Sesterterpenoids from the Sponge *Sarcotragus* sp.

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Nineteen new sesterterpenoids and eight known compounds were isolated from the sponge *Sarcotragus* sp. collected from Soheuksan Island, Korea. The structures of these compounds were determined to be linear sesterterpenoids containing furan or related oxygenated functionalities on the basis of combined chemical and spectroscopic analyses. In addition, the configurations of several previously undetermined compounds were assigned. Several compounds exhibited moderate to major antibacterial activity (compounds **1**–**3**, **17**, **18**) and cytotoxicity (**3**, **11**, **12**) against the K562 cell line and inhibitory activity against isocitrate lyase (**6**, **13**).

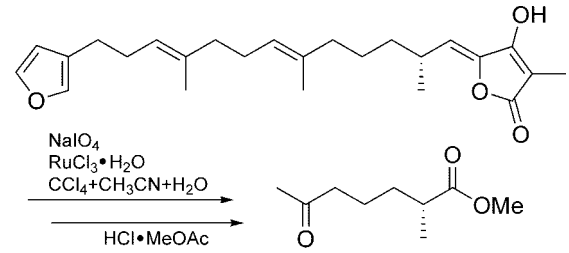
Sponges produce a wide variety of terpenoids and related metabolites containing polyprenyl moieties.[1] One of the most distinctive characteristics of sponge-derived terpenoids is the abundance and structural diversity of sesterterpenoids, a structure class rarely found in other marine or terrestrial organisms. Several compounds of this structural class exhibit various bioactivities, for example, antimicrobial, anti-inflammatory, antiviral, and cytotoxic activities, as well as inhibitory actions against diverse biosystems.[1,2] Sesterterpenoids have also been isolated from specimens of the genera *Psammocinia*, *Sarcotragus*, *Smenospongia*, and *Spongia* from Korean waters.[3–9] During the course of our search for bioactive metabolites, we encountered a sponge of the genus *Sarcotragus*, the organic extracts of which showed considerable toxicity against brine shrimp larvae (LC_{50} 87 ppm). We describe the isolation and structure determination of 19 new sesterterpenoids and eight previously reported compounds of the same structural class. All of the compounds contained furan and/or related oxygenated functionalities such as γ-lactone, γ-lactam, or corresponding methyl acetals at one end of the linear molecule, while the other end was occupied by tetronic acid, carboxylic acid, or dimethyl diester moieties. Additionally, the absolute stereochemistry of several previously reported compounds was examined by chemical degradation and CD measurements. Several compounds exhibited moderate to significant antibacterial activity, and cytotoxicity and inhibitory activity against isocitrate lyase (ICL) from *Candida albicans*.

Results and Discussion

Freeze-dried specimens were exhaustively extracted with CH_2Cl_2 and MeOH. Guided by the combined results of brine shrimp lethality and ^{1}H NMR analyses, the crude extract was sequentially separated by solvent partitioning, ODS vacuum flash chromatography, and ODS HPLC to yield 27 compounds.

The structures of compounds **1**, **2a**, **2b** (unseparable mixture), and **3**, the major constituents, were identified to be (7*E*,12*E*,18*R*,20*Z*)-variabilin, (7*E*,13*Z*,18*R*,20*Z*)-felixinin, (8*E*,13*Z*,18*R*,20*Z*)-strobilinin, and (8*Z*,13*Z*,18*R*,20*Z*)-strobilinin, respectively, on the basis of combined spectroscopic analyses and comparison of the NMR data with those in the literature.[10–12] In addition, compounds **4**–**6** and **10** were also structurally defined and found to have been previously reported.[7,12,13] The sesterterpenoids **1**–**5** and **10** have frequently been isolated from sponges of the genera *Ircinia*, *Psammocinia*, and *Sarcotragus,* while compound **6**, previously reported as a synthetic analogue, was isolated for the first time as a natural product.[1,13] Compounds **1**–**6** possess a common asymmetric carbon center at C-18. The stereochemistry at this center in **1**–**3** was assigned to be *R*, as in the known compounds, by chemical degradation followed by comparison of the optical rotations with those in the literature (Scheme 1).[7,14–19] The absolute configuration at C-18 of compounds **4** and **6**, previously unassigned, was also determined to be *R* in both cases. Although the limited amounts of compound **5** prevented a stereochemical assignment, comparison of the optical rotation with those of the other compounds suggested the 18*R* configuration. Compound **10**, a furan-containing C_{21} norsesterterpene, also possessed an asymmetric center at the same position. The absolute configuration at this center was determined to be *R* using the PGME method for a β,β-dialkyl carboxylic acid (Scheme 2).[20]

Scheme 1. Oxidative Cleavage of **1** to 2-Methyl-6-oxo-heptanoic Acid



The molecular formula of compound **7** was deduced to be $C_{25}H_{34}O_6$ by combined HRFABMS and ^{13}C NMR analyses. The NMR data for this compound were very similar to those of known sesterterpenoids. In particular, the hydroxy-γ-lactone and tetronic acid moieties of compound **6** were found to be intact in **7** on the basis of ^{1}H COSY, TOCSY, HSQC, and gHMBC experiments (Table 1). Thus, the structural difference had to be in the prenyl chain connecting these terminal functionalities. Two-dimensional (2-D) NMR data placed the double bonds at C-8 and C-13 of the carbon framework. From the characteristic chemical shifts of C-9 and C-14 in the ^{13}C NMR data at δ 23.5 and 23.7, respectively, we assigned the *Z* geometry for both double bonds. Although the

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Scheme 2. PGME Amidation of **10** and $\Delta\delta$(**10*R*** − **10*S***) Values (ppm)

