# Cytotoxic Pyrrolo- and Furanoterpenoids from the Sponge *Sarcotragus* Species

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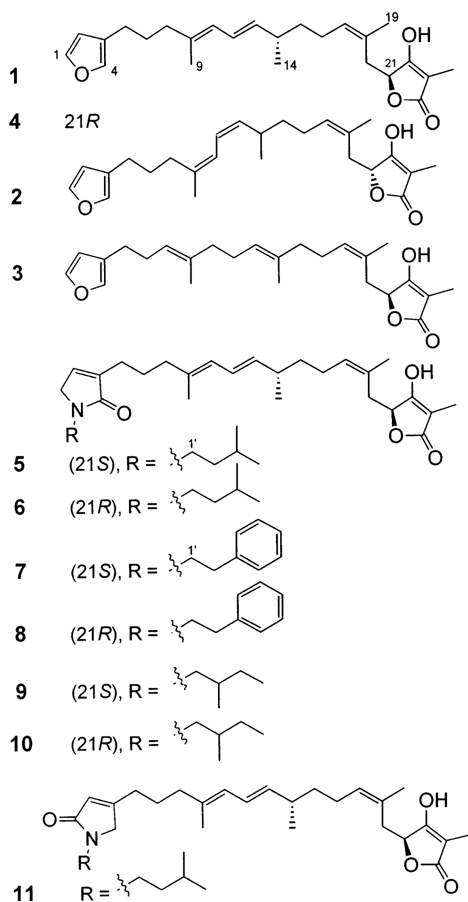
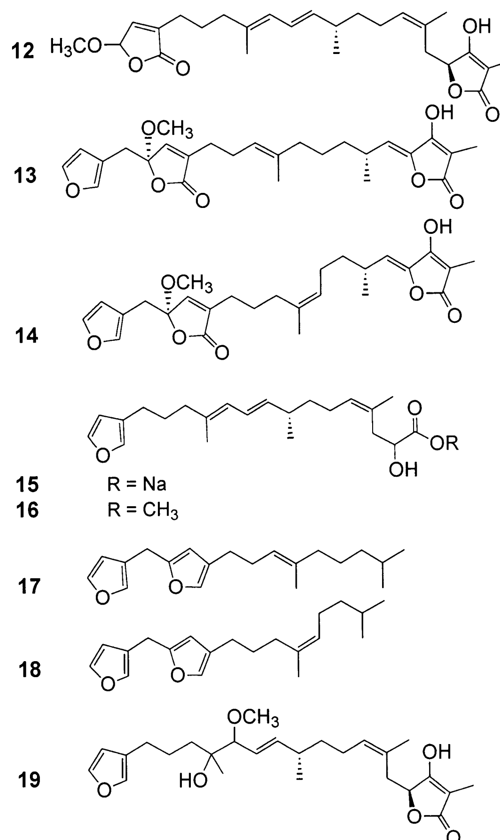
Reexamination of the configuration of sarcotins A−C, first isolated from the marine sponge *Sarcotragus* sp., revealed that the proposed stereochemistry of the tetronic acid moiety needs to be revised as shown in **1**−**3**. Additional new pyrrolosesterterpenes (**5**−**11**), furanosesterpene derivatives (**4**, **12**−**14**, **19**), and furanoterpenoids, including two trinorsesterterpenes (**15**, **16**) and two diterpenes (**17**, **18**), were isolated from the same sponge by bioactivity-guided fractionation. The planar structures were established on the basis of NMR and MS analysis. The stereochemistry was defined by combined use of NMR, CD spectroscopy, and chemical degradation. The compounds were evaluated for cytotoxicity against five human tumor cell lines and were found to exhibit moderate to significant activity.

