Scalarane Sesterterpenes from the Sponge *Smenospongia* sp.

Jueun Song,[†] Wooyeol Jeong,[†] Nan Wang,[†,‡] Hyi-Seung Lee,[⊥] Chung J. Sim,[§] Ki-Bong Oh,*[,∥] and Jongheon Shin*[,†]

Natural Products Research Institute, College of Pharmacy, Seoul National University, San 56-1, Sillim, Gwanak, Seoul 151-742, Korea, Department of Biological Science, College of Life Science and Nano Technology, Hannam University, 461-6 Jeonmin, Yuseong, Daejeon 305-811, Korea, Marine Natural Products Laboratory, Korea Ocean Research & Development Institute, Ansan P.O. Box 29, Seoul 425-600, Korea, and Department of Agricultural Biotechnology, College of Agriculture and Life Science, Seoul National University, San 56-1, Sillim, Gwanak, Seoul 151-921, Korea

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Ten new sesterterpene compounds (**4**−**13**) and seven known metabolites (**1**−**3**, **14**−**17**) were isolated from the sponge *Smenospongia* sp. collected from Korean coastal waters. On the basis of combined spectroscopic analyses, the new compounds exhibited diverse functionalities on a common scalarane sesterterpene structure. The presence of a 23-aldehyde on compound **6** and a 20-carboxylic acid on **11** is unprecedented among sponge-derived scalarane sesterterpenes. Several of the new compounds showed significant cytotoxicity and antimicrobial activity, as well as moderate inhibitory activity against isocitrate lyase.

The variety of sesterterpenoids is one of the most conspicuous characteristics of sponge-derived terpenoids and distinguishes them from those of other marine or terrestrial organisms.[1,2] Among these sesterterpenoids, the tetracyclic scalarane and related carbon frameworks are the most frequently encountered classes. Since the isolation of scarlarin from *Cacospongia scalaris* in early 1970s, numerous compounds of this skeletal class have been isolated from the order Dictyoceratida and continue to serve as chemical markers of these animals.[3−5] In general, members of this class of compounds exhibit antimicrobial,[6−8] cytotoxic,[9−28] anti-inflammatory,[29−32] platelet-aggregation inhibitory,[33] and farsenoid X receptor (FXR) antagonistic activities,[34,35] in addition to other important ecological functions including antifeedant,[36,37] antifouling,[38] and ichthyotoxic activities.[32,39]

Previously, we reported the structures and bioactivity of five linear and scalarane-based sesterterpenoids obtained from *Smenospongia* sp.[24] These compounds had moderate to significant cytotoxicity against K562 cells. Further examination of this sponge re-collected from the same area, using bioactivity-guided separation, led to the isolation of 17 compounds, including seven known sesterterpenes. Here, we report the structures of 10 new scalarane sesterterpenes that exhibit moderate to significant cytotoxicity against K562 cells. Several of these compounds also showed moderate antimicrobial activity and inhibitory activity against the microbial enzyme isocitrate lyase (ICL).

Results and Discussion

The major constituents of the sponge extract, compounds **1** and **2**, and an additional metabolite, compound **3**, were identified as 12-deacetoxy-23-hydroxyheteronemin, 12-deacetoxy-23-acetoxy-19-*O*-acetylscalarin, and 12-deacetoxy-23-acetoxyscalarin, respectively, based on combined spectroscopic analyses and comparison of the NMR data with previous results.[24] Four linear furanosesterterpenes (**14**−**17**) were also isolated. Compounds **1**−**3** and **14** were previously isolated as cytotoxic constituents of *Smenospongia* sp.,[24] while **15**−**17** were obtained from *Sarcotragus* sp.[40] Linear furanosesterterpenes with a tetronic acid moiety, such as **15**−**17**, have been frequently isolated from the genera *Ircinia*, *Psammocinia*, and *Sarcotragus*, yet had never been obtained from the genus *Smenospongia*.[2] Therefore, it is unclear whether these are true metabolites of *Smenospongia* or those from a contaminant sponge with similar morphological features.

Compound **4** ($C_{31}H_{46}O_7$) was isolated as an amorphous solid. The ^{1}H and ^{13}C NMR data of this compound were very similar to those of **1** with an additional acetyl group: δ_H 2.10 (3 H, s), δ_C 171.0 (C), 21.1 (CH_3) (Tables 1 and 2). The downfield shifts of the H-23 methylene protons to δ 4.55 and 4.13 from δ 4.02 and 3.88 in **1** placed this acetyl group at C-23. This assignment was

