New Cytotoxic Sesterterpenoids and Norsesterterpenoids from Two Sponges of the Genus *Sarcotragus*

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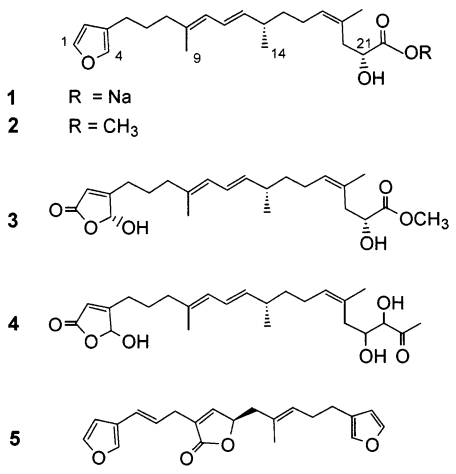
New norsesterterpenoids (**3** and **4**), a sesterterpenoid (**6**), pyrroloterpenoids (**7**−**10**), and a stereoisomer of kurospongin (**5**) were isolated, along with known furanosesterterpenes (**11**−**15**), from two marine sponges of the genus *Sarcotragus*. The gross structures were established on the basis of NMR and MS analysis. The stereochemistry was defined by combined use of NMR and CD spectroscopy. The compounds were evaluated for cytotoxicity against five human tumor cell lines and were found to exhibit marginal to moderate activity.

Marine sponges of the order Dictyoceratida have frequently provided a large number of linear furanoterpenoids.[1] The structural conciseness and diverse bioactivity of the compounds have made them attractive targets for both biomedical and synthetic purposes. In the course of our study on cytotoxic constituents of a sponge *Sarcotragus* sp., 23 terpenoids and three cyclitol derivatives were isolated.[2−4] In a continuing study on minor terpenoids from the same sponge, two new norsesterterpenoids (**3** and **4**) and a C_{21} furanoterpene (**5**) were isolated. Unlike other common furanosesterterpenes, compounds **3** and **4** were carrying an oxidized furan ring similar to that found in manoalide.[5] Compound **5** is the first C_{21} furanoterpene to be reported from this genus.



For a successive chemical study on a sponge of the genus *Sarcotragus*, we collected another sponge of the same genus. Guided by ^{1}H NMR-monitoring and brine shrimp lethality, a new furanosesterterpenoid (**6**) and four new pyrroloterpenoids (**7**−**10**) were isolated, along with five known furanosesterterpenes, sarcotin A (**11**),[2,3] *epi*-sarcotin A (**12**),[3] *epi*-sarcotrine B (**13**),[3] (7*E*,12*E*,18*R*,20*Z*)-variabilin (**14**),[6] and (8*E*,13*Z*,18*R*,20*Z*)-strobilinin (**15**).[6] The occurrence of pyrroloterpenoids, possibly biosynthesized by condensation of a sesterterpene and an amino acid derived unit,[7,8] is highly unusual among marine natural products. Sarcotin A (**11**) and *epi*-sarcotin A (**12**) were isolated in high yield (0.07 and 0.03%, wet weight) from this species. Variabilin (**14**) was previously isolated from this genus, while strobilinin (**15**) was first isolated from this genus. The gross structures of the compounds were elucidated by the aid of COSY, HSQC, and HMBC experiments, while the stereochemistry was proposed by comparison of the NMR and CD spectral data. The isolation, structure elucidation, and cytotoxicity evaluation of the new compounds are described herein.

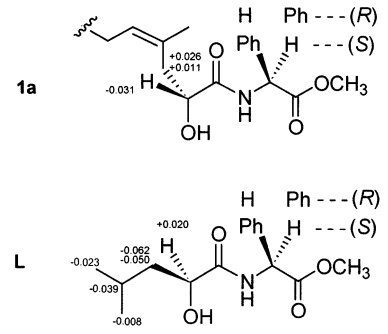


Figure 1. Conformations and $\Delta\delta$ ($\delta_S - \delta_R$) values of the PGME amides of **1** (**1a**) and L-leucic acid (**L**).[9]

Results and Discussion

The MeOH extract of the first *Sarcotragus* sponge that we have been studying was successively fractionated employing reversed-phase flash column chromatography and HPLC to afford cytotoxic components from the active fractions.[2−4] From the less active fractions, compounds **3**−**5** were isolated as minor components. The MeOH extract of the second *Sarcotragus* sponge showed significant toxicity to brine shrimp larvae (LD_{50}, 149 μg/mL). The MeOH extract was successively fractionated employing reversed-phase flash column chromatography and ODS HPLC to afford compounds **6**−**15**.

In the previous report of sarcotin I (**1**), the configuration of the C-21 hydroxyl group was undetermined due to rapid

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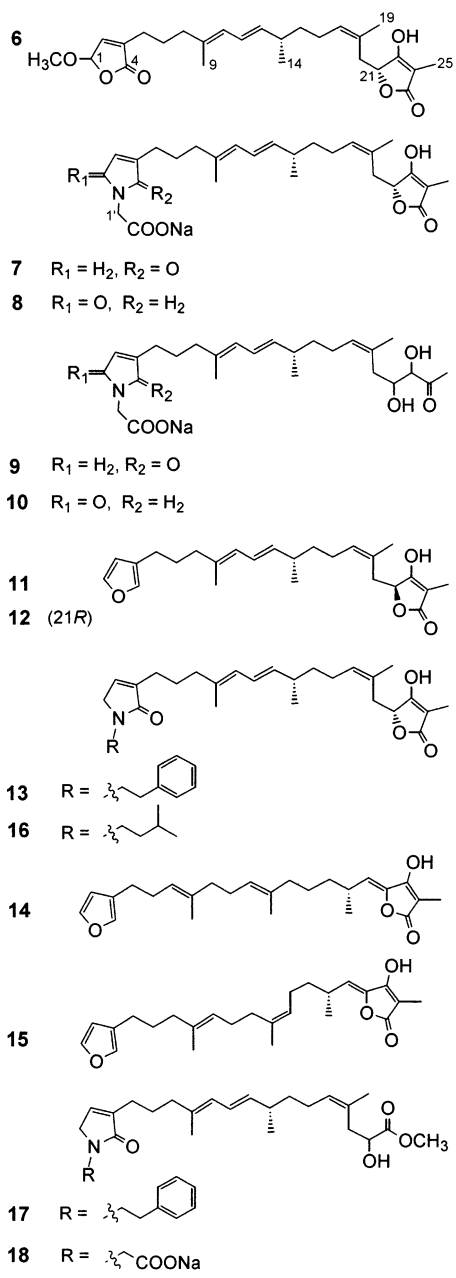