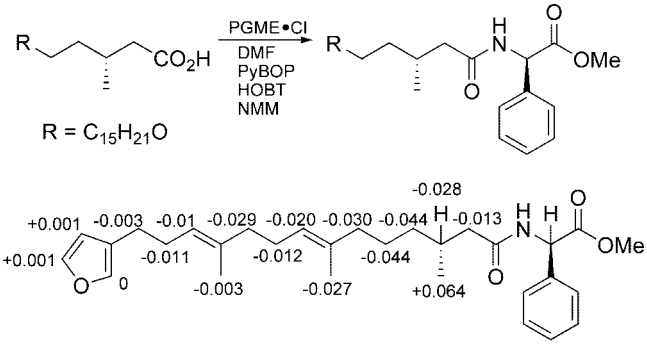


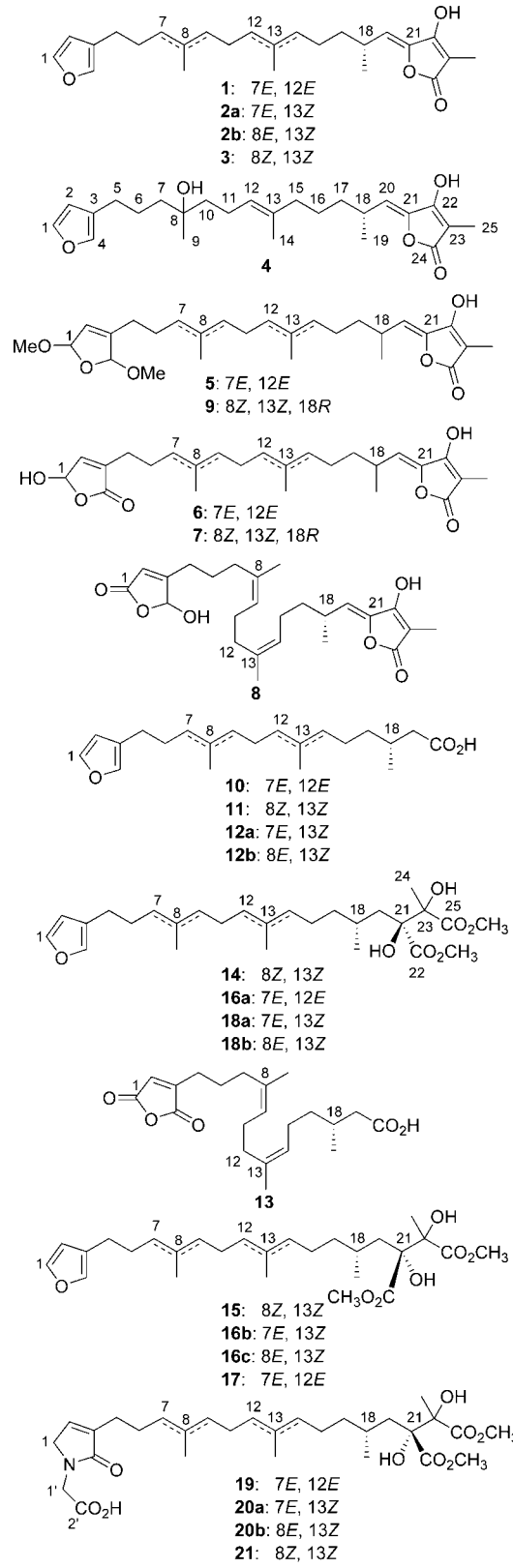
Table 1. ^{13}C NMR Assignments for Compounds **7**–**9** and **11**–**13** (in CD_3OD solutions)

position	**7**	**8**	**9**	**11**	**12a**	**12b**	**13**
1	99.0	173.7	109.1	144.0	142.8[d]	143.9[d]	173.7
2	146.3	117.7	124.9	111.8	112.0	111.9	117.8
3	138.2	172.5	146.8	126.3	126.2	126.4	172.4
4	174.0	101.1	108.1	140.1	140.0[c]	140.1[c]	101.5
5	25.9	26.1	27.2	25.6	26.0	25.2	28.3
6	26.8	28.3	26.7	29.7	29.6	29.5	26.1
7	32.1	32.1	32.3	32.3	125.2	40.3	32.2
8	135.6	135.4	135.8	136.3[a]	136.6	136.0	135.4
9	23.5	23.4	23.5	23.7[b]	16.1	16.0	23.4
10	126.8	127.0	126.6	126.3	40.6	125.6	127.1
11	27.4	27.5	27.5	27.5	27.4	27.4	27.5
12	33.1	33.1	33.2	33.2	32.2	32.8	33.1
13	136.3	136.3	136.3	136.2[a]	136.6	136.2	136.0
14	23.7	23.7	23.7	23.6[b]	23.6	23.6	23.7
15	126.1	126.2	126.1	126.3	126.0	126.3	126.5
16	27.1	27.1	27.1	26.4	26.4[e]	26.3[e]	26.5
17	38.7	38.7	38.7	38.2	38.2	38.2	38.3
18	31.8	31.8	31.8	31.3	31.2	31.2	31.4
19	21.1	21.2	21.1	20.0	20.1	20.1	20.1
20	115.4	114.9	114.9	42.9	42.8	42.8	43.7
21	145.5	145.9	145.9	177.4	177.4	177.4	178.5
22	166.0	168.0	167.1				
23	98.2	98.2	97.4				
24	174.0	174.5	174.5				
25	6.1	6.1	6.1				
1-OMe			54.2				
4-OMe			54.2				

[a] Assignments may be interchanged. [b] Assignments may be interchanged. [c] Assignments may be interchanged. [d] Assignments may be interchanged. [e] Assignments may be interchanged.

limited amounts of the isolated material prevented application of a chemical degradation method, the 18*R* configuration was assigned to this compound on the basis of a comparison of the optical rotation with other metabolites and biogenetic considerations. Because of interference by the tetronic acid moiety, however, the absolute stereochemistry at C-1 could not be assigned using CD measurements.[5]

The molecular formula of compound **8** was found to be $C_{25}H_{34}O_6$, identical to **7**, by HRFABMS and ^{13}C NMR analyses. The NMR data of this compound were also very similar to those of **7**. However, close inspection of the ^{13}C NMR data revealed noticeable differences in the carbon signals of the γ-hydroxy-γ-lactone ring: δ 173.7 (C), 172.5 (C), 117.7 (CH), and 101.1 (CH) (Table 1). Corresponding differences were also observed in the ^{1}H NMR spectra in which the lactone proton signals were moved from δ 6.96 (1 H, br s) and 6.06 (1 H, br s) in **7** to δ 6.00 (1 H, br s) and 5.87 (1 H, s) in **8** (Table 2). These changes were accommodated by the exchange of the carbonyl and hemiacetal carbons and supported by a combination of 2-D experiments. Thus, the carbonyl and hemiacetal carbons were placed at C-4 and C-1, respectively, on the basis of long-range correlations between these carbons and neighboring protons in the gHMBC data. The absolute configuration



at C-18 was assigned as *R*, as in the other compounds, on the basis of chemical degradation, followed by measurement of specific rotation.

The molecular formula of compound **9** was determined to be $C_{27}H_{40}O_6$ by HRFABMS and was consistent with the ^{13}C NMR data. The NMR data for this compound were very similar to those of compound **5**. Combined 2-D NMR analyses revealed that the C-7 and C-12 double bonds of **5** were instead at C-8 and C-13 in **9**. The *Z* configuration was assigned at both double bonds on the

Table 2. ^{1}H MMR Assignments for Compounds **7–9** and **11–13** (in CD_3OD solutions)

position	7	8	9	11	12a	12b	13
1	6.06, br s		5.39, br s	7.38, br s	7.36, br s[a]	7.35, br s[a]	
2	6.96, br s	5.87, s	5.66, br s	6.29, br s	6.29, br s[b]	6.27, br s[b]	5.88, br s
4		6.00, br s	5.52, br s	7.26, br s	7.23, br s	7.23, br s	6.01, br s
5	2.23, t (7.7)	2.37, m	2.09, m	2.44, t (7.5)	2.44, t (7.5)	2.37, t (7.5)	2.39, t (7.5)
6	1.65, m	1.69, m	1.57, m	1.65, m	2.24, q (7.5)	1.64, m	1.72, m
7	2.09, t (7.7)	2.11, t (7.5)	2.06, m	2.07, t (7.5)	5.17, t (7.5)	2.03, m	2.15, t (7.5)
9	1.67, s	1.67, s	1.65, br s	1.68, s	1.58, s	1.61, s	1.69, br s
10	5.15, t (6.6)	5.16, t (6.8)	5.13, m	5.15, t (7.0)	1.97, m	5.17, m	5.21, t (7.0)
11	2.03, m	2.03, m	2.02, m	2.03, m	1.47, m	2.07, m	2.08, m
12	2.00, m	2.00, m	1.99, m	2.02, m	1.97, m	2.07, m	2.05, m
14	1.67, s	1.66, s	1.66, br s	1.66, s	1.67, s	1.68, s	1.67, s
15	5.12, t (6.9)	5.11, t (6.6)	5.12, t (7.7)	5.11, t (6.5)	5.10, m	5.10, m	5.13, t (7.0)
16	1.97, m	1.95, m	1.97, m	2.00, m	2.00, m	2.00, m	2.02, m
17	1.38, m; 1.46, m	1.44, m; 1.35, m	1.44, m; 1.38, m	1.21, m; 1.37, m	1.35, m; 1.22, m	1.35, m; 1.22, m	1.35, m; 1.22, m
18	2.73, m	2.72, m	2.72, m	1.92, m	1.91, m	1.91, m	1.92, m
19	1.06, d (6.7)	1.05, d (6.7)	1.05, d (6.7)	0.94, d (6.6)	0.94, d (6.6)	0.94, d (6.6)	0.94, d (6.6)
20	5.28, d (10.1)	5.26, d (10.0)	5.26, d (10.0)	2.27, dd (14.7, 6.0) 2.07, m	2.28, m; 2.05, m	2.28, m; 2.05, m	2.25, dd (14.3, 5.8) 2.03, m
25	1.73, s	1.71, s	1.71, s				
1-OMe			3.35, s				
4-OMe			3.34, s				