Table 1. ^{13}C NMR Assignments for Compounds **4**−**13**

position	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
1	34.9	40.0	34.3	34.4	34.4	34.33[g]	34.9	34.6	34.8	34.8
2	18.4	18.3	19.4	18.5	18.5	18.6	18.5	18.3	18.4	18.4
3	41.7	42.0[c]	41.6	41.8	41.8	41.8	41.7	41.5	41.6	41.6
4	33.0	33.3	33.6	33.0	33.0	33.0	33.0	32.9	32.9	33.0
5	56.9	56.6	53.5	56.8	56.8	57.0	57.2	56.5	56.6	56.8
6	18.0	18.6	17.5	17.8	17.9	18.0	18.1	17.8	17.9	17.9
7	42.4	42.1[c]	40.7	42.2	42.37[f]	42.39[h]	42.3	41.7	41.8	42.1
8	38.1	37.6	37.8	37.7	37.8	37.9	37.9	37.4	37.5	37.2
9	61.7	61.4	61.2	62.0	62.0	61.9	61.7	61.4	61.6	61.4
10	40.8	38.0	55.0	42.4	42.39[f]	42.5	40.8	40.6	40.7	40.7
11	19.7	17.1	17.4	20.3	20.6	21.1	20.3	19.9	19.9	19.7
12	41.6	40.8	40.2	41.3	42.1	42.41[h]	42.2	39.4	39.5	42.4
13	36.8	36.8	36.6	33.9	33.5	34.27[g]	34.3	38.0	38.0	37.8
14	56.5	56.4	55.0	54.8	55.4	57.2	57.1	48.8	49.1	54.2
15	28.3	32.1	28.5	24.3	22.9	18.2	18.2	24.2	24.0	23.5
16	69.4	68.1	69.2	137.5	116.6	20.8	20.8	146.0	143.2	142.9
17	114.1	119.0	113.9	126.0	136.3	119.9	119.8	124.9	125.6	126.6
18	63.5	63.6	63.4	56.3	60.6	137.4	137.3	60.4	60.9	62.3
19	98.3	98.6	98.3	93.6	106.1	135.1	135.1	203.1	203.2	202.2
20	134.8	134.6	135.0	166.6	69.0	136.7	136.7	171.1	166.8	167.2
21	33.7	33.3	31.8	34.0	33.9	33.9	33.8	33.7	33.7	33.7
22	21.8	21.3	20.7	21.9	21.9	21.8	21.8	21.8	21.9	21.8
23	64.7	16.3	206.0	62.8	63.1	62.3	64.9	64.8	64.8	64.8
24	16.7	17.3	17.8	15.8	16.0	17.0	17.0	16.2	16.3	16.5
25	14.4	14.8	14.4	14.6	14.5	25.9	25.9	22.2	22.2	15.7
16-OAc	169.9[a]		169.9[d]							
	21.2[b]		21.1[e]							
19-OAc	170.1[a]	170.0	170.1[d]	169.0						
	21.2[b]	21.2	21.2[e]	20.9						
19-OMe					55.5					
20-OMe									51.9	51.9
23-OAc	171.0[a]						171.1	171.3	171.0	171.1
	21.1[b]						21.3	21.3	21.2	21.2

[a−e] Assignments may be interchanged. [f−h] Due to the similar chemical shifts, values are reported with two decimal points.

* To whom correspondence should be addressed. (J.S.) Tel: 82 2 880 2484. Fax: 82 2 762 8322. E-mail: shinj@snu.ac.kr. (K.-B.O.) Tel: 82 2 880 4646. Fax: 82 2 873 3112. E-mail: ohkibong@snu.ac.kr.

[†] College of Pharmacy, Seoul National University.

[‡] Visiting Student from Shenyang Pharmaceutical University, Shenyang, China. Present address: Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, China.

[⊥] Korea Ocean Research and Development Institute, Seoul National University.

[∥] College of Agriculture and Life Sciences, Seoul National University.

[§] Department of Biology, Hannam University.

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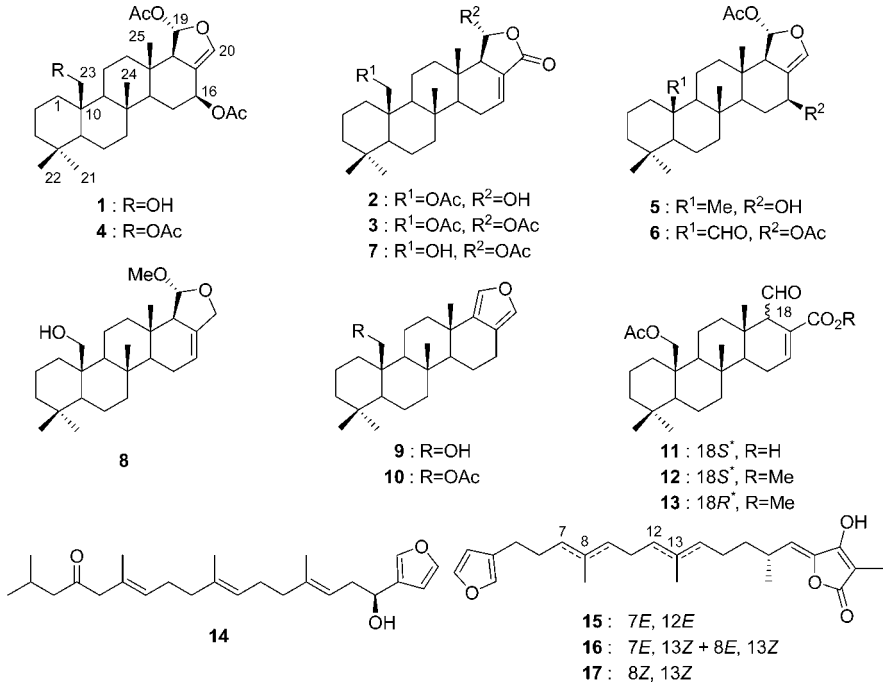