Marine sponges of the order Dictyoceratida have frequently afforded a wide variety of linear sesterterpenes, many of which contain furanyl and tetronic acid termini.[1] In our previous study on the cytotoxic compounds of the sponge *Sarcotragus* sp. (family Thorectidae, order Dictyoceratida), seven cytotoxic furanosesterterpenes were reported.[2] In a continuing study, new pyrrolosesterterpenes (**5**−**11**) and furanosesterterpenes (**4**, **12**−**14**, **19**) were




isolated from the same sponge. The pyrrolosesterterpenes were chemically unique, incorporating a pyrrole ring in place of the furan ring. Unlike other common furanosesterterpenes, compounds **12**−**14** were carrying an oxidized furan ring similar to that found in manoalide.[3] Additional furanoterpenoids including a sodium salt of trinorsesterterpene acid (**15**) and its methyl ester (**16**), and two bisfuranoditerpenes (**17**, **18**), were also isolated. The gross structures of the compounds were elucidated by the aid of COSY, HMQC, and HMBC experiments, while the absolute

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**Table 1.** $^1$H NMR Data of Compounds **4**−**11** ($CD_3OD$, 500 MHz)[a]

| position | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** |
|---|---|---|---|---|---|---|---|---|
| 1 | 7.37 (brs) | 3.94 (brs) | 3.94 (brs) | 3.78 (brs) | 3.77 (brs) | 3.95 (brs) | 3.98 (brs) | |
| 2 | 6.29 (brs) | 6.83 (brs) | 6.82 (brs) | 6.74 (brs) | 6.74 (brs) | 6.84 (brs) | 6.87 (brs) | 5.79 (brs) |
| 4 | 7.24 (brs) | | | | | | | 3.98 (brs) |
| 5 | 2.37 (t, 8.0) | 2.20 (t, 7.0) | 2.20 (t, 7.0) | 2.19 (t, 7.0) | 2.19 (t, 7.0) | 2.21 (t, 7.0) | 2.24 (t, 7.0) | 2.37 (t, 7.0) |
| 6 | 1.66 (m) | 1.66 (m) | 1.66 (m) | 1.66 (m) | 1.65 (m) | 1.67 (m) | 1.68 (m) | 1.66 (m) |
| 7 | 2.04 (t, 7.0) | 2.07 (t, 7.5) | 2.07 (t, 7.5) | 2.07 (t, 7.5) | 2.04 (t, 7.5) | 2.08 (t, 7.4) | 2.10 (t, 7.5) | 2.07 (t, 7.5) |
| 9 | 1.70 (s) | 1.70 (s) | 1.69 (s) | 1.70 (s) | 1.70 (s) | 1.71 (s) | 1.72 (s) | 1.73 (s) |
| 10 | 5.75 (d, 11.0) | 5.77 (d, 11.0) | 5.76 (d, 11.0) | 5.76 (d, 11.0) | 5.75 (d, 11.0) | 5.78 (d, 11.0) | 5.79 (d, 11.0) | 5.78 (d, 11.0) |
| 11 | 6.18 (dd, 15.0, 11.0) | 6.18 (dd, 15.0, 11.0) | 6.17 (dd, 15.0, 11.0) | 6.18 (dd, 15.0, 11.0) | 6.18 (dd, 15.0, 11.0) | 6.18 (dd, 15.0, 11.0) | 6.20 (dd, 15.0, 11.0) | 6.17 (dd, 15.0, 11.0) |
| 12 | 5.38 (dd, 15.0, 8.5) | 5.37 (dd, 15.0, 8.5) | 5.38 (dd, 15.0, 8.5) | 5.37 (dd, 15.0, 8.5) | 5.39 (dd, 15.0, 8.0) | 5.37 (dd, 15.0, 8.5) | 5.41 (dd, 15.0, 8.0) | 5.39 (dd, 15.0, 8.5) |
| 13 | 2.15 (m) | 2.15 (m) | 2.15 (m) | 2.15 (m) | 2.15 (m) | 2.15 (m) | 2.16 (m) | 2.15 (m) |