[a] Assignments may be interchanged. [b] Assignments may be interchanged.

basis of downfield shifts of the vinyl methyl carbons: δ 23.5 (C-9) and 23.7 (C-14). The 18*R* configuration was assigned by comparing the optical rotation (+23.2) with that of other sesterterpene tetronic acids, compound **5** (+21.2) in particular.

The molecular formula of compound **11** was deduced to be $C_{21}H_{32}O_3$ by combined HRFABMS and ^{13}C NMR analyses. The NMR data for this norsesterterpene were similar to those of compound **10**. Structural differences were traced to the replacement of the 7*E* and 12*E* double bonds in **10** with the 8*Z* and 13*Z* double bonds in **11** on the basis of combined 2-D NMR experiments. The 18*R* configuration was assigned by the PGME method.

A mixture of compounds **12a** and **12b** was isolated as a colorless oil, which was analyzed as $C_{21}H_{32}O_3$ by HRFABMS and ^{13}C NMR spectrometry. Although these compounds were obtained as an unseparable mixture, detailed examination of 2-D NMR data coupled with chemical shifts of vinyl methyl carbons revealed that they were indeed 7*E*, 13*Z* and 8*E*, 13*Z* isomers, respectively, of **10** and **11**. The configuration at the common asymmetric center at C-18 was determined to be *R*, the same as the other compounds, after applying the PGME method to the mixture.

The molecular formula of compound **13** was established to be $C_{21}H_{32}O_5$ by HRFABMS and ^{13}C NMR spectrometry. Although a norsesterterpene carboxylic acid was evident from its ^{1}H and ^{13}C NMR spectra, carbon signals for the furan ring of other compounds were markedly changed in both chemical shifts and multiplicity. Combined 2-D NMR analyses, including long-range correlations of the newly appearing downfield carbons at δ 173.7 (C) and 172.4 (C) with the neighboring protons in the gHMBC data, established a γ-hydroxy-γ-lactone ring. The 8*Z*, 13*Z*, 18*R* configuration was assigned by the downfield shifts of the vinyl methyls and PGME analysis. In addition, the absolute stereochemistry at the C-4 hemiacetal was assigned to be *S* by CD measurement: a positive Cotton effect, 204 (−1.82), 212 (−0.07), 219 (+0.53), 228 (+0.56) nm.[21] Thus, the structure of compound **13** was determined to be a C_{21} norsesterterpene acid containing a γ-lactone moiety.

Compound **14** was isolated as a colorless oil, which was analyzed as $C_{27}H_{42}O_7$ by HRFABMS and ^{13}C NMR spectrometry. Although the sesterterpene nature of this compound was evident from its spectroscopic data, the loss of the C-20 tetronic acid moiety, present in compounds **1–9**, was readily observed in the ^{13}C NMR spectra. Carbon signals of this functional group were replaced with others appearing at δ 175.5 (C), 175.3 (C), 84.4 (C), 81.8 (C), 52.8 (2 × CH_3), and 21.3 (CH_3) in the ^{13}C NMR spectra (Table 3). Corresponding differences were also observed in the ^{1}H NMR spectra, in which signals of singlet methyl protons appeared at δ 3.73 (×2) and 1.45 (Table 4). In addition, the IR and UV spectra indicated the presence of a hydroxyl-bearing group (ν_{max} 3500 cm^{-1}) and the loss of the tetronic acid moiety (λ_{max} 250 and 210 nm), respectively.

Aided by this information, the structure of **14** was determined by combined 2-D NMR analyses. The ^{1}H COSY, TOCSY, gHSQC, and gHMBC experiments showed that **14** possessed the same furan ring and 8*Z* and 13*Z* double bonds as compounds **3** and **11**. Starting at the furan ring, the proton spin system was traced to the H-19 methyl protons at δ 0.94 and the H-20 methylene protons at δ 1.94 and 1.83 on the basis of TOCSY and gHMBC data (Table 4). The remaining part consisted of newly appearing NMR signals that were also examined in the gHMBC experiment. Specifically, the long-range correlations of the methyl proton at δ 1.45 with the carbons at δ 175.5, 84.4, and 81.8 indicated the presence of a carbonyl-bearing isopropyl equivalent at the terminus of the molecule. The linkage of this group to the prenyl chain was determined by long-range couplings of the H-20 protons with the carbons at δ 84.4 and 81.8. An additional coupling of these protons with the carbonyl carbon at δ 175.3 revealed the presence of an additional methyl equivalent, a rearranged terpene skeleton. Finally, long-range couplings of the two methyl protons at δ 3.75 with the carbonyl carbons at δ 175.5 and 175.3 showed the presence of two methyl esters. Consideration of the molecular formula, coupled with the hydroxyl absorption band in the IR data, revealed that each of the quaternary carbons at δ 84.4 and 81.8 carried a hydroxyl group. Thus, the structure of compound **14** was determined to be a rearranged furanosesterterpene containing a 1,2-dicarbomethoxy-1,2-dihydroxy group. A literature study showed that a compound possessing the same terminal structure was reported as a chemical degradation product of a tetronic acid using a strong base.[22] To our knowledge, however, this is the first isolation of a natural product with this carbon skeleton. The stereochemistry of the asymmetric centers at C-18, C-21, and C-23 is discussed later.

The molecular formula of compound **15** was determined to be $C_{27}H_{42}O_7$ by HRFABMS and was consistent with the ^{13}C NMR data. The spectroscopic data for this compound were very similar to those of **14** (Tables 3 and 4). Because the 2-D NMR data demonstrated that **15** had the same planar structure as **14**, this compound was defined to be an epimer of **14**.