Table 2. ^{1}H NMR Assignments for Compounds **4**−**8**

position	4	5	6	7	8
1α	0.72, ddd (13.2, 13.2, 2.5)	0.76, ddd (13.7, 13.7, 2.4)	0.63, ddd (12.6, 12.6, 3.0)	0.72, ddd (13.2, 13.2, 3.3)	0.71, ddd (13.2, 13.2, 3.0)
1*β*	2.10, br d (13.2)	1.70, br d (13.7)	2.54, br d (12.6)	2.22, br d (13.2)	2.21, br d (13.2)
2α	1.45, m	1.36, m	1.28, m	1.48, m	1.47, m
2*β*	1.59, m	1.55, m	1.45, m	1.59, br ddd (13.5, 13.2, 13.2)	1.59, br ddd (13.8, 13.2, 13.2)
3α	1.19, ddd (13.2, 12.2, 4.3)	1.10, ddd (13.3, 13.2, 4.4)	1.19, ddd (12.6, 12.6, 3.6)	1.17, m	1.18, m
3*β*	1.45, br d (12.2)	1.36, br d (13.2)	1.36, br d (12.6)	1.42, br d (13.2)	1.42, br d (13.2)
5	1.03, br d (12.8)	0.91, br d (12.7)	1.04, br d (13.2)	0.94, br d (13.2)	0.93, br d (13.2)
6α	1.58, br d (13.7)	1.38, br d (13.7)	1.78, m	1.49, m	1.48, m
6*β*	1.43, dddd (13.7, 13.2, 12.8, 3.0)	1.52, dddd (13.7, 13.2, 12.7, 3.0)	1.12, m	1.37, m	1.34, dddd (13.8, 13.2, 12.6, 3.0)
7α	1.00, ddd (13.2, 12.8, 3.9)	0.91, ddd (13.2, 12.6, 3.6)	1.12, m	1.02, ddd (13.2, 12.7, 3.6)	1.18, m
7*β*	1.82, br d (12.8)	1.80, br d (12.7)	1.93, br d (12.6)	1.79, br d (12.7)	1.77, br d (12.6)
9	0.95, br d (13.2)	1.12, br d (13.2)	1.12, br d (13.2)	0.95, br d (13.2)	0.95, br d (13.2)
11α	1.84, m	1.59, br d (13.2)	1.76, br d (13.2)	1.77, br d (13.2)	1.75, br d (12.6)
11*β*	1.77, m	1.36, m	1.12, m	1.71, ddd (13.2, 13.2, 12.3)	1.69, m
12α	1.16, m	1.15, m	1.12, m	1.16, m	1.02, m
12*β*	1.73, br d (13.2)	1.80, br d (13.2)	1.82, br d (13.2)	1.67, br d (13.2)	1.75, br d (13.2)
14	1.07, br d (12.0)	1.08, br d (11.7)	1.08, br d (11.7)	1.33, dd (11.2, 5.7)	1.25, dd (11.4, 5.4)
15	2.07, m	2.02, m	2.10, m	2.38, ddd (20.4, 5.7, 3.6)	2.09, m
	1.32, m	1.26, m	1.28, m	2.13, br dd (20.4, 11.2)	1.93, br dd (20.4, 11.4)
16	5.40, dd (8.3, 8.2)	4.35, br dd (7.9, 7.7)	5.41, dd (8.4, 6.6)	6.91, br dd (3.6, 3.6)	5.49, dd (3.4, 3.4)
18	2.33, br s	2.29, br s	2.32, br s	2.64, m	2.18, br s
19	6.32, d (1.8)	6.35, d (1.8)	6.32, d (1.2)	6.42, d (5.7)	4.78, d (3.6)
20	6.10, d (1.8)	6.10, d (1.8)	6.10, d (1.2)		4.34, d (11.4)
					4.15, d (11.4)
21	0.87, s	0.84, s	0.92, s	0.86, s	0.86, s
22	0.82, s	0.80, s	0.77, s	0.77, s	0.77, s
23	4.55, d (12.0)	0.82, s	10.06, s	4.03, d (11.4)	4.02, d (11.4)
	4.13, d (12.0)			3.89, d (11.4)	3.93, d (11.4)
24	0.91, s	0.85, s	0.72, s	1.09, s	1.03, s
25	0.85, s	0.83, s	0.79, s	0.82, s	0.76, s
16-OAc	2.07, s		2.10, s		
19-OAc	2.06, s	2.08, s	2.07, s	2.13, s	
19-OMe					3.39, s
23-OAc	2.10, s				

confirmed by combined 2D NMR data, in particular, long-range correlations between H-23 and the carbonyl carbon in the gHMBC data. The stereochemical configuration was identical to that of other scalarane sesterterpenes based on NOESY cross-peaks at H-5/H-9, H-9/H-14, H-14/H-16, H-14/H-18, H-16/H-18, H-19/H-25, H-22/H-23, and H-24/H-25. Therefore, compound **4** was identified as the 23-acetyl derivative of **1**.