| 14 | 0.99 (d, 7.0) | 0.98 (d, 7.0) | 0.98 (d, 6.0) | 0.98 (d, 6.5) | 0.98 (d, 6.5) | 0.98 (d, 7.0) | 1.00 (d, 7.0) | 0.98 (d, 6.5) |
| 15 | 1.31 (m) | 1.34 (m) | 1.32 (m) | 1.33 (m) | 1.32 (m) | 1.34 (m) | 1.33 (m) | 1.34 (m) |
| 16 | 1.99 (q, 8.0) | 1.99 (q, 7.0) | 2.01 (q, 7.0) | 1.99 (q, 7.0) | 2.00 (q, 7.5) | 1.99 (q, 7.0) | 2.01 (q, 7.0) | 1.99 (q, 7.0) |
| 17 | 5.25 (t, 7.5) | 5.28 (t, 7.0) | 5.25 (t, 7.0) | 5.28 (t, 7.0) | 5.24 (t, 7.0) | 5.28 (t, 7.0) | 5.27 (t, 7.0) | 5.29 (t, 7.0) |
| 19 | 1.76 (s) | 1.75 (s) | 1.75 (s) | 1.75 (s) | 1.75 (s) | 1.75 (s) | 1.76 (s) | 1.76 (s) |
| 20 | 2.58 (dd, 14.5, 2.5) | 2.61 (dd, 14.0, 4.0) | 2.58 (dd, 14.0, 3.0) | 2.60 (dd, 14.0, 3.0) | 2.59 (dd, 14.5, 3.0) | 2.60 (dd, 14.0, 3.0) | 2.59 (dd, 14.0, 3.0.0) | 2.60 (dd, 14.0, 3.0) |
| 21 | 2.17 (dd, 14.5, 9.5) | 2.25 (dd, 14.0, 8.5) | 2.16 (dd, 14.0, 9.5) | 2.24 (dd, 14.0, 8.0) | 2.18 (dd, 14.5, 9.5) | 2.25 (dd, 14.0, 8.5) | 2.18 (dd, 14.0, 8.0) | 2.24 (dd, 14.0, 8.5) |
| | 4.47 (dd, 9.5, 2.5) | 4.72 (dd, 8.5, 4.0) | 4.52 (dd, 9.5, 3.0) | 4.69 (dd, 8.0, 3.0) | 4.48 (dd, 9.5, 3.0) | 4.72 (dd, 8.5, 3.0) | 4.46 (dd, 8.0, 3.0) | 4.69 (dd, 8.5, 3.0) |
| 25 | 1.57 (s) | 1.64 (s) | 1.57 (s) | 1.62 (s) | 1.57 (s) | 1.64 (s) | 1.59 (s) | 1.64 (s) |
| 1′ | | 3.48 (t, 7.0) | 3.47 (t, 7.5) | 3.69 (t, 7.5) | 3.69 (t, 7.0) | 3.34 (m) | 3.34 (m) | 3.44 (t, 7.0) |
| 2′ | | 1.48 (q, 7.0) | 1.48 (q, 7.0) | 2.90 (t, 7.0) | 2.89 (t, 7.0) | 1.76 (m) | 1.76 (m) | 1.48 (q, 7.0) |
| 3′ | | 1.55 (m) | 1.55 (m) | | | 0.85 (d, 6.5) | 0.87 (d, 6.0) | 1.55 (m) |
| 4′ | | 0.94 (d, 6.5) | 0.94 (d, 6.5) | 7.22 (m) | 7.20 (m) | 1.42 (m) 1.17 (m) | 1.42 (m) 1.17 (m) | 0.94 (d, 6.5) |
| 5′ | | 0.94 (d, 6.5) | 0.94 (d, 6.5) | 7.27 (m) | 7.25 (m) | 0.93 (t, 6.5) | 0.94 (d, 6.5) | 0.94 (d, 6.5) |
| 6′ | | | | 7.22 (m) | 7.20 (m) | | | |

[a] Multiplicities and coupling constants in parentheses.

configuration of the tetronic acid moiety was proposed by comparison of the NMR and CD data of each diastereomeric pair. In accordance with the recent observation on the stereochemistry of the tetronic acid derivatives,[4] the reported C-21 configuration of sarcotins A−C[2] was revised as shown in structures **1**−**3**. The isolation, structure elucidation, and cytoxicity of the new compounds are described herein.

## Results and Discussion

The MeOH extract of the sponge displayed cytotoxicity against a set of five human tumor cell lines (see Experimental Section) and showed toxicity to brine shrimp larvae ($LD_{50}$, 93 $\mu$g/mL). Guided by the brine shrimp assay, the MeOH extract was successively fractionated employing reversed-phase flash column chromatography and HPLC to afford compounds **4**−**19** as the new bioactive components.

