] (**8c**) was available from **8a** via direct acetylation.[19] 12-*O*-deacetyl-12-oxoscalaradial (**8d**) and 12-*O*-deacetyl-18-epi-12-oxoscalardial (**8e**) were prepared by treating 12-oxoheteronemin (**3**) with potassium carbonate in methanol. The physical data of the above compounds are in accordance with those reported.[24]

The dimethylacetals 12-*O*-deacetyl-24,25-dimethoxyscalarins **9a** and **9b** were readily obtained by treatment of 12-*O*-deacetyl-12-episcalaradial (**8a**) with an acidic ion-exchange resin in MeOH with the major product (**9a**) having both methoxy groups α-oriented. The ^{1}H NMR chemical shifts of H-24 in **9a** and **9b** were similar to those reported,[19] confirming *syn*- and *anti*-1,3-bismethoxy groups for **9a** (H-24 = 5.17) and **9b** (H-24 = 5.47), respectively. Dimethylacetals of dialdehydes have been reported as natural products, although the authors have often considered them artifacts due to the use of MeOH during extraction and/or isolation.[19,25]

The natural and synthesized scalaranes were evaluated for their cytotoxicity against SK-MEL melanoma, SKOV3 ovarian, and BT549 breast cancer cell lines, as well as their antimicrobial activity against *Mycobacterium intracellulare* and methicillin-resistant *Staphylococcus aureus* (MRSA). The IC_{50} values are presented in Table 1. It was originally suggested by Crews and Bescana that the oxygenation pattern at C-25 of the scalarane sesterterpene exerts a strong influence on their biological activities.[19] Such an influence was evident among the scalaranes tested, in which the furanosesterterpenes **6a−6d**, lacking an oxygen functionality at C-25, were not cytotoxic at doses up to 45 μM. Our data also suggest that the oxygenation pattern at C-16 is similarly influential on the activity since the 16-deoxy sesterterpenes **4a** and **4b** lack cytotoxicity at doses up to 45 μM. Alternatively, the C-17−C-24 double bond slightly affects the cytotoxicity of the scalaranes, as evident in compound **7a**. The dialdehydes **8a−8d** exhibited cytoxicity against all the cancer cell lines tested. Natural products with an 1,4-unsaturated dialdehyde functionality have been proposed to exert their cytotoxicity by reacting with proteins in the cell membrane via the formation of adducts between the unsaturated dialdehyde moieties and the amino groups of amino acid residues.[26] Heteronemin (**1**) and pentacylic sesterterpenes, lacking the dialdehyde moiety, exhibit their cytotoxicity via a different mechanism. However, heteronemin (**1**) may act as a prodrug of the dialdehyde that may be unmasked via hydrolysis of the C-25 ester functionality.

Evaluation of the antimicrobial activities of the scalaranes showed that sesterterpene **8b** is a potent inhibitor of MRSA with an IC_{50} value of 3.8 μM, while its epimer (**8a**) was less potent at 6.3 μM. This is in agreement with the suggestion that the extent of antimicrobial activity of bicyclic sesquiterpenes with dialdehyde groups such as warburganal and polygodial depends on the stereochemical arrangement of B-ring-oxygenated substituents.[27] It has been suggested that the unsaturated dialdehyde functionality and its reactivity toward biological nucleophiles is responsible for the general antibiotic activity of these compounds.[25] Unsaturated dialdehydes are sensitive; for example, **8a** and **8b** interconvert via epimerization at alkaline pH, which may affect their reactivity and bioactivity.

Scalarane sesterterpenes were reported to possess nonselective moderate to significant cytotoxic activities, which, in many cases, inhibited further biological studies. Here we report the preparation and biological testing of 20 natural and semiynsthetic sesterterpene analogues. Our data indicate that oxygenation at both C-16 and C-25 of the scalarane skeleton exerts a strong influence on their cytotoxicities. Recent studies reported scalaranes with an oxygen

functionality at C-3 of the A ring from the sponge *Hyrtios* with selective activity on the P-388 murine lymphatic leukemia cell line at nanomolar concentrations.[28] This data suggests that the structure of ring A may play a significant but less obvious role in their mechanism of action. Our results also identified 12-*O*-deacetyl-12,18-diepiscalaradial (**8b**) and 12-episcalaradial (**8c**) as potent inhibitors of MRSA at concentrations that are not significantly cytotoxic to mammalian cells.