decomposition of the compound in MTPA esterification.[3] Phenylglycine methyl ester (PGME) has recently been used as a chiral anisotropic reagent for determination of the absolute configuration of α-substituted chiral carboxylic acids.[9] The $\Delta\delta$ ($\delta_S - \delta_R$) values of the PGME amides of **1** (Figure 1) were opposite in sign of those of the model compound L-leucic acid,[9] so the configuration at C-21 was defined as *R*. Co-occurring sarcotin J (**2**)[3] was assumed to have the same configuration at C-21.

Sarcotin N (**3**) was isolated as a colorless oil. The molecular formula of **3** was established as $C_{23}H_{34}O_6$ on the basis of HRFABMS data. Analysis of its NMR data suggested that it shares the same carbon framework as sarcotin J (**2**), but includes a 5-hydroxy-2(5*H*)-furanone moiety instead of the furan ring found in **2**. The presence of the 5-hydroxy-2(5*H*)-furanone moiety was deduced from the characteristic NMR chemical shifts. The signals corresponding to a lactone carbonyl group (δ_C 171.4, C-1), a hemiacetal moiety (δ_H 6.04, δ_C 99.2, C-4), and two olefinic carbons (δ_C170.0 and 117.3, C-3 and -2, respectively) were observed.[10] The long-range correlation between H-5 and C-4

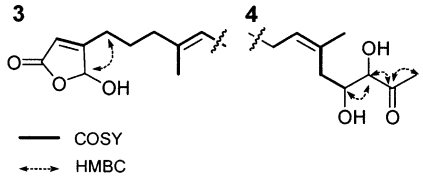

Figure 2. Key HMBC and COSY correlations of **3** and **4**.

was observed in the HMBC spectrum (Figure 2). The ^{1}H NMR spectrum also featured two vinylic methyl singlets overlapping at δ 1.73 (δ_C 16.0 and 24.2), a secondary methyl doublet at δ 0.99 (δ_C 21.3), a trisubstituted olefin (δ 5.25), and a 1,1,4-trisubstituted diene (δ 6.20, 5.81, and 5.41). The geometry of the trisubstituted double bond (C-8) was assigned as *E* on the basis of the upfield resonance of the vinylic methyl carbon (δ_C 16.0, C-9),[11] while the geometry of the disubstituted double bond (C-11) was determined to be *E* on the basis of the coupling constant of the respective olefinic protons ($J = 15.0$ Hz). The downfield-shifted carbon signal of the C-19 methyl (δ_C 24.4) indicated a *Z* geometry of this trisubstituted double bond, which was also supported by the upfield-shifted signal of C-20 (δ 36.3) compared to that of palinurin (δ 41.6, C-20).[12] It appears that the lactone ring readily epimerizes in a polar solvent. The signals of H-2 and -4 appeared as rather sharp singlets in acetone-d_6. However, they each changed into broad singlets and shifted in CD_3OD, possibly due to enhanced epimerization at C-4, as observed in manoalide derivatives.[13] The stereochemistry of the major epimer could be deduced from CD data.[14,15] It has been reported that the absolute configuration of heterosubstituted 2(5*H*)-furanone is related to the sign of the Cotton effect of $\pi-\pi^*$ (200−220 nm) and $n-\pi^*$ (235−250 nm) transitions. The positive Cotton effect at 228 nm ($\pi-\pi^*$) and the negative Cotton effect at 244 nm (n-π^*) of compound **3** suggested an *R* configuration at C-4.[14,15] The configurations at C-13 and -21 were assumed to be the same as those of sarcotin I (**1**).

Sarcotin O (**4**) was isolated as a colorless oil. The molecular formula of **4** was established as $C_{24}H_{36}O_6$ on the basis of HRFABMS data. Most of the ^{1}H and ^{13}C NMR spectra of **4** were in accordance with those of **3**. However, the signals corresponding to the α-hydroxycarboxylic group were replaced by an α,β-dihydroxybutanone moiety. Corresponding HMBC correlations of the α,β-dihydroxybutanone moiety were observed (Figure 2). As in the case of **3**, the NMR spectra of **4** were highly solvent-dependent, possibly due to a facile epimerization at C-4.[13] In CD_3OD, only a signal at δ 5.92 (H-2) was observed for the moiety. In acetone-d_6, both signals at δ 5.90 and 6.09 were observed, the latter as a very broad one. The configuration at C-13 was assumed to be the same as that of sarcotin A (**11**).[3] An attempt to convert **4** to its MTPA ester by the modified Mosher's method was unsuccessful due to a rapid decomposition of the reactant.

ent-Kurospongin (**5**) was isolated as a yellow oil. The NMR and MS data of **5** were identical to those reported for kurospongin isolated from a Japanese sponge *Spongia* sp.[16] However, the optical rotation of **5** was opposite in sign of that reported (**5**: $[\alpha]^{21}_D$ +33.4°, $CHCl_3$; kurospongin: $[\alpha]_D$ −16.8°, $CHCl_3$). This suggested that **5** is enantiomeric to kurospongin. The stereochemistry at C-11 was further corroborated by CD spectroscopy. The CD spectrum of **5** revealed a negative Cotton effect at 226 nm ($\pi-\pi^*$) and a positive Cotton effect at 242 nm ($n-\pi^*$), which suggests an *R* configuration.