Concerning the stereochemistry of the tetronic acid derivatives, Gawronski et al.[4] stated a relationship between the stereochemistry and CD data. Previous assignments[5] of the stereochemistry of the furanosesterterpenes were based on the observation of n−$\pi$* and $\pi$−$\pi$* Cotton effects of polycyclic homosubstituted 2(5*H*)-furanones.[5] However, the monocyclic heterosubstituted 2(5*H*)-furanones in Gawronski's report were more close in structure to the tetronic acid moiety of the furanosesterterpenes. The contradictory assignment of the stereochemistry of the homosubstituted 2(5*H*)-furanones and the heterosubstituted 2(5*H*)-furanones prompted us to reconsider the stereochemistry of sarcortins A−C, leading to revision of stereochemistry as shown in **1**−**3**, where the stereochemical assignments were reversed compared to those previously reported.[2]

The configuration at C-13 of sarcortin A (**1**) was now determined to be *S* by chemical degration.[6] Treatment of **1** with $NaIO_4$ in the presence of $RuCl_3{\cdot}xH_2O$ as a catalyst yielded (*S*)-2-methylglutaric acid, which was confirmed by comparison of the $^1$H NMR and optical rotation data with those reported.[6]

On standing, **1** decomposed to give several degradation products. One (**4**) of the major degradation products was identified as the 21*R* epimer of **1**. The molecular formula of **4** was established as $C_{25}H_{34}O_4$ on the basis of HRFABMS. The $^1$H and $^{13}$C NMR data indicated that **4** shared the same gross structure with **1**. However, notable differences were observed in the NMR data of the tetronic acid terminus. An upfield shift of the H-21 oxymethine signal from $\delta$ 4.75 to 4.47 and slight shifts of the H-20 and 25 proton signals were observed as well (Table 1). The $^{13}$C NMR spectral data of **4** were also very similar to those of **1** except for the downfield shifts of C-22 ($\delta_C$ 177.2 → 183.7) and C-24 ($\delta_C$ 178.1 → 192.5) and upfield shift of C-23 ($\delta_C$ 96.8 → 88.4),[2] which indicate apparent chemical change in the tetronic acid moiety. Since it was observed that the diastereomeric furanosesterterpene pairs with C-21 stereoisomerism exhibit a characteristic NMR pattern for each tetronic acid moiety, the C-21 configuration of **4** was proposed as *R* by comparison of the NMR data with those of **1**−**3**.[2] The epimeric relationship between **4** and **1** was affirmed by the CD spectral data. The CD spectrum of **4** revealed Cotton effects opposite in sign to those of **1** (Figure 1). A negative Cotton effect at 220 nm ($\pi$−$\pi$*) and a positive Cotton effect at 248 nm (n−$\pi$*) were observed. *epi*-Sarcotin A (**4**) might be produced as an artifact from **1** by an $A_{AL}1$ mechanism,[7] prompted by the formation of an allylic cation at C-21, which can then be captured by water to give either diastereomer with 21*S* or 21*R* configuration.

Sarcotrine A (**5**) was isolated as a colorless oil. The molecular formula of **5** was established as $C_{30}H_{45}NO_4$ on the basis of HRFABMS. The presence of an unconjugated tetronic acid moiety was established by analysis of NMR