The spectroscopic data of compound **5** ($C_{27}H_{42}O_4$) were similar to those of **1** and **4**. Preliminary examination of the ^{1}H and ^{13}C NMR data revealed that the dihydroacetoxyfuran moiety present in **1** and **4** was also present in **5**. These data also indicated the presence of an additional methyl group and the loss of at least one acetyl group. Furthermore, the oxygenated functional groups of **1** and **4** were reduced in **5**. Long-range correlations of an isolated methyl proton at δ_H 0.82 (δ_C 16.3) with C-1, C-5, C-9, and C-10 (preassigned by 2D NMR experiments) in the gHMBC data placed this methyl group at C-23. An allylic correlation between the oxymethine proton at δ 4.35 and a furan proton at δ 6.10 in the ^{1}H correlation spectroscopy (COSY) spectrum indicated a hydroxyl group at C-16. This assignment was corroborated by long-range carbon−proton correlations of the oxymethine proton with C-14, C-15, C-18, and C-20. The stereochemical configuration at this center was identical to those of **1** and **4**, as indicated by cross-peaks H-14/H-16, H-14/H-18,

Table 3. ^{1}H NMR Assignments for Compounds **9**−**13**

position	**9**	**10**	**11**	**12**	**13**
1α	0.72, ddd (13.2, 13.2, 3.6)	0.76, ddd (13.2, 13.2, 3.4)	0.70, ddd (13.0, 12.8, 2.0)	0.72, ddd (13.2, 13.2, 2.4)	0.76, ddd (13.2, 13.2, 2.5)
1*β*	2.23, br d (13.2)	2.08, br d (13.2)	2.02, br d (13.0)	2.04, br d (13.2)	2.10, br d (13.2)
2α	1.52, m	1.43, m	1.46, m	1.43, m	1.46, m
2*β*	1.57, m	1.58, m	1.53, m	1.57, m	1.57, m
3α	1.16, m	1.15, m	1.14, m	1.16, m	1.16, m
3*β*	1.41, br d (13.2)	1.43, m	1.46, m	1.43, m	1.46, m
5	0.95, br d (12.6)	1.19, br d (12.2)	0.97, br d (12.4)	0.98, br d (12.6)	0.97, br d (12.4)
6α	1.52, br d (13.2)	1.58, br d (13.2)	1.53, br d (13.2)	1.55, br d (13.2)	1.56, br d (13.2)
6*β*	1.41, m	1.43, m	1.33, m	1.38, m	1.37, m
7α	1.02, ddd (13.2, 12.6, 3.6)	1.09, m	1.03, ddd (13.2, 12.4, 3.6)	1.04, ddd (13,2, 12.6, 3.6)	0.99, m
7*β*	1.95, br d (13.2)	1.95, br d (12.2)	1.79, br d (12.4)	1.78, br d (12.6)	1.77, br d (12.4)
9	0.98, br d (12.6)	0.96, br d (13.2)	0.89, br d (13.2)	0.88, br d (13.2)	0.87, br d (12.6)
11α	1.81, br d (13.2)	1.76, br d (13.2)	1.72, m	1.73, m	1.79, m
11*β*	1.78, m	1.78, m	1.67, m	1.69, m	1.70, m
12α	1.02, m	1.02, m	1.36, m	1.35, m	1.37, m
12*β*	2.06, br d (12.6)	1.94, br d (12.2)	1.78, br d (12.6)	1.78, br d (12.6)	1.92, br d (13.2)
14	1.08, br d (11.4)	1.02, br d (11.7)	1.46, dd (12.0, 5.4)	1.48, dd (11.4, 5.4)	1.21, dd (12.0, 0.5.2)
15	1.81, m	1.76, m	2.40, ddd (20.4, 5.4, 4.8)	2.36, ddd (20.4, 5.4, 3.4)	2.27, ddd (20.0, 5.2, 3.2)
	1.57, m	1.43, m	2.14, br dd (20.4, 12.0)	2.14, br dd (20.4, 11.4)	2.19, br dd (20.0, 12.0)
16	2.77, dd (16.2, 5.4)	2.77, dd (16.1, 6.1)	7.40, dd (2.4, 1.5)	7.28, dd (2.4, 1.5)	7.22, dd (2.7, 1.5)
	2.43, m	2.43, m			
18			3.17, br d (1.5)	3.18, br d (1.5)	2.89, br d (1.5)
19	7.07, d (1.5)	7.07, d (1.5)	9.82, d (2.9)	9.83, d (4.1)	9.53, d (4.1)
20	7.04, d (1.5)	7.04, d (1.5)			
21	0.86, s	0.87, s	0.85, s	0.87, s	0.87, s
22	0.78, s	0.84, s	0.81, s	0.82, s	0.82, s
23	4.03, d (11.4)	4.57, d (11.7)	4.54, d (12.0)	4.56, d (11.4)	4.56, d (12.0)
	3.94, d (11.4)	4.18, d (11.7)	4.14, d (12.0)	4.16, d (11.4)	4.15, d (12.0)
24	1.08, s	0.96, s	0.95, s	0.96, s	0.99, s
25	1.21, s	1.20, s	0.94, s	0.95, s	0.91, s
20-OMe				3.70, s	3.72, s
23-OAc		2.08, s	2.06, s	2.06, s	2.05, s

and H-16/H-18 in the NOESY data. Therefore, compound **5** was a 12-deacetyl derivative of scalarin.