epi-Sarcotin F (**6**) was isolated as a yellow oil. The molecular formula of **6** was deduced as $C_{26}H_{36}O_6$ on the

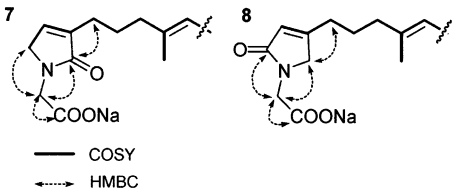

Figure 3. Key HMBC and COSY correlations of **7** and **8**.

basis of NMR and MS data. The ^{1}H and ^{13}C NMR spectral data of **6** showed a close similarity to those of sarcotin F[3] except those corresponding to the tetronic acid terminus. The presence of a 5-methoxy-2(5*H*)-furanone moiety was deduced from the characteristic NMR signals at δ_C 173.4 (C-4), 144.6 (C-2), 139.5 (C-3), 104.0 (C-1), δ_H 6.96 (H-2), 5.83 (H-1), and 3.51 (s, 3H, OMe, δ_C 56.8).[10] The long-range correlation between H-5 (δ 2.23) and C-4 (δ 173.4) was observed in the HMBC spectrum. The ^{1}H and ^{13}C NMR data of the tetronic acid terminus of **6** exhibited typical chemical shifts for H-21 (δ 4.34), H-20 (δ 2.57, 2.25), H-17 (δ 5.23), H-25 (δ 1.54), C-22 (δ 182.6), C-24 (δ 192.1), and C-23 (δ 88.0), which were very close to those of (21*R*)-isomers such as *epi*-sarcotin A (**12**).[3] The stereochemistry at C-1 could not be deduced from CD data since **6** contained two similar chromophores [2(5*H*)-furanones] in its structure.[15] However, it is assumed to be the same as that of sarcotin F since the CD spectrum of **6** showed Cotton effects opposite in sign of those of sarcotin F, which has alternative stereochemistry at C-21. The configuration at C-13 was assumed to be the same as that of the sarcotin F.[3]

Sarcotrine E (**7**) was isolated as a colorless oil. The molecular formula of **7** was established as $C_{27}H_{36}NO_6Na$ on the basis of HRFABMS data. Most of the ^{1}H and ^{13}C NMR data of **7** were in accordance with those of *epi*-sarcotrine A (**16**).[3] However, the signals corresponding to the *N*-isopentyl moiety were replaced by those of the *N*-acetate moiety. The H-1′ signal (δ 4.01) displayed HMBC correlations to the signals of C-1 (δ 53.1), C-4 (δ 172.9), and C-2′ carboxylic carbon (δ 176.3). Further evidence for the location of the C-4 carbonyl group was obtained from its HMBC correlation with the H-5 methylene proton signal at δ 2.22 (Figure 3).[7] The ^{1}H and ^{13}C NMR data of the tetronic acid terminus of **7** exhibited typical chemical shifts for H-21 (δ 4.34), H-20 (δ 2.60, 2.14), H-17 (δ 5.23), H-25 (δ 1.54), C-22 (δ 187.1), C-24 (δ 193.2), and C-23 (δ 88.4), which were very close to those of (21*R*)-isomers such as *epi*-sarcotin A (**12**).[3] The CD spectrum of **7** (Supporting Information) displayed a pattern similar to those of *epi*-sarcotrine A (**16**).[3] Thus the configuration at C-21 was proposed as *R* on the basis of NMR and CD spectral data. The configuration at C-13 was assumed to be the same as that of *epi*-sarcotrine A (**16**).[3]

Isosarcotrine E (**8**) was isolated as a yellow oil. In the FABMS, compound **8** showed a $[M + Na]^+$ ion at *m*/*z* 516, indicating the same molecular mass as **7**. The ^{1}H and ^{13}C NMR spectral data of **8** showed a close homology to those of **7**. The singlet methylene proton at δ 4.12, which was correlated to the carbon signal at δ 55.8, was assigned to H-4. The H-4 signal showed HMBC correlation to the methylene carbon signals at δ_C 47.0 (C-1′) and 29.8 (C-5). The H-5 signal showed long-range coupling to the signal at δ_C 55.8 (C-4) instead of the carbonyl carbon signal (Figure 3). Therefore, isosarcotrine E (**8**) can be differentiated from **7** as carrying a β-substituted lactam ring instead of the α-substituted one. A (21*R*)-configuration was proposed on the basis of ^{1}H and ^{13}C NMR spectral data (Tables 2 and 3). The configuration at C-13 was assumed to be the same as that of *epi*-sarcotrine A (**16**).

Table 1. ^{1}H NMR Data of Compounds **3** and **4** (CD_3OD, 500 MHz)[a]

position	**3**	**3**[b]	**4**[b]
1			
2	5.90 (brs)	5.90 (s)	5.90 (brs)
4	6.04 (brs)	6.09 (s)	6.09 (brs)
5	2.39 (t, 7.5)	2.39 (t, 7.5)	2.40 (t, 7.5)
6	1.76 (m)	1.76 (m)	1.76 (m)
7	2.13 (t, 7.0)	2.15 (t, 7.0)	2.14 (m)
9	1.73 (s)	1.74 (s)	1.75 (s)
10	5.81 (d, 11.0)	5.85 (d, 11.0)	5.85 (d, 11.0)
11	6.20 (dd, 15.0, 11.0)	6.25 (dd, 15.0, 11.0)	6.25 (dd, 15.0, 11.0)
12	5.41 (dd, 15.0, 7.5)	5.46 (dd, 15.0, 7.5)	5.48 (dd, 15.0, 7.5)
13	2.19 (m)	2.19 (m)	2.19 (m)
14	0.99 (d, 7.0)	0.99 (d, 7.0)	0.99 (d, 6.5)
15	1.32 (m)	1.32 (m)	1.32 (m)
16	1.97 (q, 7.0)	1.99 (q, 7.0)	2.12 (m)
17	5.25 (t, 7.0)	5.25 (t, 7.0)	5.26 (t, 7.0)
18			
19	1.73 (s)	1.73 (s)	1.76 (s)
20	2.43 (dd, 13.0, 6.0)	2.44 (13.5, 5.5)	2.42 (dd, 13.0, 6.5)
	2.39 (dd, 13.0, 8.0)	2.41 (13.5, 8.0)	2.30 (dd, 13.0, 7.5)
21	4.22 (dd, 8.0, 6.0)	4.25 (dd, 8.0, 5.5)	4.14 (t, 6.5)
22			4.00 (brs)
23			2.21 (s)
OCH_3	3.64 (s)	3.68 (s)	