Also obtained by HPLC were compounds **16a–16c** (mixture of three), **17**, and **18a** and **18b** (mixture), all having the same molecular formula as **14** and **15**. Combined 2-D NMR analyses of these compounds revealed that they were 7*E*, 12*E* (**16a** and **17**), 7*E*, 13*Z*

Table 3. ^{13}C NMR Assignments for Compounds **14–21** (in CD_3OD solutions)

position	14	15	16a	16b	16c	17	18a	18b	19	20a	20b	21
1	144.0	144.0	143.8[b]	143.8[b]	143.9[b]	143.8	143.9[i]	143.8[i]	53.2	53.2[q]	53.3[q]	53.3
2	109.6	111.9	112.0[c]	112.0[c]	111.9[c]	112.0	112.0[j]	111.9[j]	138.2	138.2	138.0	137.9
3	126.4	126.3	126.2[d]	126.2[d]	126.4[d]	126.2	126.4[k]	126.2[k]	139.9	139.9	140.3	140.2
4	140.1	140.1	140.1	140.1	140.1	140.1	140.1	140.1	174.1	174.1	174.1	174.0
5	25.6	25.6	26.0	26.0	25.2	26.0	26.0	25.2	27.1	27.1	26.4	26.7
6	29.7	29.7	29.6	29.6	29.5	29.6	29.6	29.5	27.1	27.1	27.1	27.3
7	32.3	32.3	125.2	125.2	40.3	125.3[g]	125.2	40.3	124.8	124.8	40.4	32.4
8	136.3	136.2	136.5	136.7	135.9	136.6	136.6	136.0	137.1	137.1	135.9	136.0
9	23.7	23.66	16.1	16.1	16.0	16.1	16.0	16.0	16.2	16.0	16.1	23.6
10	126.3	126.3	40.8[e]	40.7	125.6	40.8[h]	40.7	125.6	40.8	40.7	125.8	126.47
11	27.5	27.5	27.5	27.5	27.5	27.5	27.5	27.5	27.6	27.5[r]	27.6[r]	27.5
12	33.2	33.2	125.4	32.2	32.8	125.2[g]	32.3	32.9	125.4	32.4	32.9	33.2
13	135.9	135.7	136.0	136.1	135.8	136.1	136.3	136.0	136.1	136.3	136.0	135.9
14	23.7	23.71	16.0	23.6	23.7	16.0	23.7	23.6	16.0	23.7	23.7	23.7
15	126.5	126.7	40.9[e]	126.5	126.7	40.9[h]	126.3	126.5	40.8	126.3	126.5	126.54
16	26.3	26.4	26.2	26.3[f]	26.4[f]	26.3	26.3[l]	26.2[l]	26.2	26.2[s]	26.3[s]	26.3
17	39.5	39.9	38.4	40.0	40.0	39.1	39.5	39.5	38.4	39.5	39.5	39.5
18	30.6	29.4	30.7	29.4	29.4	29.4	30.7[m]	30.6[m]	30.7	30.6[t]	30.5[t]	30.6
19	22.0	20.1	22.1	20.1	20.1	20.2	21.9[n]	22.0[n]	22.1	22.0	22.0	22.0
20	40.6	40.7	40.6	40.6	40.6	40.6	40.6	40.6	40.6	40.6	40.6	40.6
21	84.4	83.3	84.3	83.3	83.3	83.3	84.4	84.4	84.3	84.4	84.4	84.4
22	175.5[a]	175.6	175.5	175.6	175.6	175.7	175.3[o]	175.3[o]	175.5	175.5	175.5	175.5
23	81.8	82.0	81.9	82.0	82.0	82.0	81.8	81.8	81.8	81.8	81.8	81.8
24	21.3	21.3	21.3	21.3	21.3	21.2	21.3	21.3	21.3	21.4	21.4	21.3
25	175.3[a]	175.4	175.4	175.4	175.4	175.4	175.5[p]	175.5[p]	175.4	175.3	175.3	175.3
22-OMe	52.8	52.7	52.8	52.7	52.7	52.7	52.8	52.8	52.8	52.9	52.9	52.8
25-OMe	52.8	52.9	52.8	52.9	52.9	52.9	52.8	52.8	52.8	52.8	52.8	52.8
1′									47.1	47.1	47.1	47.1
2′									174.1	174.1	174.1	174.0

[a] Assignments may be interchanged. [b] Assignments may be interchanged. [c] Assignments may be interchanged. [d] Assignments may be interchanged. [e] Assignments may be interchanged. [f] Assignments may be interchanged. [g] Assignments may be interchanged. [h] Assignments may be interchanged. [i] Assignments may be interchanged. [j] Assignments may be interchanged. [k] Assignments may be interchanged. [l] Assignments may be interchanged. [m] Assignments may be interchanged. [n] Assignments may be interchanged. [o] Assignments may be interchanged. [p] Assignments may be interchanged. [q] Assignments may be interchanged. [r] Assignments may be interchanged. [s] Assignments may be interchanged. [t] Assignments may be interchanged.

Table 4. ^1H NMR Assignments for Compounds **14–18b** (in CD_3OD solutions)

position	14	15	16a	16b	16c	17	18a	18b
1	7.38, br s	7.37, br s	7.36, br s	7.36, br s	7.37, br s	7.36, br s	7.37[b]	7.36[b]
2	6.29, br s	6.29, br s	6.29, br s	6.29, br s	6.28, br s	6.29, br s	6.29[c]	6.28[c]
4	7.25, br s	7.25, br s	7.24, br s	7.24, br s	7.24, br s	7.23, br s	7.24, br s	7.24, br s
5	2.40, t (7.5)	2.40, t (7.6)	2.44, t (7.3)[a]	2.43, t (7.4)[a]	2.37, t (7.6)	2.43, t (7.4)	2.43, t (7.6)	2.37, t (7.6)
6	1.65, m	1.65, m	2.24, m	2.24, m	1.65, m	2.23, q (7.4)	2.25, m	1.65, m
7	2.07, t (7.5)	2.06, t (7.6)	5.17, m	5.17, m	2.01, m	5.16, t (6.6)	5.18, t (7.6)	2.01, m
9	1.68, s	1.67, s	1.58, s	1.58, s	1.61, s	1.58, s	1.58, s	1.61, s
10	5.15, m	5.15, m	1.99, m	1.98, m	5.15, m	1.99, t (7.2)	1.98, m	5.14, t (7.4)
11	2.01, br s	2.01, br s	2.08, m	1.47, m	2.08, m	2.07, q (7.2)	1.46, m	2.08, m
12	2.01, br s	1.99, m	5.08, m	1.96, m	2.06, m	5.08, t (6.6)	1.96, m	2.06, m
14	1.65, s	1.64, s	1.57, s	1.65, s	1.67, s	1.59, s	1.65, s	1.67, s
15	5.06, t (6.9)	5.09, t (7.7)	1.90, t (7.8)	5.09, m	5.11, m	1.92, t (7.3)	5.06, t (7.2)	5.08, t (7.7)
16	1.91, m	1.93, m	1.37, m	1.95, m	1.95, m	1.38, m	1.93, m	1.93, m
17	1.21, m	1.37, m	1.15, m	1.39, m	1.39, m	1.33, m	1.21, m	1.21, m
	1.10, m	1.14, m	1.03, m	1.15, m	1.15, m	1.12, m	1.10, m	1.10, m
18	1.38, m	1.57, m	1.38, m	1.57, m	1.57, m	1.57, m	1.38, m	1.38, m
19	0.94, d (6.6)	0.76, d (6.7)	0.94, d (6.6)	0.76, d (6.6)	0.75, d (6.6)	0.75, d (6.6)	0.94, d (6.6)	0.94, d (6.6)
20	1.94, dd (14.2, 4.4)	2.16, dd (14.1, 4.4)	1.93, m	2.17, dd (14.1, 3.4)	2.17, dd (14.1, 3.4)	2.17, dd (14.0, 4.1)	1.94, m	1.94, m
	1.83, dd (14.2, 7.2)	1.72, dd (14.1, 7.9)	1.83, dd (14.2, 7.1)	1.72, dd (14.1, 8.0)	1.72, dd (14.1, 8.0)	1.69, dd (14.0, 8.1)	1.83, dd (14.1, 7.2)	1.83, dd (14.1, 7.2)
24	1.45, s	1.45, s	1.45, s	1.45, s	1.45, s	1.45, s	1.46, s	1.46, s
22-OMe	3.73, s	3.74, s	3.73, s	3.74, s	3.74, s	3.74, s	3.73, s	3.73, s
25-OMe	3.73, s	3.72, s	3.73, s	3.72, s	3.72, s	3.72, s	3.73, s	3.73, s

[a] Assignments may be interchanged. [b] Assignments may be interchanged. [c] Assignments may be interchanged.