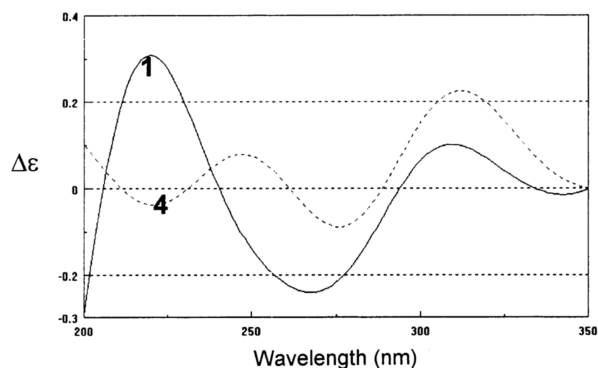


**Figure 1.** CD spectra of compounds **1** and **4**.

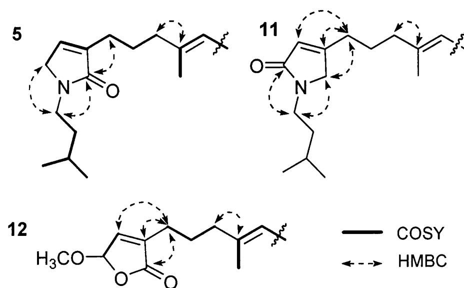


**Figure 2.** Key HMBC and COSY correlations of **5**, **11**, and **12**.

data as well as by comparison of the data with that of sarcotin A (**1**).[2] The NMR spectra also featured two vinylic methyl singlets at $\delta$ 1.70 and 1.75 ($\delta_C$ 16.4 and 24.4, respectively), a secondary methyl doublet at $\delta$ 0.98 ($J$ = 7.0 Hz, $\delta_C$ 21.5), a trisubstituted olefin ($\delta$ 5.28, H-17), and a 1,1,4-trisubstituted diene ($\delta$ 6.18, H-11; 5.77, H-10; 5.37, H-12). In addition, the presence of a 2(5*H*)-pyrrolone moiety was indicated. The broad singlet at $\delta$ 3.94 (H-1, $\delta_C$ 52.2) was coupled to the broad olefinic singlet at $\delta$ 6.83 (H-2, $\delta_C$ 137.6). Both H-1 and H-2 displayed weak HMBC correlations to the quaternary carbonyl carbon resonating at $\delta_C$ 173.6. The H-1′ signal ($\delta$ 3.48) displayed HMBC correlations to C-1 ($\delta$ 52.2) and C-4 ($\delta$ 173.6). 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 $\delta$ 2.20 (Figure 2).[8] The *E* geometry of the trisubstituted double bond (C-8) was assigned on the basis of the upfield resonance ($\delta_C$ 16.4, C-9) of the vinylic methyl carbon,[9] 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 protons ($J$ = 15.0 Hz). The downfield carbon chemical shift of the C-19 methyl ($\delta_C$ 24.4) indicated *Z* geometry of this trisubstituted double bond, which was also supported by the upfield shift of the C-20 signal ($\delta_C$ 35.5) compared to that of palinurin ($\delta_C$ 41.6, C-20).[10] The $^1$H and $^{13}$C NMR data of the tetronic acid terminus of **5** exhibited typical chemical shifts for H-21 ($\delta$ 4.72), H-20 ($\delta$ 2.61, 2.25), H-17 ($\delta$ 5.28), H-25 ($\delta$ 1.64), C-22 ($\delta_C$ 178.0), C-24 ($\delta_C$ 178.3), and C-23 ($\delta_C$ 96.3), which were very close to those of (21*S*)-furanosesterterpenes such as sarcotins A (**1**) and C (**3**).[2] Thus the configuration at C-21 was proposed as *S*. The CD spectral pattern of the pyrrolosesterterpenes appeared to be modulated by the presence of the pyrrolone moiety. The configuration at C-13 was determined to be *S* by chemical degration.[6] As in the case of **1**, treatment of **5** with $NaIO_4$ in the presence of $RuCl_3 \cdot xH_2O$ as a catalyst yielded (*S*)-2-methylglutaric acid (see Experimental Section).

On standing, **5** decomposed to give several degradation products. One (**6**) of the major degradation products was