[a] Multiplicities and coupling constants are in parentheses. [b] Measured in acetone-d_6.

Table 2. ^{13}C NMR Data of Compounds **3**, **4**, and **6**−**10** (CD_3OD, 50 MHz)

position	**3**	**4**[c]	**6**[c]	**7**	**8**	**9**[c]	**10**[c]
1	171.4	172.0	104.0	53.1	173.8	53.1	173.6
2	117.3	117.1	144.6	137.7	121.8	137.7	121.8
3	170.0	170.0	139.5	140.0	136.5	140.2	136.0
4	99.2	99.2	173.4	172.9	55.8	173.6	55.8
5	27.6	27.5	25.6	26.3	29.8	26.3	29.5
6	25.6	25.4[a]	26.6	27.1[a]	27.0[a]	27.0[a]	27.0[a]
7	40.0	39.7	40.2	40.5	40.2	40.4	40.2
8	135.6	135.6	136.0	136.5	136.0	136.6	136.0
9	16.0	16.0	16.4	16.5	16.4	16.4	16.3
10	126.0	126.0	126.5[a]	126.3[b]	126.5[b]	126.4[b]	126.4[b]
11	125.2	125.2	127.0[a]	126.7[b]	127.0[b]	126.6[b]	126.6[b]
12	139.1	139.4	139.5	139.4	140.2	140.1	139.5
13	37.7	38.1	38.0	38.0	38.0	38.1	38.1
14	21.3	20.9	21.4	21.2	21.5	21.3	21.3
15	38.4	37.6	38.2	38.5	38.5	38.2	38.3
16	26.0	25.9	27.0	26.9[a]	26.8[a]	26.9[a]	26.9[a]
17	129.0	129.0	130.4	128.9	128.9	129.6	129.6
18	131.8	132.0	130.7	132.6	132.7	132.5	132.5
19	24.2	23.8	24.4	24.3	24.3	24.3	24.3
20	37.7	36.0	36.3	36.1	36.1	35.9	35.9
21	70.4	70.5	81.0	81.4	81.4	71.9	71.9
22	175.3	79.0	182.6	187.1	183.6	80.3	80.3
23		211.0	88.0	88.4	88.0	212.3	212.3
24	24	25.3[a]	192.1	193.2	193.2	25.6	25.6
25	25		6.0	6.0	6.0		
1′				47.1	47.0	46.8	46.8
2′				176.3	174.0	176.0	174.0
OCH_3	52.3		56.8				

[a,b] Assignments with the same superscript in the same column may be interchanged. [c] Assignments were supported by HMQC and HMBC experiments.

Sarcotrine F (**9**) and isosarcotrine F (**10**) were isolated as an inseparable mixture. The FABMS of **9** and **10** showed a single $[M + Na]^+$ ion at *m*/*z* 506. The molecular formulas of **9** and **10** were established as $C_{26}H_{38}NO_6Na$ on the basis of HRFABMS data. The carbon skeletons of **9** and **10** were recognized as linear pyrroloterpenes by analysis of the ^{1}H and ^{13}C NMR spectra. Most of the ^{1}H and ^{13}C NMR signals

Table 3. ^{1}H NMR Data of Compounds **6**−**10** (CD_3OD, 500 MHz)[a]

position	6	7	8	9	10
1	5.83 (brs)	4.05 (brs)		4.04 (brs)	
2	6.96 (brs)	6.83 (brs)	5.81 (brs)	6.83 (brs)	5.83 (brs)
4			4.12 (d, 1.5)		4.11 (d, 1.5)
5	2.23 (t, 7.5)	2.22 (t, 7.5)	2.38 (t, 7.5)	2.22 (t, 7.5)	2.38 (t, 7.5)
6	1.67 (m)	1.69 (m)	1.68 (m)	1.68 (m)	1.68 (m)
7	2.07 (t, 7.5)	2.08 (t, 7.0)	2.06 (t, 7.0)	2.09 (t, 7.0)	2.06 (t, 7.0)
9	1.71 (s)	1.72 (s)	1.70 (s)	1.72 (s)	1.72 (s)
10	5.77 (d, 11.0)	5.79 (d, 11.0)	5.78 (d, 11.0)	5.79 (d, 11.0)	5.79 (d, 11.0)
11	6.18 (dd, 15.0, 11.0)	6.19 (dd, 15.0, 11.0)	6.18 (dd, 15.0, 11.0)	6.19 (dd, 15.0, 11.0)	6.19 (dd, 15.0, 11.0)
12	5.40 (dd, 15.0, 8.5)	5.39 (dd, 15.0, 8.0)	5.40 (dd, 15.0, 8.0)	5.41 (dd, 15.0, 8.0)	5.41 (dd, 15.0, 8.0)
13	2.15 (m)	2.15 (m)	2.12 (m)	2.16 (m)	2.16 (m)
14	0.98 (d, 7.0)	0.98 (d, 6.0)	0.98 (d, 7.0)	0.99 (d, 7.0)	0.99 (d, 7.0)
15	1.33 (m)	1.31 (m)	1.32 (m)	1.33 (m)	1.33 (m)
16	1.99 (q, 7.0)	2.01 (q, 8.0)	2.00 (q, 8.0)	2.05 (m)	2.05 (m)
17	5.23 (t, 7.0)	5.23 (t, 7.0)	5.23 (t, 7.0)	5.27 (t, 7.0)	5.27 (t, 7.0)
19	1.76 (s)	1.76 (s)	1.76 (s)	1.75 (s)	1.75 (s)
20	2.57 (dd, 14.0, 2.5)	2.60 (dd, 14.0, 2.0)	2.58 (dd, 14.0, 2.5)	2.40 (dd, 13.5, 7.0)	2.40 (dd, 13.5, 7.0)
	2.25 (dd, 14.0, 9.5)	2.14 (dd, 14.0, 10.0)	2.14 (dd, 14.0, 10.0)	2.28 (dd, 13.5, 7.0)	2.28 (dd, 13.5, 7.0)
21	4.34 (dd, 9.5, 2.5)	4.34 (dd, 10.0, 2.0)	4.34 (dd, 10.0, 2.5)	4.05 (td, 7.0, 2.0)	4.05 (td, 7.0, 2.0)
22				3.93 (d, 2.0)	3.93 (d, 2.0)
24				2.20 (s)	2.22 (s)
25	1.54 (s)	1.54 (s)	1.54 (s)		
OCH_3	3.51 (s)				
1′		4.01 (s)	3.97 (s)	4.02 (s)	3.98 (s)