(**16b** and **18a**), and 8*E* and 13*Z* (**16c** and **18b**) configurational isomers of **14** and **15**, respectively.

Close examination of the ^1H and ^{13}C NMR data of compounds **14–18** revealed that chemical shifts of the carbons and protons in the region of C-17 to C-25 were unambiguously divided into two groups. That is, chemical shifts of carbons and protons in compounds **14**, **16a**, **18a**, and **18b** were almost identical to each other, while they were noticeably shifted from those of compounds **15**, **16b**, **16c**, and **17**, the chemical shifts of which were also very similar to each other (Tables 3 and 4). In trying to assign the stereochemistry at C-18, C-21, and C-23, several attempts at converting the natural products to the corresponding cyclic ketals or lactones were unsuccessful.[23] This problem was addressed by NOESY experiments; cross-peaks were found at H-18/H-20β (δ 2.16), H-19/H-20α (δ 1.72), H-19/22-OMe, H-20α/22-OMe, and H-24/22-OMe for **15** (Figure 1). In contrast, cross-peaks were found at H-18/22-OMe, H-19/H-20α (δ 1.83), and H-20α/H-24 for **14**. Assuming the 18*R* configuration, based on biogenetic consider-

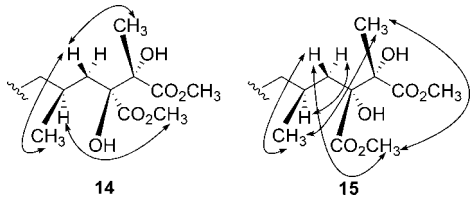


Figure 1. NOE correlations of the 1,2-dicarbomethoxy-1,2-dihydroxy moiety of **14** and **15**.

ations, these NOESY results indicated 21*S* and 21*R* configurations for **14** and **15**, respectively. Because of the lack of reliable NOE correlations, however, the configuration at the terminal C-23 could not be assigned.

The molecular formula of compound **19** was deduced to be $C_{29}H_{45}NO_9$ by combined HRFABMS and ^{13}C NMR analyses. Although the terpenoid nature of this compound was evident from its spectroscopic data, the ^{13}C NMR spectra showed the appearance of additional carbon signals at δ 174.1 (C) and 47.1 (CH_2) (Table 3). Because a preliminary examination of the ^1H and ^{13}C NMR data revealed that this compound possessed the same C-5 to C-25 portion as compound **14**, the structural difference was thought to be related to the remaining portion having the partial formula of $C_6H_6NO_3$. A small spin coupling between the olefinic proton at δ 6.84 and methylene protons at δ 4.05, coupled with the HMBC correlations of these protons with the carbonyl carbon at δ 174.1, allowed us to construct a five-membered carbonyl-bearing ring (Table 5). The IR absorption band at 1670 cm^{-1} suggested this moiety to be an α,β-unsaturated γ-lactam. Furthermore, isolated methylene protons at δ 4.04 exhibited long-range couplings with another carbonyl carbon at δ 175.5. The linkage of this carboxymethylene with the lactam moiety was determined by the mutual HMBC couplings of the methylene protons at δ 4.05 and 4.04 with the carbons bearing these protons (Figure 2). The configurations of the C-7 and C-12 double bonds were assigned as *E* for both on the basis of the upfield shifts of vinyl methyl carbons. Thus, the structure of compound **19** was determined to be a sesterterpene lactam containing a carboxymethylene group. Sesterterpenoids containing this kind of lactam moiety were previously isolated from Korean sponges of the genus *Sarcotragus*.[3,5]

The molecular formula of three closely related compounds, **20a**, **20b** (mixture), and **21**, were all found to be $C_{29}H_{45}NO_9$, identical to **19**, by HRFABMS, and consistent with their ^{13}C NMR data. The spectroscopic data of these compounds were also very similar to those of **19**. Combined 2-D NMR experiments defined that these were configurational isomers of each other, and on the basis of the ^{13}C chemical shifts of the vinyl methyls, 7*E*, 13*Z* (**20a**), 8*E*, 13*Z* (**20b**), and 8*Z*, 13*Z* (**21**) geometries were assigned for these compounds. Comparison of the ^1H and ^{13}C NMR data of these compounds in the region of C-18 to C-25 revealed a very close similarity with those of **14**, **16a**, **18a**, and **18b**, suggesting 18*R*, 21*S* configurations for these compounds. Because of the lack of relevant NOESY cross-peaks, however, the configuration at the terminal C-23 asymmetric center was not assigned.

Sesterterpenoids containing tetronic acid and/or related functional groups frequently exhibit diverse bioactivities.[1] In our measurements of bioactivity, compounds **1**–**3**, **17**, and **18** exhibited moderate to major activity against diverse Gram-positive and Gram-negative strains, including MRSA (Supporting Information). Although none

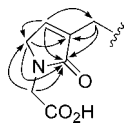


Figure 2. Key HMBC correlations (H → C) of the α,β-unsaturated γ-lactam moiety of **19**.

were active against the fungal strains *Aspergillus fumigatus* HIC6094, *A. niger* ATCC9642, *A. niger* IFO6661, or *C. albicans* SC5314, several compounds, in particular, **6** and **13**, showed moderate inhibitory activity against isocitrate lyase (ICL), an enzyme that plays a key role in fungal metabolism derived from *C. albicans*.[24] In addition, **3**, **11**, and **12** displayed moderate cytotoxicity against the K562 cell line. Although the bioactivity tests displayed somewhat scattered results, a general trend was revealed; the bioactivity of these sesterterpenoids was apparently attributable to the presence of tetronic acid or carboxylic acid moieties.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO P-1020 polarimeter using a 1 cm cell. CD data were obtained on a JASCO J-715 spectropolarimeter in MeOH solutions, UV spectra were recorded on a Hitachi U-3010 spectrophotometer, and IR spectra were recorded on a JASCO 300E FT-IR spectrometer. NMR spectra were recorded in CD_3OD solutions containing Me_4Si as an internal standard on Bruker Avance 500 and Varian Gemini 2000 spectrometers. Proton and carbon NMR were measured at 500 and 125 MHz, respectively. Mass spectra were obtained on a JEOL JMS-700 high-resolution mass spectrometer for FAB experiments, provided by the Basic Science Research Institute, Seoul Branch, Seoul, Korea. All solvents used were spectral grade or distilled from glass prior to use.

Animal Material. Specimens of *Sarcotragus* sp. (voucher number 97J-4) were collected by hand using scuba (25 m) off the shore of Jeju Island in July 1997. The morphological characteristics of this sponge have been reported previously. Similar specimens (voucher number 01SH-15) were also collected (25–30 m) off the shore of Soheuksan Island, Korea, in June 2001. Gross morphological features, such as color and shape of this sponge, were very similar to those collected previously at a nearby area. The voucher specimens are deposited at the Sponge Collection, KORDI, under the curatorship of H.-S.L.

Extraction and Isolation. Freshly collected specimens were immediately frozen and kept at –25 °C until chemical investigation. The combined specimens were lyophilized (dry wt 560 g), macerated, and repeatedly extracted with CH_2Cl_2 (4 L × 3) and MeOH (4 L × 3). The combined crude extracts were successively partitioned between H_2O and *n*-BuOH, and then the latter was repartitioned between 15% aqueous MeOH (26.4 g) and *n*-hexane (10.1 g). The aqueous MeOH layer was separated by C_{18} reversed-phase vacuum flash chromatography using a sequential mixture of MeOH and H_2O as eluents (elution order: 50%, 40%, 30%, 20%, 10% aqueous MeOH, 100% MeOH), acetone, and finally EtOAc.