The spectroscopic data obtained for compound **6** ($C_{29}H_{42}O_6$) were similar to those of **1** and **5**. However, the appearance of aldehyde signals (δ_H 10.06, δ_C 206.0) in the NMR spectra indicated an oxidized methyl group. This group was placed at C-23 based on long-range correlations in the gHMBC data between the aldehyde proton and the neighboring C-5 and C-10. The stereochemical configuration, determined by NOESY cross-peaks with H-22 and H-24, of this aldehyde was identical to the other scalaranes. To our best knowledge, this is the first example of a scalarane sesterterpene with a C-23 aldehyde functional group.

The spectroscopic data of **7** ($C_{27}H_{40}O_5$) were closely related to those of the other scalaranes. The presence of an α,*β*-unsaturated ester group {δ_H 6.91 (1 H, br dd, J = 3.6, 3.6 Hz), δ_C 166.6 (C), 137.5 (CH), 126.0 (C)} in the NMR data (Tables 1 and 2), coupled with a strong absorption band at 1770 cm^{-1} and a maximum at 220 nm in the IR and UV absorption spectra, respectively, indicated that **7** possessed the same unsaturated *γ*-lactone moiety as **2** and **3**. Combined ^{1}H COSY, TOCSY, and gHSQC experiments showed that this compound had the same general structure as **2** and **3**, with the only differences being the presence of oxygenated functional groups at C-19 and C-23. The NMR spectral similarities with **2** and **3**, together with long-range proton−carbon correlations at these centers, indicated the presence of an acetoxyl and hydroxy group at C-19 and C-23, respectively.

The spectroscopic data of **8** ($C_{26}H_{42}O_3$) indicated the loss of carbonyl carbon atoms and the appearance of methoxy (δ_H 3.39, δ_C 55.5) and oxymethylene (δ_H 4.34 and 4.15, δ_C 69.0) groups (Tables 1 and 2). A combination of 2D NMR data revealed that the oxymethylene was derived from a tetrahydrofuran ring. This was evidenced by an allylic coupling between the methylene protons and an olefinic proton at C-16 in the ^{1}H COSY data and long-range correlations of the oxymethylene with neighboring protons and carbon atoms in the gHMBC data. Similarly, the replacement of the C-19 acetoxyl group found in other scalaranes with a methoxy group was evident based on mutual gHMBC correlations between the methoxy and C-19 acetal methine groups. The stereochemical configuration at C-19 was identical to that of the other compounds based on the NOESY cross-peaks at H-18/H-OMe and H-19/H-25. Combined spectroscopic analyses showed that the remainder of the molecule, including the stereochemistry of its asymmetric centers, was the same as **7**. Therefore, the structure of **8** was that of a scalarane sesterterpene acetal.

In compound **9** ($C_{25}H_{38}O_2$), NMR signals corresponding to the C-17−C-20 lactone moiety were replaced with peaks corresponding to the presence of two oxygenated double bonds: δ_H 7.07 (1 H, d, J = 1.5 Hz), 7.04 (1 H, d, J = 1.5 Hz), δ_C 137.4 (C), 136.7 (CH), 135.1 (CH), 119.9 (C) (Tables 1 and 3). A comparison of these NMR characteristics with those in the literature suggested the presence of a furan moiety. This was confirmed by long-range proton−proton and proton−carbon couplings between the furan moiety and neighboring atoms in ^{1}H COSY and gHMBC experiments. The presence of a hydroxyl group at C-23 and the stereochemistry of the asymmetric carbon centers were identified in the gHMBC and NOESY experiments. Therefore, the structure of **9** was determined to be a furanosesterterpene of the scalarane class.

The spectroscopic data of **10** ($C_{27}H_{40}O_3$) were almost identical to those of **9**, with the exception of an acetyl group in the NMR spectra: δ_H 2.08 (3 H, s), δ_C 171.1 (C), 21.3 (CH_3). The acetyl group was assigned to C-23 based on long-range correlations between H-23 and the carbonyl carbon in the gHMBC data and a comparison with the NMR spectra obtained on the 23-acetoxylated scalaranes **2**−**4**.

The spectroscopic data of **11** ($C_{27}H_{40}O_5$) indicated a 23-acetoxyscalarane structure. However, preliminary examination of the ^{1}H and ^{13}C NMR data revealed significant modifications to the furan-type E ring generally found in other scalaranes. Additional carbonyl carbon atoms were evidenced by peaks at δ 203.1 and 171.1 in the ^{13}C NMR spectrum (Table 1). The occurrence of a corresponding signal at δ 9.82 (1 H, d, J = 2.9 Hz) revealed that

Table 4. Results of Bioactivity Tests[a]