[a] Multiplicities and coupling constants are in parentheses.

of **9** were in accordance with those of **7**. However, the signals corresponding to the unconjugated tetronic acid terminus of **7** were replaced by an α,*β*-dihydroxybutanone moiety. Signals of two oxymethine groups and a methyl ketone group were observed. As in the case of **4**, HMBC correlations corresponding to an α,*β*-dihydroxybutanone moiety were observed. The proton signals of H-1, -1′, -21, and -22 were crowded around *δ* 4.0, hindering signal analysis. Therefore, the splitting patterns and coupling constants were analyzed by a homonuclear 2D *J*-resolved experiment. The configuration at C-13 was assumed to be the same as that of *epi*-sarcotrine A (**16**). An attempt to convert **9** and **10** to their MTPA esters by the modified Mosher's method was unsuccessful due to a rapid decomposition of the reactant.

With the exception of the signals corresponding to the pyrrolone ring, the ^{1}H and ^{13}C NMR signals of **10** appeared to be very similar to those of **9**. The methylene singlet at *δ* 4.11 (H-4), which correlated to the carbon signal at δ_C 55.8, showed HMBC correlations to the methylene carbon signals at *δ* 46.8 (C-1′) and 29.5 (C-5). The H-5 signal showed long-range coupling to the carbon signal at δ_C 55.8 (C-4) instead of the carbonyl carbon signal. Therefore, isosarcotrine F (**10**) can be differentiated from **9** as carrying a *β*-substituted lactam ring instead of the α-substituted one. The configuration at C-13 was assumed to be the same as that of *epi*-sarcotrine A (**16**).

Sarcotin A (**11**),[2,3] *epi*-sarcotin A (**12**),[3] *epi*-sarcotrine B (**13**),[3] (7*E*,12*E*,18*R*,20*Z*)-variabilin (**14**),[6] and (8*E*,13*Z*,18*R*,-20*Z*)-strobilinin (**15**)[6] were identified on comparison of spectral data with those reported.

Two C_{22} trinorsesterterpene derivatives (**17** and **18**) have been isolated from the sponge of the same genus collected from Korean waters.[8] Sarcotrines (**7**−**10**) and the sarcotragins A and B (**17** and **18**) undoubtedly share the same biosynthetic precursor, and the latter are derived from the former by loss of two or three carbons. Sarcotin O (**4**), sarcotrine F (**9**), and isosarcotrine F (**10**) are new C_{24} norsesterterpenes that possess a hitherto unknown α,*β*-dihydroxybutanone moiety. Three chlorinated C_{24} norsesterterpenes have been previously isolated from the North Adriatic sponge *Ircinia oros*[17] and an unidentified Senegalese marine sponge.[18] The finding of C_{24} norsesterterpenes supports the biogenetic hypothesis that C_{21} furanoterpenes are derived from sestersterpenes by loss of four carbons, C_{24} norsesterterpenes being the first-stage degradation products of the sesterterpenes.[17] A biogenetic route from sesterterpene to C_{24} norsesterterpene has been proposed.[18]

Table 4. Cytotoxicity Data of Compounds **3**−**10**[a]

compound	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
3	19.4	>30.0	10.9	>30.0	21.7
4	6.8	14.9	3.0	11.5	4.6
5	7.9	32.3	4.5	11.8	4.2
6	>30.0	>30.0	10.9	>30.0	33.0
7	>30.0	>30.0	>30.0	>30.0	>30.0
8	>30.0	>30.0	>30.0	>30.0	>30.0
9, **10**[b]	>30.0	>30.0	>30.0	>30.0	>30.0
doxorubicin	0.04	0.15	0.06	0.19	0.24

[a] Data expressed in ED_{50} values (*μ*g/mL). A549, human lung cancer; SK-OV-3, human ovarian cancer; SK-MEL-2, human skin cancer; XF498, human CNS cancer; HCT 15, human colon cancer. [b] Obtained as an inseparable mixture.

The artifactual origin of several of the isolated compounds should be considered. It is known that 3-alkylfurans could react with singlet oxygen to produce 3-alkyl-4-hydroxybutenolides such as **3** and **4**, to which solvent might be added to produce esters such as **6**.[19] Oxidized alkylfurans may also react with free amino acids to produce pyrroloterpenoids such as **7**−**10** and **13**.[20]

The compounds were evaluated for cytotoxicity against a small panel of five human tumor cell lines (Table 4). Furanoterpenoids **3**−**6** showed marginal to moderate cytotoxicity, while the pyrroloterpenoids **7**−**10** showed no activity.