Experimental Section

General Experiment Procedures. Optical rotations were measured with a JASCO DIP-370 digital polarimeter. UV spectra were recorded on an Agilent Chemstation with 8453 spectrophotometer. NMR spectra were recorded on a Bruker Avance DRX-400 spectrometer. ^{1}H and ^{13}C NMR spectra were measured and reported in ppm by using the $CDCl_3$ and DMSO-d_6 solvent peaks (δ_H 7.24 and δ_C 77.2 and δ_H 2.50 and δ_C 39.5, respectively) as internal standards. ESIFTMS were recorded on a Bruker-Magnex BioAPEX 30es ion cyclotron HR HPLC-FT spectrometer by direct injection into an electrospray interface. Chromatographic separations were carried out by conventional column chromatography on Sorbent silica gel 60 (230 × 450 mesh).

Animal Material. The sponge *Hyrtios* sp. was collected from American Samoa in February 2004 and identified by one of the authors (M.S.). A voucher specimen (AS40204070) was deposited at the NOAA Ocean Biotechnology Center and Repository, Oxford, MS.

12-Oxoheteronemin (3). Pyridinium chlorochromate (8.6 mg) was ground to a fine powder with 10 mg of silica gel, and the light orange mixture was suspended in 2 mL of CH_2Cl_2. Heteronemin **1** (10 mg in 1 mL of CH_2Cl_2) was added in one portion to the orange suspension, and the mixture was stirred for 2 h at rt. The reaction mixture was filtered through Celite and the solvent removed under vacuum. The residue was dissolved in $CHCl_3$ (10 mL), washed with H_2O (2 × 10 mL), dried over anhydrous Na_2SO_4, filtered, and evaporated to give a crude residue, which after column chromatography (hexanes/EtOAc, 8:2) afforded **3**[19] as a white solid (6 mg, 65%).

12-*O*-Deacetyl-25-deoxy-12-episcalarin (4b). To a solution of the triol **5** (8 mg) in CH_2Cl_2 (2 mL) was added $Ba(MnO_4)_2$ (40 mg), and the mixture was stirred for 24 h at rt. Filtration through a Celite pad and removal of the solvent gave a residue, which after column chromatography (hexanes/EtOAc, 8:2) afforded **4b**[20] as a white solid (4 mg, 50%).

Triol 5. To a solution of heteronemin (**1**) (25 mg) in MeOH (5 mL) was added an excess of $NaBH_4$. The mixture was stirred at rt for 1 h. After acidification with HOAc (1 mL) and addition of H_2O (20 mL), the aqueous mixture was extracted with CH_2Cl_2 (2 × 10 mL) and dried over anhydrous Na_2SO_4, and the solvent was evaporated under vacuum. The resultant residue was chromatographed over silica gel, eluting with 5% MeOH in $CHCl_3$ to afford **5**[19] as a white solid (16 mg, 87%).

Scalarafuran (6a). Heteronemin (**1**) (10 mg) was stirred with *p*-TsOH (4 mg, 1 equiv) in CH_2Cl_2 (5 mL) for 30 min at rt. The reaction mixture was diluted with H_2O (20 mL) and extracted with $CHCl_3$ (3 × 10 mL). The combined organic phases were dried over anhydrous Na_2SO_4, filtered, and evaporated to afford a crude residue, which after column chromatography (hexanes/EtOAc, 9:1) gave **6a**[22] as a white solid (5.2 mg, 60%).