On the basis of the combined results of the bioassay and ^1H NMR analysis, the fractions eluted with 10% aqueous MeOH (6.22 g) from flash chromatography were separated by Sephadex LH-20 gel-permeation chromatography using MeOH as an eluent. The fractions containing sesterterpenoids were combined and separated by semi-preparative silica HPLC (YMC-Pack silica column, 1 cm × 25 cm, 20% EtOAc/*n*-hexane). Purification of these metabolites was then accomplished by reversed-phase HPLC (15% aqueous MeCN and then 15% aqueous MeOH) to yield compounds **11**, **12**, and **10**, in order of elution, as colorless oils. Another terpenoid-containing LH-20 fraction was also separated and purified by reversed-phase HPLC (20% aqueous MeCN for **4**, 17.5% aqueous MeOH for **5**, **9**, **14**–**18**, 15% aqueous MeOH for **1**–**3**). The purified metabolites were isolated in the following amounts: 100.2, 109.3, 107.6, 16.5, 2.8, 4.6, 14.7, 20.2, 17.1, 8.5, 10.9, 15.2, 8.2, and 8.4 mg of **1**–**5**, **9**–**12**, and **14**–**18**, respectively.

The fractions eluted with 20% MeOH(aq) (2.76 g) were separated by Sephadex LH-20 gel-permeation chromatography (4 × 35 cm) using MeOH (20 mL/fraction) as an eluent to yield 25 fractions. Fraction LH3 was separated by reversed-phase HPLC (YMC-Pack CN column, 60% MeOH(aq)) to yield compounds **19**–**21**, in order of elution, as pale yellow oils. On the basis of the TLC results, fractions LH9–11 were combined and separated by semipreparative reversed-phase HPLC (YMC-Pack ODS-A column, 1 cm × 25 cm, 22.5% MeOH(aq)) to yield compounds **13**, **8**, **7**, and **6**, in order of elution, as colorless oils. The purified metabolites were obtained in the following amounts: 11.8, 12.7, 3.8, 5.8, 23.5, 23.3, and 22.9 mg of **6**–**8**, **13**, and **19**–**21**, respectively.

Table 5. ^{1}H NMR Assignments for Compounds **19**–**21** (in CD_3OD solutions)

position	**19**	**20a**	**20b**	**21**
1	4.05, br s	4.06, br s	4.06, br s	4.06, br s
2	6.84, br s	6.85, br s	6.85, br s	6.85, br s
5	2.26, m	2.27, m	2.21, t (7.7)	2.23, t (7.4)
6	2.26, m	2.06, m	1.67, m	1.65, m
7	5.16, t (6.0)	5.17, m	2.04, m	2.11, t (7.5)
9	1.61, s	1.61, s	1.62, s	1.68, br s
10	2.01, m	1.96, m	5.17, m	5.16, t (6.8)
11	2.07, m	1.49, m	2.08, m	2.06, m
12	5.09, t (6.5)	1.97, m	2.06, m	2.03, m
14	1.57, s	1.65, s	1.67, s	1.66, br s
15	1.91, m	5.07, m	5.07, m	5.07, t (6.8)
16	1.37, m	1.93, m	1.93, m	1.92, m
17	1.15, m	1.21, m	1.21, m	1.21, m
	1.03, m	1.11, m	1.11, m	1.11, m
18	1.38, m	1.38, m	1.38, m	1.38, m
19	0.93, d (6.6)	0.94, d (6.6)	0.94, d (6.6)	0.94, d (6.6)
20	1.93, dd (14.1, 4.7)	1.94, m	1.94, m	1.94, dd (14.1, 4.7)
	1.83, dd (14.1, 7.2)	1.83, dd (14.2, 7.2)	1.83, dd (14.2, 7.2)	1.83, dd (14.1, 7.1)
24	1.45, s	1.46, s	1.46, s	1.45, s
1′	4.04, s	4.03, br s	4.03, br s	4.04, s
22-OMe	3.73, s[a]	3.73, s	3.73, s	3.73, s
25-OMe	3.74, s[a]	3.73, s	3.73, s	3.72, s

[a] Assignments may be interchanged.

Compound 1: colorless oil; $[\alpha]_D^{20}$ +36.4 (*c* 1.15, MeOH) {lit. + 33.3 (*c* 1.1, MeOH)};[12] IR (KBr) ν_{max} 2950, 1760, 1635, 1500, 1425, 1285 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 290 (3.71), 253 (4.04) nm.

Compound 2 (2a and 2b): colorless oil; $[\alpha]_D^{20}$ +50.7 (*c* 1.14, MeOH) {lit. + 44.6 (*c* 0.01, MeOH)};[11] IR (KBr) ν_{max} 2950, 1760, 1630, 1505, 1425, 1280 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 297 (3.73), 255 (3.97) nm.

Compound 3: colorless oil; $[\alpha]_D^{20}$ +46.4 (*c* 0.87, MeOH); IR (KBr) ν_{max} 2950, 1760, 1635, 1500, 1425, 1280 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 307 (3.74), 253 (3.98) nm.

Compound 4: colorless oil; $[\alpha]_D^{20}$ +9.4 (*c* 0.48, MeOH) {lit. –24.5 (*c* 0.22, MeOH)};[13] IR (KBr) ν_{max} 3400 (br), 2945, 1815, 1760, 1640, 1460, 1260, 1125 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 307 (4.05), 250 (4.32), 209 (4.24) nm.

Compound 5: colorless oil; $[\alpha]_D^{20}$ +21.2 (*c* 0.52, MeOH) {lit. + 16.3 (*c* 0.1, MeOH)};[14] IR (KBr) ν_{max} 2955, 1760, 1640, 1545, 1455, 1310 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 313 (3.60), 250 (3.93), 209 (3.87) nm.

Compound 6: colorless oil; $[\alpha]_D^{20}$ +38.6 (*c* 0.52, MeOH); IR (KBr) ν_{max} 3400 (br), 2955, 2925, 1735, 1640, 1455, 1280 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 307 (3.80), 254 (3.98) nm.

Compound 7: colorless oil; $[\alpha]_D^{20}$ +36.4 (*c* 0.37, MeOH); IR (KBr) ν_{max} 3400 (br), 2925, 1735, 1645, 1545, 1310, 1140 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 307 (3.79), 253 (3.97) nm; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRFABMS *m/z* 453.2250 [M + Na]$^+$ (calcd for $C_{25}H_{34}O_6Na$, 453.2253).

Compound 8: colorless oil; $[\alpha]_D^{20}$ +21.2 (*c* 0.46, MeOH); IR (KBr) ν_{max} 3400 (br), 2925, 1740, 1645, 1545, 1455, 1310, 1135 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 310 (3.73), 210 (4.85) nm; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRFABMS *m/z* 453.2233 [M + Na]$^+$ (calcd for $C_{25}H_{34}O_6Na$, 453.2253).

Compound 9: colorless oil; $[\alpha]_D^{20}$ +23.2 (*c* 0.55, MeOH); IR (KBr) ν_{max} 2960, 2925, 1760, 1640, 1545, 1455, 1305, 1140 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 308 (3.70), 250 (3.96), 213 (3.70) nm; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRFABMS *m/z* 483.2716 [M + Na]$^+$ (calcd for $C_{27}H_{40}O_6Na$, 483.2723).