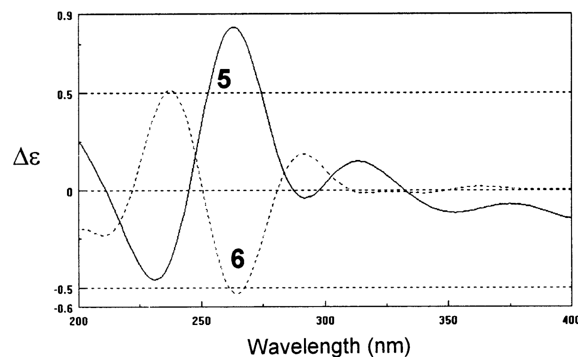


**Figure 3.** CD spectra of compounds **5** and **6**.

identified as the 21*R* epimer of **5**. Compound **6** showed the same molecular mass as **5** (*m*/*z* 506 [M + Na]$^+$, 484 [M + H]$^+$). The molecular formula of **6** was established as $C_{30}H_{45}NO_4$ on the basis of HRFABMS. The COSY and HMBC data of **6** revealed that it shares the same gross structure with **5**. However, the NMR data of the tetronic acid moiety of **6** was comparable to those of (21*R*)-furanosesterterpenes **2** and **4**. The CD spectrum of **6** revealed Cotton effects opposite in sign to those of **5** (Figure 3). Hence, the identity of **6** was proposed as the 21*R* epimer (*epi*-sarcotrine A) of sarcotrine A (**5**). The absolute configuration at C-13 was determined to be the same as **5** because chemical degradation of **6** again afforded (*S*)-2-methylglutaric acid (see Experimental Section).[6]

Sarcotrine B (**7**) was isolated as a colorless oil. The molecular formula of **7** was established as $C_{33}H_{43}NO_4$ on the basis of HRFABMS. Both the $^1$H and $^{13}$C NMR spectra of **7** showed a close similarity to those of **5**, except for the additional presence of five aromatic proton signals. The phenyl terminus of the molecule could be elucidated from the $^1$H NMR signals observed at $\delta$ 7.22−7.27 and the $^{13}$C NMR signals observed at $\delta_C$ 140.2, 129.8, 129.6, and 127.6. The terminal phenyl group was joined to the nitrogen of the 2(5*H*)-pyrrolone ring by a two-carbon chain. The corresponding long-range couplings were observed in the HMBC spectrum. The absolute configuration at C-21 was proposed as *S* on the basis of the NMR data of the relevant carbons and protons (Tables 1 and 3). The configuration at C-13 was assumed to be the same as that of sarcotrine A (**5**).

As in the case of **1**, compound **7** decomposed to give several degradation products on standing. One (**8**) of the major degradation products was identified as the 21*R* epimer of **7**. The molecular formula of **8** was established as $C_{33}H_{43}NO_4$ on the basis of HRFABMS. The $^1$H and $^{13}$C NMR data indicated that **8** shares the same gross structure with **7**. However, the NMR data of the tetronic acid terminus corresponded to that of the typical 21*R* isomer (Tables 1 and 3). The stereoisomerism between **7** and **8** was affirmed by the CD spectral data. The CD spectrum of **8** revealed Cotton effects opposite in sign to those of **7** (see Experimental Section). Hence, the identity of **8** was proposed as the 21*R* epimer of **7**. The configuration at C-13 was assumed to be the same as that of sarcotrine A (**5**).

Sarcotrine C (**9**) was isolated as a yellow oil. Both the $^1$H and $^{13}$C NMR spectra of **9** showed a close similarity to those of **5**, except for the presence of an anteiso branched chain in place of the iso branched chain. The COSY and HMBC gave the evidence for the gross structure. Being the same as **5** and **6**, compound **9** showed the [M + H]$^+$ ion at *m*/*z* 484 in the FABMS spectrum. The molecular formula of **9** was established as $C_{30}H_{45}NO_4$ on the basis of HRFABMS. The absolute configuration at C-21 was defined