16-Deacetoxy-15,16-dehydroscalarafuran (6b). Heteronemin (**1**) (20 mg) was stirred with *p*-TsOH (38 mg) in CH_2Cl_2 (5 mL), and the mixture was heated to reflux for 30 min. The reaction mixture was diluted with H_2O (20 mL) and extracted with $CHCl_3$ (3 × 10 mL). The combined organic phases were dried over anhydrous Na_2SO_4, filtered, and evaporated to afford a crude residue, which after column chromatography (hexanes/EtOAc, 9:1) gave **6a** (1 mg, 10%) and **6b** (6.2 mg, 45%) as white solids. Compound **6b**: $[\alpha]^{25}_D$ −9.2 (*c* 0.5, $CHCl_3$); IR (film) ν_{max} 3429, 2931, 1735, 1383, 1213, 763 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.42 (1H, s, H-25), 7.24 (1H, s, H-24), 6.50 (1H, dd, J = 9.8, 3 Hz, H-16), 5.79 (1H, dd, J = 9.8, 2.4 Hz, H-15), 3.88 (1H, bd, J = 10 Hz, H-12), 1.94 (1H, bs, H-14), 1.91 (1H, m, H-7), 1.84 (1H, dd, J = 10, 2 Hz, H-11), 1.71 (1H, m, H-1), 1.65 (1H, m, H-6), 1.59 (1H, m, H-2), 1.55 (1H, m, H-11), 1.45 (1H, m, H-6), 1.43 (1H, m, H-2), 1.37 (1H, m, H-3), 1.15 (1H, m, H-3), 1.04 (3H, s, H-23), 1.00 (3H, s, H-21), 0.96 (1H, m, H-9), 0.92 (1H, m, H-7), 0.88 (3H, s, H-22), 0.85 (3H, s, H-19), 0.84 (1H, m, H-1), 0.83 (3H, s, H-20) 0.79 (1H, m, H-5); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 136.3 (C-25), 135.6 (C-24), 132.6 (C-18), 127.6 (C-15), 121.3 (C-17), 119.2 (C-16), 78.9 (C-12), 58.4 (C-9), 57.0 (C-14), 56.9 (C-5), 42.2 (C-3), 40.7 (C-7), 40.5 (C-13), 39.8 (C-1), 37.2 (C-10), 37.0 (C-8), 33.5 (C-4, C-19), 28.1 (C-11), 21.5 (C-20), 18.8 (C-6), 18.7 (C-21), 18.1 (C-2), 16.3 (C-22), 16.2 (C-23); HRESIMS *m/z* 369.2782 $[M + H]^+$ (calcd for $C_{25}H_{37}O_2$, 369.2793).

16-*O*-Methylsesterstatin 4 (6c). A solution of scalarafuran (**6a**) in MeOH (3 mg in 1 mL) was stirred with KOH (7 mg) at rt for 24 h. The reaction was diluted with water (5 mL) and extracted with CH_2Cl_2 (3 × 5 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered, and evaporated to afford a crude residue, which after column chromatography (hexanes/EtOAc, 8:2) gave **6c** (1 mg, 35%) and **6d** (1.2 mg, 45%). Compound **6c**: $[\alpha]^{25}_D$ −6.3 (*c* 0.2, $CHCl_3$); IR (film) ν_{max} 3439, 2932, 1461, 1387, 1087, 756 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.57 (1H, d, J = 1.2 Hz, H-25), 7.36 (1H, d, J = 1.2 Hz, H-24), 4.36 (1H, d, J = 1.6 Hz, H-16), 3.70 (1H, bd, J = 10 Hz, H-12), 3.35 (3H, s, OMe), 1.86 (1H, m, H_α −15), 1.55 (1H, m, H_β-15); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 138.9 (C-24), 137.6 (C-25), 133.4 (C-18), 120.5 (C-17), 79.4 (C-12), 70.6 (C-16), 58.5 (C-5), 56.5 (OMe), 55.5 (C-14), 49.3 (C-13), 42.0 (C-3), 41.5 (C-7), 40.5 (C-8), 39.8 (C-1), 37.4 (C-9), 36.9 (C-10), 33.3 (C-4), 33.2 (C-19), 28.0 (C-11), 24.2 (C-15), 21.2 (C-20), 18.7 (C-6), 18.5 (C-21), 18.1 (C-2), 16.4 (C-22), 16.2 (C-23); HRESIMS *m/z* 400.2924 $[M]^+$ (calcd for $C_{26}H_{40}O_3$, 400.2977).