	MIC (μ/mL)						ICL	K562
	Gram (+) bacteria			Gram (−) bacteria			IC_{50}	LC_{50}
compound	A	B	C	D	E	F	(μ/mL)	(μ/mL)
1	>100	0.78	100	>100	6.25	>100	>100	0.13
2	>100	>100	>100	>100	>100	>100	67.2	7.2
3	>100	3.12	>100	>100	>100	>100	>100	4.9
4	>100	>100	>100	>100	>100	>100	>100	6.8
5	>100	3.12	>100	>100	12.5	>100	>100	5.8
6	>100	>100	>100	>100	>100	>100	>100	17.5
7	>100	1.56	3.12	>100	6.25	>100	>100	22.5
8	>100	50	>100	>100	6.25	>100	42.0	0.11
9	>100	1.56	6.25	>100	12.5	>100	45.7	0.97
10	>100	3.12	25	>100	>100	>100	>100	2.3
11	>100	>100	>100	100	>100	>100	>100	4.2
12	>100	>100	>100	>100	>100	>100	>100	3.7
13	25	0.78	12.5	6.25	12.5	>100	55.0	8.6
14	>100	6.25	>100	>100	>100	>100	>100	5.7
15	12.5	6.25	6.25	6.25	6.25	>100	27.0	43.7
16	6.25	3.12	6.25	3.12	12.5	>100	24.1	16.9
17	6.25	6.25	3.12	6.25	12.5	>100	31.2	7.3
ampicillin	1.56	1.56	1.56	3.12	3.12	12.5		
3-nitropropinate							6.05	
doxorubicin								4.9

[a] A: *Staphylococcus aureus* (ATCC 65389). B: *Bacillus subtilis* (ATCC 6633). C: Micrococcus leuteus (IFC 12708). D: *Proteus vulgaris* (ATCC 3851). E: *Salmonella typhimurium* (ATCC 14028). F: *Escherichia coli* (ATCC 25922).

one of the carbonyl groups was an aldehyde (Table 3). The other carbonyl group was identified as a carboxylic acid based on the molecular formula and a broad IR absorption band at 3400 cm^{-1}. In addition, downfield-shifted olefinic NMR signals at δ_H 7.40 (1 H, dd, J = 2.4, 1.5 Hz) and δ_C 146.0 (CH) and 124.9 (C), and a UV absorption maximum at 210 nm, indicated a double bond conjugated to one of these carbonyl groups. The strong IR absorption bands at 1721 cm^{-1} suggested that the conjugation occurred at the carboxylic acid.

The structure of **11** was further determined by a combination of 2D NMR experiments. The presence of an aldehyde group at C-19 was secured by mutual long-range correlations of the aldehyde group with the C-18 methine group (preassigned by 2D NMR) in the gHMBC experiments. Similarly, a carboxylic acid at C-20 was identified based on long-range couplings of the carboxylic carbon atom with the neighboring H-16 and H-18 protons. The stereochemical configuration at C-18 was assigned as *S** by a NOESY cross-peak at H-18/H-25 that was linearly attached to the trans-ring junctures at H-5/H-9, H-9/H-14, H-22/H-23, H-23/H-24, and H-24/H-25. Therefore, the structure of **11** was a scalarane sesterterpene carboxylic acid. Despite the frequent occurrence of sesterterpenes with furan moieties with various levels of oxidations,[2] a literature survey revealed that aldehyde-unsaturated carboxylic acids, such as that found in compound **11**, are unprecedented among sponge-derived scalarane sesterterpenes.

The spectroscopic data of compounds **12** and **13** ($C_{28}H_{42}O_5$ isomers) were very similar to those of **11**. On the basis of 2D NMR analyses, both compounds were methyl esters of the sesterterpene carboxylic acid **11**. Therefore, **12** and **13** were presumably diastereomers. The stereochemical center was traced to C-18 due to considerable differences in the chemical shifts of the protons and carbons at this center and in the immediate vicinity (Tables 1 and 3). Compound **12** exhibited NOESY cross-peaks identical to those found for compound **11** at H-18/H-25 and H-24/H-25, while cross-peaks at H-19/H-25 and H-24/H25 were found for **13**. Therefore, **12** and **13** were methyl esters of **11** possessing a 18 *S** and 18 *R** stereochemical configuration, respectively.

Scalarane sesterterpenes typically have diverse bioactivities.[2] The compounds described here showed moderate to significant cytotoxicity[41] against a K562 cell line, and some were even more potent than doxorubicin (Table 4). Several compounds exhibited moderate to significant antibacterial activity[42] against both Gram-positive and -negative bacteria, with the exception of *E. coli*. Although none of these compounds were active against pathogenic fungi, some compounds moderately inhibited isocitrate lyase, a key enzyme in fungal metabolism.[43]

Experimental Section

General Experimental Procedures. Melting points were measured on a Buchi B-540 apparatus. Optical rotation was measured on a JASCO P-1020 polarimeter with a 1 cm cell. UV absorption spectra were recorded on a Hitachi U-3010 spectrophotometer; IR absorption was recorded on a JASCO 300E FT-IR spectrometer. NMR spectra were recorded on a Bruker Avance 600 spectrometer in $CDCl_3$ with a Me_4Si internal standard. Proton and carbon NMR spectra were measured at 600 and 150 MHz, respectively. Mass spectroscopic data were obtained at the Korea Basic Science Institute (Daegu) on a JEOL JMS 700 high-resolution mass spectrometer. Molecular formulas were determined from ^{13}C NMR and high-resolution mass spectroscopic analyses. All solvents used were spectroscopic grade or distilled from glass before use.