Experimental Section

General Experimental Procedures. Optical rotations were obtained using a JASCO DIP-370 digital polarimeter. CD spectra were measured using a JASCO J-715 spectropolarimeter (sensitivity 50 mdeg, resolution 0.2 nm). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC200 and Varian Inova 500. Chemical shifts were reported with reference to the respective residual solvent peaks (δ_H 3.30 and δ_C 49.0 for CD_3-

OD, δ_H 2.05 and δ_C 29.8 for acetone-d_6). FABMS data were obtained on a JEOL JMS-700 double focusing (B/E configuration) instrument. HPLC was performed with an YMC ODS-H80 (semipreparative, 250 × 10 mm, 4 μm, 80 Å; preparative, 250 × 20 mm, 4 μm, 80 Å) column using a Shodex RI-71 detector.

Animal Material. The first sponge was collected in July 1998 (15−25 m in depth), off the coast of Jeju Island, Korea. The specimen was identified as *Sarcotragus* sp. (Irciniidae) by Prof. Chung Ja Sim, Hannam University. A voucher specimen (registry No. Por. 33) was deposited in the Natural History Museum, Hannam University, Daejon, Korea, and has been described elsewhere.[2]

The second sponge was collected by hand using scuba (25 m in depth) in October 2002 off the coast of Jeju Island, Korea. It was identified as *Sarcotragus* sp. by Prof. Chung Ja Sim. It was a massive sponge (10.5 × 6 and 4 cm thick) with oscules (2−4 mm in diameter) irregularly scattered on the surface. The surface was a shade of gray in life, and the texture was elastic. The surface was covered with sharply pointed conules (2−5 mm height and 2−6 mm apart). The heavily fasciculated primary fibers (500−700 μm in diameter) were devoid of sands. Slightly fasciculated secondary fibers (80−400 μm in diameter) were devoid of sands. Filaments (3−6 μm thick) were very tightly arranged in a matrix and had terminal knobs (10−12 μm in diameter). A voucher specimen (registry No. Spo. 40) was deposited at the Natural History Museum, Hannam University, Daejon, Korea.

Extraction and Isolation. The first sponge (7 kg) was extracted with MeOH at room temperature. The MeOH extract of the sponge displayed moderate cytotoxicities against five human tumor cell lines (ED_{50} values for A549, SK-OV-3, SK-MEL-2, XF498, and HCT15 were 19.0, 20.3, 11.8, 15.5, and 12.6 μg/mL, respectively). The MeOH extract was partitioned between water and CH_2Cl_2. The CH_2Cl_2 layer was further partitioned between 90% methanol and *n*-hexane to yield 90% methanol- (54 g) and *n*-hexane-soluble (13 g) fractions. As described in our previous report,[2] the 90% methanol fraction was subjected to reversed-phase flash column chromatography (YMC Gel ODS-A, 60 Å 500/400 mesh), eluting with a solvent system of 25 to 0% H_2O/MeOH, to afford 20 fractions (Fg1−Fg20). The fraction Fg4 was further separated by reversed-phase flash column chromatography (YMC Gel ODS-A, 60 Å, 500/400 mesh), eluting with 25 to 0% H_2O/MeOH, to afford 14 fractions. Compound **3** (3.5 mg) was obtained by purification of fraction Fg4-7 by ODS HPLC, eluting with a solvent system of 75% MeOH. Compounds **4** (1.1 mg) and **5** (0.9 mg) were obtained by purification of subfraction Fg4-8 by ODS HPLC, eluting with a solvent system of 70% MeOH.

The second sponge (3 kg) was extracted with MeOH at room temperature. The MeOH extract was partitioned between water and CH_2Cl_2. The CH_2Cl_2 layer was further partitioned between 90% methanol and *n*-hexane to yield 90% methanol- (26 g) and *n*-hexane-soluble (6 g) fractions. The 90% methanol fraction was subjected to reversed-phase flash column chromatography (YMC Gel ODS-A, 60 Å, 500/400 mesh), eluting with a solvent system of 25 to 0% H_2O/MeOH, to afford 13 fractions (FJ1−FJ13). Compound **6** (1.3 mg) was obtained by purification of fraction FJ4 by ODS HPLC, eluting with a solvent system of 60% MeOH. Compounds **7** (5.4 mg) and **8** (2.7 mg) were obtained by purification of fraction FJ2 by ODS HPLC, eluting with a solvent system of 50% MeOH. Compounds **9** and **10** (1.4 mg) were obtained by purification of fraction FJ3 by ODS HPLC, eluting with a solvent system of 50% MeOH. Compound **11** (2 g) was obtained by purification of fraction FJ8 by ODS HPLC, eluting with a solvent system of 85% MeOH. Compound **12** (1 g) was obtained by purification of fraction FJ7 by ODS HPLC, eluting with a solvent system of 75% MeOH. Compound **13** (10 mg) was obtained by purification of fractions FJ5 and FJ6 by ODS HPLC, eluting with a solvent system of 70% MeOH. Compounds **14** (2.0 mg) and **15** (2.1 mg) were obtained by purification of fraction FJ9 by ODS HPLC, eluting with a solvent system of 88% MeOH.

Sarcotin N (3): light yellow oil; $[\alpha]^{21}_D$ +66.7° (*c* 0.06, MeOH); CD (*c* 1 × 10^{-4} M, MeOH) Δε 0 (216.4), +0.075 (228.0), 0 (240.5), −0.016 (244.0), 0 (249.2), +0.051 (262.3), 0 (284.6), −0.025 (300.1), 0 (337.5); ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; FABMS *m/z* 429 $[M + Na]^+$ (12), 407 $[M + H]^+$ (2), 381 (5); HRFABMS *m/z* 429.2259 (calcd for $C_{23}H_{34}O_6$-Na, 429.2253).