17,24-Dihydroheteronemin (7a), 16,25-Deacetoxy-17,24-dihydroheteronemin (7b), and 16-Deacetoxy-25-methoxy-17,24-dihydroheteronemin (7c). A solution of heteronemin (**1**) in anhydrous MeOH (19 mg 2 mL) containing 10% palladium on charcoal catalyst was stirred under an atmosphere of H_2 for 24 h. The catalyst was removed by filtration and the solvent evaporated to obtain a mixture, which after column chromatography (hexanes/EtOAc, 9:1) afforded **7a** (2.8 mg, 16%), **7b** (1.8 mg, 13%), and **7c** (6.4 mg, 45%) as white solids. Compound **7a**: $[\alpha]^{25}_D$ −2.4 (*c* 0.1, $CHCl_3$); IR (film) ν_{max} 3404, 2938, 1676, 1440, 1387, 1205, 1142 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.39 (1H, s, H-25), 5.07 (1H, t, J = 6 Hz, H-16), 3.92 (1H, dd, J = 11, 8.8 Hz, H_α-24), 3.39 (1H, bd, J = 11 Hz, H_β-24), 3.10 (1H, bd, J = 9 Hz, H-12), 2.07 (3H, s, AcO-16), 2.04 (3H, s, AcO-25); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.1 (Ac-25), 170.3 (Ac-16), 104.9 (C-25), 83.1 (C-12), 72.9 (C-16), 70.5 (C-24), 59.3 (C-18), 58.9 (C-9), 56.7 (C-5), 54.6 (C-15), 42.1 (C-3), 41.8 (C-1), 40.8 (C-8), 39.9 (C-7), 37.8 (C-10), 37.6 (C-13), 37.4 (C-17), 33.3 (C-4), 33.2 (C-19), 26.0 (C-11), 21.4 (Ac-25), 21.2 (Ac-16), 21.0 (C-20), 18.6 (C-6), 18.2 (C-2), 18.0 (C-21), 16.2 (C-22), 11.5 (C-23); HRESIMS *m/z* 431.3236 $[M - OAc]^+$ (calcd for $C_{27}H_{43}O_4$, 431.3161). Compound **7b**: $[\alpha]^{25}_D$ −8.6 (*c* 0.2, $CHCl_3$); IR (film) ν_{max} 2924, 1683, 1463, 1206 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.37 (1H, dd, J = 9, 2 Hz, H_α-24) 3.82 (2H, brd, J = 9 Hz, H_β-24, H_α-25), 3.43 (1H, dd, J = 12, 9 Hz, H_β-25), 3.33 (1H, bd, J = 9 Hz, H-12); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 84.8 (C-12), 72.0 (C-25), 71.1 (C-24), 58.7 (C-9), 56.6 (C-5), 56.3 (C-18), 53.0 (C-14), 42.1 (C-3), 41.6 (C-1), 41.1 (C-8), 39.9 (C-7), 37.9 (C-10), 37.6 (C-17), 37.4 (C-13), 33.2 (C-4, C-19), 27.8 (C-11), 24.2 (C-15), 21.2 (C-20), 18.6 (C-6), 18.2 (C-2), 17.4 (C-21), 16.4 (C-16), 16.2 (C-22), 11.7 (C-23); HRESIMS *m/z* 375.3257 $[M + H]^+$ (calcd for $C_{25}H_{43}O_2$, 375.3263). Compound **7c**: $[\alpha]^{25}_D$ −12 (*c* 0.5, $CHCl_3$); IR (film) ν_{max} 3421, 2927, 2874, 1466, 1390, 1203, 1093, 1039 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.32 (1H, s, H-25), 4.00 (1H, t, J = 8.4 Hz, H_α-24), 3.55 (1H, t, J = 8.4 Hz, H_β-24), 3.43 (1H, bd, J = 9 Hz, H-12), 3.37 (3H, s, OMe); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 108.5 (C-25), 84.6 (C-12), 71.7 (C-24), 58.7 (C-9), 58.6 (C-18), 56.6 (C-5), 55.2 (OMe), 54.9 (C-13), 42.1 (C-3), 41.6 (C-1), 40.3 (C-8), 39.9 (C-7), 37.8 (C-14), 37.4 (C-10), 34.8 (C-17), 33.3 (C-19), 33.2 (C-4), 27.2 (C-15), 24.0 (C-16), 21.3 (C-20), 18.6 (C-6), 18.2 (C-2), 17.5 (C-21), 16.4 (C-22), 12.2 (C-23); HRESIMS *m/z* 427.3067 $[M + Na]^+$ (calcd for $C_{26}H_{44}O_3Na$, 427.3188).