Animal Material. Specimens of *Smenospongia* sp. (voucher number 06SH-14) were collected by hand in June 2006 using scuba equipment (25−30 m depth) off the shore of Soheuksan Island, Korea. Morphologically, the sponges were very similar to those collected previously.[24] The specimens were large (80 × 105 × 70 mm) and had several locally opened oscules (1−5 mm in diameter). They were dark reddish-brown in life and turned dark brown on storage in alcohol. The sponges were firm and compressible, yet easily cut or torn, with surfaces covered in sharply pointed, low conules (<1 mm in height, 1−3 mm apart). A voucher specimen (registry no. Spo. 43) is currently on deposit at the Natural History Museum, Hannam University, Korea, under the curatorship of C.J.S.

Extraction and Isolation. Freshly collected specimens were frozen immediately and kept at −25 °C until chemical investigation. The combined specimens were lyophilized (dry wt 190 g), macerated, and extracted repeatedly with CH_2Cl_2 (3 L × 3) and MeOH (3 L × 3). The combined crude extracts were successively partitioned between H_2O and *n*-BuOH; the latter fraction was repartitioned in 15% MeOH(aq) (21.33 g) and *n*-hexane (17.13 g). The MeOH layer was separated by C_{18} reversed-phase vacuum flash chromatography and eluted with a sequential mixture of MeOH and H_2O (elution order: 50, 40, 30, 20, and 10% aqueous MeOH and 100% MeOH), acetone, and EtOAc.

On the basis of ^1H NMR analyses, the fractions eluted with 100% MeOH (5.2 g) were further separated using semipreparative silica high-performance liquid chromatography (HPLC; YMC-Pack silica column, 1 × 25 cm, 20% EtOAc/*n*-hexane). Eight fractions, identified by eight peaks in the HPLC chromatogram, were rich with secondary metabolites. Further purification of the first and fifth fractions by reversed-

phase HPLC (YMC-Pack ODS column, 1 × 25 cm, 15% MeOH(aq)) afforded compounds **16** and **1**, respectively, as amorphous solids. Similar purification of the sixth and eighth fractions (10% MeOH(aq)) yielded **15** and **2**, respectively.

The remaining fractions were separated and purified using reversed-phase HPLC (12% MeOH(aq) for the second, third, and fourth fractions, 7% MeOH(aq) for the seventh fraction), yielding compounds **3**−**14** and **17** as pure compounds. Compounds **7**, **9**, **10**, and **17** were obtained from the third fraction, **3**−**6**, **12**, and **13** from the fourth, and **8**, **11**, and **14** from the seventh. The purified metabolites of **1**−**17** were isolated in the following amounts: 641.5, 284.4, 45.5, 22.0, 27.1, 21.0, 59.8, 12.3, 4.7, 11.6, 12.8, 47.9, 5.8, 11.6, 6.8, 5.1, and 11.4 mg, respectively.

12-Deacetoxy-23-hydroxyheteronemin (1): amorphous solid; mp 142.5−143.0 °C; $[\alpha]^{25}_D$ −51.0 (*c* 0.59, MeOH) [lit. −38.1 (*c* 0.25, MeOH)];[24] IR (KBr) ν_{max} 3500 (br), 2930, 1745, 1455, 1365, 1235 cm^{-1}.

12-Deacetoxy-23-acetoxyscalarin (2): amorphous solid; mp 206.0−208.0 °C; $[\alpha]^{25}_D$ −49.0 (*c* 0.46, MeOH) [lit. −33.1 (*c* 0.23, MeOH)];[24] UV (MeOH) λ_{max} (log ϵ) 221 (4.04) nm; IR (KBr) ν_{max} 3450 (br), 2935, 1770, 1740, 1455, 1390, and 1240 cm^{-1}.

12-Deacetoxy-23-acetoxy-19-O-acetylscalarin (3): amorphous solid; mp 194.1−194.5 °C; $[\alpha]^{25}_D$ −37.4 (*c* 0.51, MeOH) [lit. −22.9 (*c* 0.11, MeOH)];[24] UV (MeOH) λ_{max} (log ϵ) 221 (3.91) nm; IR (KBr) ν_{max} 2930, 1775, 1735, 1375, 1235 cm^{-1}.

Compound 4: amorphous solid; mp 169.1−170.8 °C; $[\alpha]^{25}_D$ −100.4 (*c* 0.51, MeOH); IR (KBr) ν_{max} 2925, 1740, 1460, 1370, 1235 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRFABMS *m*/*z* 553.3148 [M + Na]$^+$ (calcd for $C_{31}H_{46}O_7Na$, 553.3141).

Compound 5: amorphous solid; mp 139.3−141.5 °C; $[\alpha]^{25}_D$ −41.6 (*c* 0.45, MeOH); IR (KBr) ν_{max} 3450 (br), 2925, 1740, 1460, 1390 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRFABMS *m*/*z* 413.3052 [M − H_2O + H]$^+$ (calcd for $C_{27}H_{41}O_3$, 413.3056).