Sarcotin O (4): colorless oil; $[\alpha]^{21}_D$ +13.6° (*c* 0.04, MeOH); CD (*c* 1 × 10^{-4} M, MeOH) Δε 0 (223.1), −0.025 (228.3), 0 (231.6), +0.051 (272.7), 0 (311.2); ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; FABMS *m/z* 443 $[M + Na]^+$ (10), 421 $[M + H]^+$ (2), 391 (2), 63 (50); HRFABMS *m/z* 443.2426 (calcd for $C_{24}H_{36}O_6Na$, 443.2410).

***ent*-Kurospongin (5):** yellow oil; $[\alpha]^{21}_D$ +33.4° (*c* 0.03, $CHCl_3$); CD (*c* 1 × 10^{-4} M, MeOH) Δε 0 (268.5), −0.143 (258.2), 0 (250.2), +0.145 (242.0), 0 (236.5), −0.614 (226.0); ^{1}H NMR (CD_3OD, 500 MHz) δ 7.46 (1H, brs, H-1), 6.59 (1H, brs, H-2), 7.39 (1H, brs, H-4), 6.38 (1H, d, *J* = 16.0 Hz, H-5), 6.01 (1H, dt, *J* = 16.0, 7.5 Hz, H-6), 3.08 (2H, d, *J* = 7.5 Hz, H-7), 7.20 (1H, s, H-10), 5.10 (1H, m, H-11), 2.39 (1H, dd, *J* = 14.5, 6.5 Hz, H-12), 2.33 (1H, dd, *J* = 14.5, 7.0 Hz, H-12), 1.64 (3H, s, H-14), 5.29 (1H, t, *J* = 6.0 Hz, H-15), 2.27 (2H, m, H-16), 2.44 (2H, t, *J* = 7.5 Hz, H-17), 7.22 (1H, brs, H-19), 6.29 (1H, brs, H-20), 7.41 (1H, brs, H-21); ^{13}C NMR (CD_3OD, 50 MHz) δ 144.8 (CH), 108.4 (CH), 125.4 (C), 141.5 (CH), 123.6 (CH), 137.2 (CH), 29.4 (CH_2), 133.6 (C), 174.8 (C), 151.6 (CH), 82.3 (CH), 44.1 (CH_2), 131.3 (C), 16.9 (CH_3), 129.8 (CH), 29.6 (CH_2), 25.6 (CH_2), 125.4 (C), 140.2 (CH), 112.0 (CH), 143.9 (CH); FABMS *m/z* 361 $[M + Na]^+$ (5), 339 $[M + H]^+$ (1), 326 (15).

Preparation of (*R*)- and (*S*)-Phenylglycine Methyl Ester (PGME) Amides of Sarcotin I (1). Compound **1** (1.4 mg) and (*S*)-PGME (2.4 mg) were dissolved in 1 mL of DMF and cooled to 0 °C. To the solution, 6.4 mg of PyBOP [(benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate], 1.6 mg of HOBT (1-hydroxybenzotriazole), and 4 μL of *N*-methylmorpholine were added in order, and the mixture was stirred at room temperature for 1.5 h. Benzene (1 mL) and EtOAc (2 mL) were then added, and the mixture was washed with aqueous 5% HCl, saturated $NaHCO_3$ solution, and brine.[9] Then the solvent was removed by vacuum evaporation. The residue was purified on Si gel in a Pasteur pipet eluting with *n*-hexane−EtOAc (1:1) and characterized by ^{1}H NMR. The (*R*)-PGME amide of **1** was prepared from (*R*)-PGME in a similar fashion.

(*S*)-PGME amide of 1: yellow oil; ^{1}H NMR (acetone-d_6) δ 7.499−7.325 (7H, m, phenyl−, H-1 and -4), 6.357 (1H, s, H-2), 6.334 (1H, dd, *J* = 15.0, 11.0 Hz, H-11), 5.808 (1H, d, *J* = 11.0 Hz, H-10), 5.455 (1H, dd, *J* = 15.0, 8.0 Hz, H-12), 5.257 (1H, t, *J* = 7.0 Hz, H-17), 4.195 (1H, dd, *J* = 9.0, 7.0 Hz, H-21), 3,697 (3H, s), 2.433 (1H, dd, *J* = 15.0, 9.0 Hz, H-20), 2.397 (2H, t, *J* = 7.0 Hz, H-5), 2.194 (1H, dd, *J* = 15.0, 7.0 Hz, H-20), 2.156−1.911 (overlapped with solvent peak, m, H-7, H-13 and -16), 1.731 (3H, s, H-19), 1.725 (3H, s, H-9), 1.690 (2H, m, H-6), 1.311 (2H, m, H-15), 0.991 (3H, d, *J* = 6.5 Hz, H-14).

(*R*)-PGME amide of 1: yellow oil; ^{1}H NMR (acetone-d_6) δ 7.500−7.269 (7H, m, phenyl−, H-1 and -4), 6.355 (1H, s, H-2), 6.224 (1H, dd, *J* = 15.0, 11.0 Hz, H-11), 5.794 (1H, d, *J* = 11.0 Hz, H-10), 5.428 (1H, dd, *J* = 15.0, 8.0 Hz, H-12), 5.231 (1H, t, *J* = 7.0 Hz, H-17), 4.226 (1H, dd, *J* = 9.0, 7.0 Hz), 3,697 (3H, s), 2.406 (2H, t, *J* = 7.0 Hz, H-5), 2.386 (1H, dd, *J* = 15.0, 9.0 Hz, H-20), 2.156 (1H, dd, *J* = 15.0, 7.0 Hz, H-20), 2.156−1.918 (overlapped with solvent peak, m, H-7, -13, and -16), 1.712 (6H, s, H-9 and -19), 1.694 (2H, m, H-6), 1.316 (2H, m, H-15), 0.968 (3H, d, *J* = 6.5 Hz, H-14).