12-*O*-Deacetyl-12-oxoscalaradial (8d) and 12-*O*-deacetyl-12-oxo-18-episcalaradial (8e). To a solution of **3** (10 mg) in MeOH (2 mL) was added K_2CO_3 (4 mg), and the reaction mixture was stirred at room temperature for 2 h. The reaction was then quenched with H_2O, extracted with CH_2Cl_2, dried over anhydrous Na_2SO_4, and evaporated to give a crude residue, which was chromatographed over silica gel eluting with 20% EtOAc in hexanes to afford **8d** (1.5 mg, 20%) and **8e** (1 mg, 13%) as white solids.

12-*O*-Deacetyl-24,25-dimethoxyscalarins (9a and 9b). A solution of 12-*O*-deacetyl-12-episcalaradial (**8a**) (5 mg) in MeOH (1 mL) was run through a column (5 cm × 2 cm) of Dowex 50w × 8 (100−200

mesh, H^+) ion-exchange resin (prewashed with MeOH) to give a mixture of dimethylacetals. The mixture was dried under vacuum, and the residue was chromatographed over silica gel eluting with 15% EtOAc in hexanes to give the pure dimethylacetals **9a**[19] (3 mg, 53%) and **9b**[19] (1.2 mg, 20%) as white solids.

Single-Crystal X-ray Diffraction Analysis of Compounds 6c and 8b. Diffraction data for **6c** and **8b** were collected at low temperature on a Nonius KappaCCD diffractometer equipped with Mo Kα radiation and an Oxford Cryostream sample chiller. Crystal data: for **6c** (crystals from $CHCl_3$), $C_{26}H_{40}O_3$, monoclinic space group $P2_1$, $a = 11.061(4)$ Å, $b = 6.048(2)$ Å, $c = 16.603(7)$ Å, $\beta = 101.298(17)°$, $V = 1089.2(7)$ Å^3, $T = 100.0(5)$ K, $Z = 2$, $R = 0.049$ ($F^2 > 2\sigma$), $R_w = 0.110$ (all F^2) for 2067 unique data having $2\theta < 50.0°$ and 270 refined parameters; for **8b** (crystals from $CHCl_3$), $C_{25}H_{38}O_3$, orthorhombic space group $P2_12_12_1$, $a = 8.4330(8)$ Å, $b = 11.9818(14)$ Å, $c = 20.782(3)$ Å, $V = 2099.9(4)$ Å^3, $T = 90.0(5)$ K, $Z = 4$, $R = 0.036$ ($F^2 > 2\sigma$), $R_w = 0.096$ (all F^2) for 4355 unique data having $2\theta < 65.8°$ and 261 refined parameters.

Cytotoxicity Assay. The in vitro cytotoxic activity was determined against three human cancer cell lines, SK-MEL, BT-549, and SKOV-3, as well as Vero noncancerous cell lines. Vero cells were obtained from the American Type Culture Collection (ATCC, Rockville, MS). The assay was performed in 96-well tissue culture-treated microplates. Cells (25 000 cells/well) were seeded in the wells of the plate and incubated for 24 h. Samples were added, and plates were again incubated for 48 h. The number of viable cells was determined using Neutral Red according to a modification of the procedure of Borenfreund et al.[29] IC_{50} values were determined from logarithmic graphs of growth inhibition versus concentration. Doxorubicin was used as a positive control, while DMSO was used as the negative (vehicle) control.