Compound 10: colorless oil; $[\alpha]_D^{20}$ +8.8 (*c* 0.47, MeOH); IR (KBr) ν_{max} 2960, 2930, 1730, 1580, 1460, 1285 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 210 (4.03) nm.

Compound 11: colorless oil; $[\alpha]_D^{20}$ +7.0 (*c* 0.60, MeOH); IR (KBr) ν_{max} 2960, 2925, 1730, 1575, 1460, 1285 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 307 (3.74), 253 (3.99) nm; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRFABMS *m/z* 355.2249 [M + Na]$^+$ (calcd for $C_{21}H_{32}O_3Na$, 355.2249).

Compound 12 (12a and 12b): colorless oil; $[\alpha]_D^{20}$ +7.8 (*c* 0.51, MeOH); IR (KBr) ν_{max} 2925, 1735, 1575, 1455, 1285 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 274 (2.84), 209 (3.90) nm; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRFABMS *m/z* 355.2245 [M + Na]$^+$ (calcd for $C_{21}H_{32}O_3Na$, 355.2249), 355.2419 [M + H]$^+$ (calcd for $C_{21}H_{33}O_3$, 355.2430).

Compound 13: colorless oil; $[\alpha]_D^{20}$ +6.4 (*c* 0.51, MeOH); IR (KBr) ν_{max} 3400 (br), 2960, 2930, 1730, 1455, 1285 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 297 (3.08), 251 (3.42), 210 (4.15) nm; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRFABMS *m/z* 387.2148 [M + Na]$^+$ (calcd for $C_{21}H_{32}O_5Na$, 387.2147).

Compound 14: colorless oil; $[\alpha]_D^{20}$ –7.6 (*c* 0.52, MeOH); IR (KBr) ν_{max} 3500 (br), 2925, 1735, 1460, 1280, 1125 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 250 (3.15), 210 (4.00) nm; ^{1}H and ^{13}C NMR data, see Tables 3 and 4; HRFABMS *m/z* 479.2991 [M + H]$^+$ (calcd for $C_{27}H_{43}O_7$, 479.3009).

Compound 15: colorless oil; $[\alpha]_D^{20}$ +0.6 (*c* 0.45, MeOH); IR (KBr) ν_{max} 3500 (br), 2930, 1730, 1465, 1280, 1125 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 210 (3.98) nm; ^{1}H and ^{13}C NMR data, see Tables 3 and 4; HRFABMS *m/z* 479.3007 [M + H]$^+$ (calcd for $C_{27}H_{43}O_7$, 479.3009).

Compound 16 (16a, 16b, and 16c): colorless oil; $[\alpha]_D^{20}$ +13.2 (*c* 0.54, MeOH); IR (KBr) ν_{max} 3500 (br), 2960, 2925, 1730, 1460, 1280, 1125 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 211 (3.99) nm; ^{1}H and ^{13}C NMR data, see Tables 3 and 4; HRFABMS *m/z* 479.3011 [M + H]$^+$ (calcd for $C_{27}H_{43}O_7$, 479.3009).

Compound 17: colorless oil; $[\alpha]_D^{20}$ +5.2 (*c* 0.67, MeOH); IR (KBr) ν_{max} 3450 (br), 2960, 2925, 1730, 1465, 1280 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 210 (3.83) nm; ^{1}H and ^{13}C NMR data, see Tables 3 and 4; HRFABMS *m/z* 501.2821 [M + Na]$^+$ (calcd for $C_{27}H_{42}O_7Na$, 501.2828).

Compound 18 (18a and 18b): colorless oil; $[\alpha]_D^{20}$ +7.5 (*c* 0.50, MeOH); IR (KBr) ν_{max} 3450 (br), 2950, 1735, 1460, 1280, 1125 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 210 (3.99) nm; ^{1}H and ^{13}C NMR data, see Tables 3 and 4; HRFABMS *m/z* 501.2824 [M + Na]$^+$ (calcd for $C_{27}H_{42}O_7Na$, 501.2828).

Compound 19: pale yellow oil; $[\alpha]_D^{20}$ –2.4 (*c* 0.58, MeOH); IR (KBr) ν_{max} 3450 (br), 2960, 2925, 1735, 1670, 1460, 1280 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 250 (3.11), 211 (3.92) nm; ^{1}H and ^{13}C NMR data, see Tables 3 and 5; HRFABMS *m/z* 574.2993 [M + Na]$^+$ (calcd for $C_{29}H_{45}NO_9Na$, 574.2992).

Compound 20 (20a and 20b): pale yellow oil; $[\alpha]_D^{20}$ –2.5 (*c* 0.73, MeOH); IR (KBr) ν_{max} 3450 (br), 2955, 2925, 1735, 1670, 1460, 1280 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 211 (3.92) nm; ^{1}H and ^{13}C NMR data, see Tables 3 and 5; HRFABMS *m/z* 574.2995 [M + Na]$^+$ (calcd for $C_{29}H_{45}NO_9Na$, 574.2992).

Compound 21: pale yellow oil; $[\alpha]_D^{20}$ –2.8 (*c* 0.47, MeOH); IR (KBr) ν_{max} 3500 (br), 2955, 2925, 1730, 1670, 1460, 1280 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 247 (3.16), 211 (3.92) nm; ^{1}H and ^{13}C NMR data, see Tables 3 and 5; HRFABMS *m/z* 574.2994 [M + Na]$^+$ (calcd for $C_{29}H_{45}NO_9Na$, 574.2992).

Oxidative Cleavage of Sesterterpenoids to 2-Methyl-6-oxo-heptanoic Acid (1, 4, and 6). The reaction was carried out following the general procedure for compound **1**. From 6.5 mg (0.016 mmol) of **1**, we obtained 1.0 mg (0.006 mmol, 39% yield) of (*R*)-(−)-2-methyl-6-oxo-heptanoic acid: $[\alpha]_D^{20}$ –5.2 (*c* 0.08, MeOH); ^{1}H NMR (CD_3OD)

δ 2.50 (1 H, m, H-2), 2.45 (2 H, t, $J = 6.8$ Hz, H-5), 2.14 (3 H, s, H-7), 1.61 (2 H, m, H-4), 1.44 (2 H, m, H-3), 1.20 (3 H, d, $J = 7.2$ Hz, 2-CH_3). Authentic 2-methyl-6-oxo-heptanoic acid prepared from 2,6-dimethylcyclohexanone following the procedure reported previously gave identical ^{1}H NMR data.[7,12,13] The same degradation with **4** and **6** yielded (*R*)-(−)-2-methyl-6-oxo-heptanoic acid with $[\alpha]_D^{20}$ values of –6.7 (*c* 0.03, MeOH) and –8.3 (*c* 0.06, MeOH), respectively.