***epi*-Sarcotin F (6):** yellow oil; $[\alpha]^{21}_D$ +57.7° (*c* 0.04, MeOH); CD (*c* 1 × 10^{-4} M, MeOH) Δε −0.071 (218.1), 0 (222.2), +0.021 (229.5), 0 (233.4), −0.055 (245.1), 0 (255.6), +0.120 (270.8), 0 (312.3); ^{1}H NMR data, see Table 3; ^{13}C NMR data, see Table 2; ESIMS *m/z* 445 $[M + H]^+$.

Sarcotrine E (7): light yellow oil; $[\alpha]^{21}_D$ +38.8° (*c* 0.18, MeOH); CD (*c* 1 × 10^{-4} M, MeOH) Δε 0 (201.1), +0.165 (228.2), 0 (250.1), −0.125 (275.6); ^{1}H NMR data, see Table 3; ^{13}C NMR data, see Table 2; FABMS *m/z* 538 $[M + 2Na - H]^+$ (12), 516 $[M + Na]^+$ (7), 494 $[M + H]^+$ (2), 478 (4); HRFABMS *m/z* 516.2352 (calcd for $C_{27}H_{36}NO_6Na_2$, 516.2338).

Isosarcotrine E (8): light yellow oil; $[\alpha]^{21}_D$ +33.3° (*c* 0.09, MeOH); ^{1}H NMR data, see Table 3; ^{13}C NMR data, see Table 2; FABMS *m/z* 538 $[M + 2Na - H]^+$ (2), 516 $[M + Na]^+$ (1), 494 $[M + H]^+$ (1), 478 (4).

Sarcotrine F (9) and isosarcotrine F (10): yellow oil; $[\alpha]^{21}_D$ +42.0° (*c* 0.05, MeOH); ^{1}H NMR data, see Table 3; ^{13}C NMR data, see Table 2; FABMS *m/z* 506 $[M + Na]^+$ (4), 484 $[M + H]^+$ (15), 460 (1), 439 (2); HRFABMS *m/z* 506.2492 (calcd for $C_{26}H_{38}NO_6Na_2$, 506.2495).

(7*E*,12*E*,18*R*,20*Z*)-Variabilin (14): light yellow oil; $[\alpha]^{21}_D$ +40.3° (*c* 0.01, MeOH); ^{1}H NMR (500 MHz, CD_3OD) δ 7.35 (1H, brs, H-1), 6.28 (1H, brs, H-2), 7.27 (1H, brs, H-4), 2.42 (2H, t, J = 7.5 Hz, H-5), 2.22 (2H, q, J = 7.5 Hz, H-6), 5.14 (1H, t, J = 7.0 Hz, H-7), 1.56 (3H, s, H-9), 1.95 (2H, m, H-10), 2.06 (2H, m, H-11), 5.08 (1H, t, J = 6.0 Hz, H-12), 1.54 (3H, s, H-14), 1.95 (2H, m, H-15), 1.35 (2H, m, H-16), 1.32 (2H, m, H-17), 2.72 (1H, m, H-18), 1.05 (3H, d, J = 7.0 Hz, H-19), 5.23 (1H, d, J = 10.0 Hz, H-20), 1.83 (3H, s, H-25); ^{13}C NMR (50 MHz, CD_3OD) δ 143.7 (C-1), 112.0 (C-2), 126.2 (C-3), 140.1 (C-4), 26.0 (C-5), 29.6 (C-6), 125.2 (C-7), 136.5 (C-8), 16.1 (C-9), 40.4 (C-10), 27.4 (C-11), 125.6 (C-12), 135.8 (C-13), 16.0 (C-14), 40.7 (C-15), 26.8 (C-16), 37.6 (C-17), 31.9 (C-18), 21.0 (C-19), 115.6 (C-20), 145.1 (C-21), 165.1 (C-22), 98.7 (C-23), 173.7 (C-24), 6.0 (C-25).

(8*E*,13*Z*,18*R*,20*Z*)-Strobilinin (15): colorless oil; $[\alpha]^{21}_D$ +44.6° (*c* 0.01, MeOH); ^{1}H NMR (500 MHz, CD_3OD) δ 7.35 (1H, brs, H-1), 6.27 (1H, brs, H-2), 7.22 (1H, brs, H-4), 2.36 (2H, t, J = 7.5 Hz, H-5), 1.61 (2H, m, H-6), 2.05 (2H, m, H-7), 1.57 (3H, s, H-9), 5.10 (1H, m, H-10), 1.97 (2H, t, J = 7.5 Hz, H-11), 2.05 (2H, m, H-12), 1.66 (3H, s, H-14), 5.10 (1H, m, H-15), 1.92 (2H, m, H-16), 1.42 (2H, m, H-17), 2.72 (1H, m, H-18), 1.05 (3H, d, J = 7.0 Hz, H-19), 5.23 (1H, d, J = 10.0 Hz, H-20), 1.70 (3H, s, H-25); ^{13}C NMR (125 MHz, CD_3OD) δ 143.5 (C-1), 112.0 (C-2), 126.0 (C-3), 139.8 (C-4), 24.9 (C-5), 29.3 (C-6), 32.2 (C-7), 136.0 (C-8), 15.8 (C-9), 125.2 (C-10), 27.0 (C-11), 32.6 (C-12), 136.5 (C-13), 23.5 (C-14), 125.5 (C-15), 26.7 (C-16), 38.5 (C-17), 31.5 (C-18), 21.2 (C-19), 114.2 (C-20), 143.8 (C-21), 163.0 (C-22), 99.8 (C-23), 172.1 (C-24), 6.0 (C-25).

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Supporting Information Available: CD spectrum of **7**. This material is available free of charge via the Internet at http://pubs.acs.org.

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