Antimicrobial Assay. Organisms (methicillin-resistant *Staphylococcus aureus* ATCC 43300-MRSA and *Mycobacterium intracellulare* ATCC 23068) were obtained from the American Type Culture Collection (Manassas, VA). MRSA was tested using a modified version of the CLSI methods.[30] *M. intracellulare* was tested using a modified method of Franzblau et al.[31] Samples were serially diluted in 20% DMSO/saline and transferred in duplicate to 96-well flat bottom microplates. Microbial inocula were prepared by correcting the OD_{630} of microbe suspensions in incubation broth to afford final target inocula. Ciprofloxacin (ICN Biomedicals, Aurora, OH) was included in each assay. MRSA was read at 630 nm using the Biotek Powerwave XS plate reader (Bio-Tek Instruments, Winooski, VT) and *M. intracellulare* at 544ex/590em using the Polarstar Galaxy plate reader (BMG LabTechnologies, Germany) prior to and after incubation.

Acknowledgment. We are grateful to M. Wright, D. Kutrzeba, and S. Khan for performing the antimicrobial and cytotoxic assays at the National Center for Natural Products Research. This work was funded by the National Oceanic and Atmospheric Association's National Undersea Research program (NOAA NIUST NA16RU) and the USDA Agricultural Research Service Specific Cooperative Agreement No. 58-6408-2-0009.