Oxidative Cleavage of Sesterterpenoids to Dimethyl-2-methylglutarate (2, 3, and 8). The reaction was carried out following the general procedure for **3**: 26.3 mg of $RuCl_3 \cdot xH_2O$ was added to a biphasic solution of 7.8 mg (0.02 mmol) of **3** and 50.3 mg (0.24 mmol) of $NaIO_4$ in a mixture of 1 mL of CCl_4, 1 mL of MeCN, and 1.5 mL of H_2O. After vigorously stirring the mixture for 2 h at room temperature, the solvents were removed under vacuum. The residue was redissolved in acetone and filtered on an ODS Sep-Pak column. One milliliter of HCl·MeOAc was added to the filtrate under N_2 and stirred for 12 h. The solvent was removed under vacuum, and the residue was separated by reversed-phase HPLC (20% MeOH(aq)) to yield 1.4 mg (0.008 mmol, 40.0% yield) of dimethyl-2-methyl glutarate at a retention time of 7.4 min (flow rate 0.5 mL/min): $[\alpha]_D^{20}$ –9.1 (*c* 0.1, MeOH); ^{1}H NMR (CD_3OD) δ 3.63 (6 H, s, OCH_3), 2.48 (1 H, m, H-2), 1.89 (2 H, t, $J = 7.5$ Hz, H-4), 1.72 (2 H, m, H-3), 1.14 (3 H, d, $J = 7.2$ Hz, 2-CH_3). Authentic glutarates prepared similarly from (*R*)-(−)-2-methylglutaric acid and (*S*)-(+)-2-methylglutaric acid gave $[\alpha]_D^{20}$ –20.8 (*c* 0.53, MeOH) and +19.1 (*c* 0.79, MeOH), respectively. The same process with **2** and **8** gave (*R*)-(−)-dimethyl-2-methylglutarate with $[\alpha]_D^{20}$ values of –16.0 (*c* 0.07, MeOH) and –13.1 (*c* 0.15, MeOH), respectively.

PGME Amidation of 10. The reaction was carried out following the general procedure reported previously for the stereochemical assignment of β,β-dialkylcarboxylic acid.[6,20] Four milligrams of (*R*)-PGME·Cl (phenylglycine methyl ester chloride), 10.4 mg of PyBOP (benzothiazol-1-yloxy) tripyrrolidinophosphonium hexafluorophosphate, 2.7 mg of HOBT (1-hydroxybenzotriazole), and 0.2 mL of NMM (*N*-methylmorphosphate) were sequentially added to a stirred 1.7 mg (0.005 mmol) solution of **10** in 2 mL of DMF in a 3 mL vial at 0 °C. The reaction mixture was stirred at room temperature for 1 h. After removing the solvent under vacuum, the residue was separated by silica HPLC (40% EtOAc/*n*-hexane) to afford 0.9 mg of (*R*)-PGME amide (**10*R***). The same reaction with (*S*)-PGME·Cl yielded 0.9 mg of the corresponding (*S*)-PGME amide (**10*S***) from 1.7 mg of **10**: **10*R*** ^{1}H NMR data ($CDCl_3$) δ 7.397–7.338 (H-1, Ar), 7.231 (H-4), 6.283 (H-2), 5.455 (H-2′), 5.154 (H-7), 5.067 (H-12), 3.690 (OCH_3), 2.426 (H-5), 2.253 (H-6), 2.210 (H-20), 1.996 (H-20), 1.975 (H-11), 1.937 (H-10), 1.913 (H-15), 1.889 (H-18), 1.571 (H-9), 1.547 (H-14), 1.432–1.231 (H-16, H-17), 0.946 (H-19); **10*S*** ^{1}H NMR ($CDCl_3$) δ 7.378–7.336 (H-1, Ar), 7.231 (H-4), 6.282 (H-2), 5.466 (H-2′), 5.164 (H-7), 5.087 (H-12), 3.689 (OCH_3), 2.429 (H-5), 2.264 (H-6), 2.218 (H-20), 2.009 (H-20), 1.966 (H-10), 1.987 (H-11), 1.943 (H-15), 1.917 (H-18), 1.574 (H-9, H-14), 1.476–1.280 (H-16, H-17), 0.946 (H-19); $\Delta\delta$ (**10*R*** − **10*S***) H-1, Ar, +0.001 to +0.002 ppm; H-2, +0.001 ppm; H-4, 0 ppm; H-5, −0.003 ppm, H-6, –0.011 ppm, H-7, –0.010 ppm, H-9, –0.003 ppm; H-10, –0.029 ppm, H-11, –0.012 ppm; H-12, –0.020 ppm; H-14, –0.027 ppm; H-15, –0.030 ppm; H-16, H-17, –0.044 to –0.049 ppm; H-18, –0.028 ppm; H-19, +0.064 ppm; H-20, –0.013 ppm; H-20, –0.008 ppm; H-2′, –0.011 ppm; OCH_3, +0.001 ppm.

PGME Amidation of 11. Prepared as described for **10**. We obtained 1.1 and 1.4 mg of **11*R*** and **11*S*** from 2.3 and 2.3 mg of **11**, respectively. **11*R*** ^{1}H NMR (CD_3OD) δ 7.364–7.342 (H-1, Ar), 7.248 (H-4), 6.276 (H-2), 5.452 (H-2′), 5.093 (H-10, H-15), 3.687 (OCH_3), 2.390 (H-5), 2.255 (H-20), 2.219 (H-7), 2.100 (H-11), 2.055 (H-12), 2.024 (H-20), 2.006 (H-16), 1.664 (H-9), 1.660 (H-14), 1.635 (H-6), 1.412–1.154 (H-17), 0.937 (H-19); **11*S*** ^{1}H NMR data (CD_3OD) δ 7.376–7.347 (H-1, Ar), 7.249 (H-4), 6.279 (H-2), 5.467 (H-2′), 5.119 (H-10, H-15), 3.687 (OCH_3), 2.396 (H-5), 2.265 (H-20), 2.101 (H-11), 2.055 (H-12), 2.023 (H-20), 2.011 (H-16), 1.940 (H-18), 1.671 (H-9), 1.668 (H-9, H-14), 1.644 (H-6), 1.426–1.164 (H-17), 0.874 (H-19); $\Delta\delta$ (**11*R*** – **11*S***) H-1, Ar, –0.120 to –0.005 ppm; H-2, –0.003 ppm; H-4, –0.001 ppm; H-5, –0.006 ppm; H-6, –0.009 ppm; H-7, –0.046 ppm; H-9, –0.007 ppm; H-10, –0.026 ppm; H-11, –0.001 ppm; H-12, 0 ppm; H-14, –0.008 ppm; H-15, –0.026 ppm; H-16, –0.005 ppm; H-17, –0.014 to –0.010 ppm; H-18, –0.023 ppm; H-19, +0.063 ppm; H-2′, –0.015 ppm; OCH_3, 0 ppm.

PGME Amidation of 12. Prepared as described for **10**. We obtained 1.6 and 1.5 mg of **12*R*** and **12*S*** from 2.4 and 2.2 mg of **12** (mixture of **12a** and **12b**), respectively. **12*R*** ^{1}H NMR (CD_3OD) δ 7.377–7.337 (H-1, Ar), 7.231 (H-4), 6.276 (H-2), 5.452 (H-2′), 5.087 (H-15), 3.689 (OCH_3), 1.652 (H-14), 1.582 (H-9), 0.942 (H-19); **12*S*** ^{1}H NMR (CD_3OD) δ 7.379–7.348 (H-1, Ar), 7.234 (H-4), 6.279 (H-2), 5.466 (H-2′), 5.087 (H-15), 3.687 (OCH_3), 1.666 (H-14), 1.590 (H-9), 0.878 (H-19); $\Delta\delta$ (**12*R*** – **12*S***) H-1, Ar, –0.011 to –0.002 ppm; H-2, –0.003 ppm; H-4, –0.003 ppm; H-9, –0.008 ppm; H-14, –0.008 ppm; H-15, 0 ppm; H-19, +0.064 ppm; H-2′, –0.014 ppm; OCH_3, +0.020 ppm.

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Supporting Information Available: Antibacterial activity and cytotoxicity against the K562 cell line and inhibitory activity against isocitrate lyase. This material is available free of charge via the Internet at http://pubs.acs.org.

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