**Table 2.** $^{1}$H NMR Data of Compounds **12**−**19** ($CD_3OD$, 500 MHz)$^{a}$

| position | **12** | **13** | **14** | **15** | **16** | **17** | **18** | **19** |
|---|---|---|---|---|---|---|---|---|
| 1 | 5.83 (brs) | 7.35 (brs) | 7.36 (brs) | 7.36 (brs) | 7.37 (brs) | 7.42 (brs) | 7.42 (brs) | 7.35 (brs) |
| 2 | 6.95 (brs) | 6.26 (brs) | 6.27 (brs) | 6.28 (brs) | 6.29 (brs) | 6.33 (brs) | 6.33 (brs) | 6.27 (brs) |
| 4 | | 7.30 (brs) | 7.31 (brs) | 7.23 (brs) | 7.25 (brs) | 7.33 (brs) | 7.33 (brs) | 7.23 (brs) |
| 5 | 2.23 (t, 7.0) | 3.03 (s) | 3.04 (s) | 2.37 (t, 7.0) | 2.39 (t, 7.5) | 3.73 (s) | 3.73 (s) | 2.39 (t, 8.0) |
| 6 | 1.66 (m) | | | 1.66 (q, 7.0) | 1.65 (m) | | | 1.58 (m) |
| 7 | 2.08 (t, 7.5) | 6.83 (s) | 6.86 (s) | 2.05 (m) | 2.06 (t, 7.5) | 5.95 (brs) | 5.95 (brs) | 1.40 (m) |
| 9 | 1.71 (s) | 2.22 (t, 6.5) | 2.15 (t, 6.5) | 1.71 (s) | 1.71 (s) | 7.15 (brs) | 7.15 (brs) | 1.12 (s) |
| 10 | 5.78 (d, 11.0) | 2.19 (q, 7.0) | 1.49 (m) | 5.76 (d, 11.0) | 5.76 (d, 11.0) | 2.37 (t, 7.5) | 2.34 (t, 7.5) | 3.30$^{b}$ |
| 11 | 6.18 (dd, 15.0, 11.0) | 5.01 (t, 6.0) | 1.97 (m) | 6.18 (dd, 15.0, 11.0) | 6.19 (dd, 15.0, 11.0) | 2.20 (m) | 1.62 (m) | 5.55 (dd, 15.5, 8.0) |
| 12 | 5.39 (dd, 15.0, 8.5) | | | 5.38 (dd, 15.0, 8.0) | 5.38 (dd, 15.0, 8.0) | 5.15 (t, 6.6) | 2.07 (m) | 5.29 (dd, 15.5, 7.5) |
| 13 | 2.15 (m) | | | 2.15 (m) | 2.15 (m) | | | 2.18 (m) |
| 14 | 0.98 (d, 7.0) | 1.55 (s) | 1.64 (s) | 0.98 (d, 7.0) | 0.99 (d, 6.5) | 1.57 (s) | 1.68 (s) | 1.02 (d, 7.0) |
| 15 | 1.33 (m) | 1.94 (m) | 5.18 (t, 7.0) | 1.34 (m) | 1.31 (m) | 1.94 (m) | 5.13 (t, 6.6) | 1.37 (m) |
| 16 | 1.99 (q, 7.0) | 1.37 (m) | 1.98 (m) | 1.99 (m) | 1.97 (m) | 1.37 (m) | 1.90 (m) | 1.96 (q, 8.0) |
| 17 | 5.29 (t, 7.0) | 1.30 (m) | 1.47 (m)<br>1.40 (m) | 5.26 (t, 7.5) | 5.25 (t, 7.0) | 1.28 (m) | 1.40 (m)<br>1.32 (m) | 5.30 (t, 7.5) |
| 18 | 1.74 (s) | 2.72 (m) | 2.72 (m) | 1.75 (s) | 1.73 (s) | 1.96 (m) | 1.96 (m) | 1.75 (s) |
| 19 | | 1.05 (d, 7.0) | 1.06 (d, 7.0) | | | 0.98 (d, 6.6) | 0.97 (d, 6.6) | |
| 20 | 2.61 (dd, 14.0, 4.0) | 5.25 (d, 9.5) | 5.25 (d, 9.5) | 2.43 (m) | 2.44 (m) | 0.98 (d, 6.6) | 0.97 (d, 6.6) | 2.60 (dd, 14.5, 3.5) |
| 21 | 2.25 (dd, 14.0, 8.5) | | | 2.39 (m) | 2.39 (m) | | | 2.27 (dd, 14.5, 9.5) |
| | 4.73 (dd, 8.5, 4.0) | | | 4.20 (t, 6.0) | 4.23 (dd, 6.0, 8.0) | | | 4.68 (dd, 9.5, 3.5) |
| 25 | 1.64 (s) | 1.73 (s) | 1.72 (s) | | | | | 1.63 (s) |
| $OCH_3$ | 3.51 (s) | 3.19 (s) | 3.21 (s) | | 3.68 (s) | | | 3.23 (s) |

$^{a}$ Multiplicities and coupling constants in parentheses. $^{b}$ Overlapped with the solvent peak.