Supporting Information Available: CCDC 732431 and 732432 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html [or from the Cambridge Crystallographic Data Centre (CCDC), 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44(0)1223-336033; e-mail: deposit@ccdc.cam.ac.uk]. ^{1}H and ^{13}C NMR spectra of compounds **6b**, **6c**, and **7a**−**c** are available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Pettit, G. R.; Cichacz, Z. A.; Hoard, M. S.; Melody, N.; Pettit, R. K. *J. Nat. Prod.* **1998**, *61*, 13–16.
(2) Tsukamoto, S.; Miura, S.; Soest, R. W. M.; Ohta, T. *J. Nat. Prod.* **2003**, *66*, 438–440.
(3) Somerville, M. J.; Hooper, J. N. A.; Garson, A. J. *J. Nat. Prod.* **2006**, *69*, 1587–1590.
(4) Ponomarenko, L. P.; Kalinovsky, A. I.; Stonik, V. A. *J. Nat. Prod.* **2004**, *67*, 1507–1510.
(5) Fattorusso, E.; Magno, S.; Santacroce, C.; Sica, D. *Tetrahedron* **1972**, *28*, 5993–5997.
(6) Cimino, G.; De Rosa, S.; De Stefano, S.; Sodano, G. *Comp. Biochem. Physiol. B* **1982**, *73B*, 471–474.
(7) (a) Alvi, K. A.; Crews, P. *J. Nat. Prod.* **1992**, *55*, 859–865. (b) Fontana, A.; Mollo, E.; Ortea, J.; Gavagnin, M.; Cimino, G. *J. Nat. Prod.* **2000**, *63*, 727–530. (c) Kikuchi, H.; Tsukitani, Y.; Shimizu, I.; Kobayashi, M.; Kitagawa, I. *Chem. Pharm. Bull.* **1983**, *31*, 552–556.
(8) (a) Hochlowski, J. E.; Faulkner, D. J.; Bass, L. S.; Clardy, J. *J. Org. Chem.* **1983**, *48*, 1738–1740. (b) Wonganuchitmeta, S.; Yuenyongsawad, S.; Keawpradub, N.; Plubrukarn, A. *J. Nat. Prod.* **2004**, *67*, 1767–1770.
(9) Nakagawa, M.; Hamamoto, Y.; Ishihama, M.; Hamasaki, S.; Endo, M. *Tetrahedron Lett.* **1987**, *28*, 431–434.
(10) (a) Rueda, A.; Zubia, E.; Ortega, M.; Carballo, J. L.; Salva, J. *J. Org. Chem.* **1997**, *62*, 1481–1485. (b) Jaspars, M.; Jackson, E.; Lobkovsky, E.; Clardy, J.; Diaz, M. C.; Crews, P. *J. Nat. Prod.* **1997**, *60*, 556–561. (c) Paloma, L. G.; Randazzo, A.; Minale, L.; Debitus, C.; Roussakis, C. *Tetrahedron* **1997**, *53*, 10451–10458. (d) Jimenez, J. I.; Yoshida, W. Y.; Scheuer, P. J.; Lobkovsky, E.; Clardy, J.; Kelly, M. *J. Org. Chem.* **2000**, *65*, 6837–6840. (e) Song, J.; Jeong, W.; Wang, N.; Lee, H.; Sim, C. J.; Oh, K.; Shin, J. *J. Nat. Prod.* **2008**, *71*, 1866–1871.
(11) Walker, R. P.; Thompson, J. E.; Faulkner, D. *J. Org. Chem.* **1980**, *66*, 444–446.
(12) Sera, Y.; Adachi, K.; Shizuri, Y. *J. Nat. Prod.* **1999**, *62*, 152–154.
(13) Braekman, J. C.; Daloze, D.; Kaisin, M.; Moussiauz, B. *Tetrahedron* **1985**, *41*, 4603–4614.
(14) Li, H.-J.; Amagata, T.; Tenny, K.; Crews, P. *J. Nat. Prod.* **2007**, *70*, 802–807.
(15) Kulcitki, V.; Ungur, N.; Gavagnin, M.; Castelluccio, F.; Cimino, G. *Tetrahedron* **2007**, *63*, 7617–7623.
(16) (a) Yu, Z.-G.; Bi, K.-S.; Guo, Y.-W. *Helv. Chem. Act.* **2005**, *88*, 1004–1009. (b) Youssef, D. T.; Yamaki, R. K.; Kelly, M.; Scheuer, P. J. *J. Nat. Prod.* **2002**, *65*, 2–6.
(17) Kazlauskas, R.; Murphy, P. T.; Quinn, R. J.; Wells, R. J. *Tetrahedron Lett.* **1976**, *30*, 2631–2634.
(18) Ledroit, V.; Debitus, C.; Ausseil, F.; Raux, R.; Menou, J.-L.; Hill, B. *Pharm. Biol.* **2004**, *42*, 454–456.
(19) Crews, P.; Bescana, P. *J. Nat. Prod.* **1986**, *49*, 1041–1052.
(20) Pettit, G. R.; Cichacz, Z. A.; Tan, R.; Herald, D. L.; Melody, N.; Hoard, M. S.; Doubek, D. L.; Hooper, J. N. A. *Collect. Czech. Chem. Commun.* **1998**, *63*, 1671–1677.
(21) Pettit, G. R.; Tan, R.; Melody, N.; Cichacz, Z. A.; Herald, D. L.; Hoard, M. S.; Pettit, R. K.; Chapuis, J.-C. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2093–2098.
(22) Cimino, G.; De, Stefano; Luccia, A. D. *Experientia* **1979**, *35*, 1277–1278.
(23) Gavagnin, M.; Mollo, E.; Docimo, T.; Guo, Y.; Cimino, G. *J. Nat. Prod.* **2004**, *37*, 2104–2107.
(24) Kubo, I. *J. Nat. Prod.* **1988**, *51*, 22–29.
(25) Jonassohn, M. Sesquiterpenoid Unsaturated Dialdehydes. Ph.D. Thesis, Lund University, Lund, Sweden, 1996, p 12.
(26) Jonassohn, M.; Davidsson, R.; Kahnberg, P.; Sterner, O. *Tetrahedron* **1997**, *53*, 237–244.
(27) Cimino, G.; Sodano, G.; Spinella, A. *Tetrahedron* **1987**, *43*, 5401–5410.
(28) Tsuchiya, N.; Sato, A.; Hata, T.; Sato, N.; Sasagawa, K.; Kobayashi, T. *J. Nat. Prod.* **1998**, *61*, 468–473.
(29) Borenfreund, E.; Babich, H.; Martin-Alguacil, N. *In Vitro Cell. Dev. Biol.* **1990**, *26*, 1030–1034.
(30) NCCLS, NCCLS Document M7-A7; 2006; Vol. 26 (2), pp 1−64.
(31) Franzblau, S. G.; Witzig, R. S.; McLaughlin, J. C.; Torres, P.; Madico, G.; Hernandez, A.; Degnan, M. T.; Cook, M. B.; Quenzer, V. K.; Ferguson, R. M.; Gilman, R. H. *J. Clin. Microbiol.* **1998**, *36*, 362–366.

NP900326A