Compound 6: amorphous solid; mp 117.5−120.3 °C; $[\alpha]^{25}_D$ −62.6 (*c* 0.53, MeOH); IR (KBr) ν_{max} 2930, 1745, 1705, 1455, 1365, 1230 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRFABMS *m*/*z* 487.3059 [M + H]$^+$ (calcd for $C_{29}H_{43}O_6$, 487.3060).

Compound 7: amorphous solid; mp 164.0−166.3 °C; $[\alpha]^{25}_D$ −65.1 (*c* 0.76, MeOH); UV (MeOH) λ_{max} (log ϵ) 220 (4.10) nm; IR (KBr) ν_{max} 3500 (br), 2925, 1770, 1730, 1560, 1455, 1390, 1200 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRFABMS *m*/*z* 445.2958 [M + H]$^+$ (calcd for $C_{27}H_{41}O_5$, 445.2954).

Compound 8: amorphous solid; mp 193.3−194.9 °C; $[\alpha]^{25}_D$ −18.8 (*c* 0.60, MeOH; IR (KBr) ν_{max} 3500 (br), 2925, 1595, 1460, 1385 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 3; HRFABMS *m*/*z* 425.3036 [M + Na]$^+$ (calcd for $C_{26}H_{42}O_3Na$, 425.3032).

Compound 9: amorphous solid; mp unmeasured; $[\alpha]^{25}_D$ +12.3 (*c* 0.42, MeOH; UV (MeOH) λ_{max} (log ϵ) 218 (3.53) nm; IR (KBr) ν_{max} 3500 (br), 2925, 1455, 1390 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 3; HRFABMS *m*/*z* 371.2953 [M + H]$^+$ (calcd for $C_{25}H_{39}O_2$, 371.2950).

Compound 10: amorphous solid; mp 175.8−177.0 °C; $[\alpha]^{25}_D$ +5.6 (*c* 0.47, MeOH); UV (MeOH) λ_{max} (log ϵ) 219 (3.69) nm; IR (KBr) ν_{max} 2925, 1740, 1455, 1375, 1235 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 3; HRFABMS *m*/*z* 413.3050 [M + H]$^+$ (calcd for $C_{27}H_{41}O_3$, 413.3056).

Compound 11: amorphous solid; mp 126.1−128.6 °C; $[\alpha]^{25}_D$ −239.4 (*c* 0.35, MeOH); UV (MeOH) λ_{max} (log ϵ) 210 (4.03) nm; IR (KBr) ν_{max} 3400 (br), 2930, 1735, 1721, 1685, 1650, 1390, 1375, 1240 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 3; HRFABMS *m*/*z* 445.2956 [M + H]$^+$ (calcd for $C_{27}H_{41}O_5$, 445.2954).

Compound 12: amorphous solid; mp 200.1−202.7 °C; $[\alpha]^{25}_D$ −206.4 (*c* 0.53, MeOH); UV (MeOH) λ_{max} (log ϵ) 211 (4.09) nm; IR (KBr) ν_{max} 2930, 1740, 1730, 1570, 1440, 1370, 1240 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 3; HRFABMS *m*/*z* 459.3104 [M + H]$^+$ (calcd for $C_{28}H_{43}O_5$, 459.3110).

Compound 13: amorphous solid; mp unmeasured; $[\alpha]^{25}_D$ −29.3 (*c* 0.40, MeOH); UV (MeOH) λ_{max} (log ϵ) 216 (4.36) nm; IR (KBr) ν_{max} 2925, 1740, 1570, 1460, 1370, 1240 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 3; HRFABMS *m*/*z* 459.3107 [M + H]$^+$ (calcd for $C_{28}H_{43}O_5$, 459.3110).

4-Hydroxy-9-deoxoidiadione (14): amorphous solid; mp unmeasured; $[\alpha]^{25}_D$ −2.3 (*c* 0.25, MeOH) [lit. −5.5 (*c* 0.29, MeOH)];[24] UV (MeOH) λ_{max} (log ϵ) 215 (3.29) nm; IR (KBr) ν_{max} 3450 (br), 2925, 1710, 1365, 1230 cm^{-1}.

Compound 15: colorless gum; $[\alpha]^{25}_D$ +31.6 (*c* 0.85, MeOH); UV (MeOH) λ_{max} (log ϵ) 289 (3.67), 253 (4.15) nm; IR (KBr) ν_{max} 2945, 1755, 1635, 1500, 1285 cm^{-1}.

Compound 16: colorless gum; $[\alpha]^{25}_D$ +61.0 (*c* 0.32, MeOH); UV (MeOH) λ_{max} (log ϵ) 293 (3.89), 254 (4.08) nm; IR (KBr) ν_{max} 2950, 1760, 1630, 1505, 1425, 1280 cm^{-1}.

Compound 17: colorless gum; $[\alpha]^{25}_D$ +52.9 (*c* 0.41, MeOH); UV (MeOH) λ_{max} (log ϵ) 299 (3.71), 256 (4.09) nm; IR (KBr) ν_{max} 2945, 1760, 1635, 1505, 1425, 1280 cm^{-1}.

Biological Assays. Cytotoxicity assays according to the protocols in ref 41. Antimicrobial and isocitrate lyase inhibition assays were performed according to the methods given in refs 42 and 43, respectively.

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