**Table 3.** $^{13}$C NMR Data of Compounds **4**−**11** ($CD_3OD$, 50 MHz)

| position | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** |
|---|---|---|---|---|---|---|---|---|
| 1 | 143.9 | 52.2 | 52.2 | 52.9 | 52.9 | 52.9 | 52.2 | 174.6 |
| 2 | 111.9 | 137.6 | 137.6 | 137.7 | 137.7 | 137.6 | 137.6 | 121.8 |
| 3 | 126.3 | 140.4 | 140.4 | 140.3$^{a}$ | 140.2 | 140.3 | 140.4 | 136.0 |
| 4 | 140.1 | 173.6 | 173.7 | 173.7 | 173.7 | 174.1 | 173.7 | 55.4 |
| 5 | 25.2 | 26.3 | 26.3 | 26.3 | 26.3 | 26.4 | 26.3 | 24.3 |
| 6 | 29.5 | 27.0$^{a}$ | 27.0$^{a}$ | 27.0$^{b}$ | 27.0$^{a}$ | 27.0$^{a}$ | 26.9$^{a}$ | 27.0$^{a}$ |
| 7 | 40.4 | 40.4 | 40.4 | 40.3 | 40.3 | 40.4 | 40.4 | 40.2 |
| 8 | 136.6 | 136.4 | 136.4 | 136.4 | 136.4 | 136.5 | 136.4 | 136.0 |
| 9 | 16.5 | 16.4 | 16.4 | 16.5 | 16.5 | 16.4 | 16.4 | 16.3 |
| 10 | 126.5$^{a}$ | 126.6$^{b}$ | 126.5$^{b}$ | 126.6$^{c}$ | 126.5$^{b}$ | 126.6$^{b}$ | 126.5$^{b}$ | 126.5$^{b}$ |
| 11 | 126.6$^{a}$ | 126.7$^{b}$ | 126.7$^{b}$ | 126.7$^{c}$ | 126.6$^{b}$ | 126.7$^{b}$ | 126.7$^{b}$ | 127.0$^{b}$ |
| 12 | 139.2 | 139.2 | 139.3 | 139.2 | 139.3 | 139.2 | 139.3 | 139.5 |
| 13 | 38.0 | 38.0 | 38.0 | 38.0 | 38.0 | 38.5 | 38.0 | 38.0 |
| 14 | 21.4 | 21.5 | 21.4 | 21.5 | 21.4 | 21.5 | 21.4 | 21.5 |
| 15 | 38.4 | 38.2 | 38.4 | 38.3 | 38.2 | 38.3 | 38.4 | 38.3 |
| 16 | 26.9 | 26.9$^{a}$ | 26.9$^{a}$ | 26.9$^{b}$ | 26.9$^{a}$ | 26.9$^{a}$ | 27.0$^{a}$ | 26.9$^{a}$ |
| 17 | 129.4 | 130.4$^{c}$ | 129.6 | 130.2$^{d}$ | 129.8 | 130.4$^{c}$ | 129.6 | 130.2$^{c}$ |
| 18 | 132.0 | 130.8$^{c}$ | 131.8 | 131.0$^{d}$ | 132.0 | 130.9$^{c}$ | 131.8 | 131.0$^{c}$ |
| 19 | 24.3 | 24.4 | 24.3 | 24.4 | 24.3 | 24.4 | 24.3 | 24.4 |
| 20 | 35.9 | 35.5 | 35.8 | 35.7$^{e}$ | 35.7$^{c}$ | 35.5 | 35.8 | 35.6 |
| 21 | 80.9 | 78.9 | 80.3 | 79.3 | 80.0 | 79.0 | 80.3 | 79.2 |
| 22 | 183.7 | 178.0 | 181.2 | 179.1 | 181.0 | 178.5 | 182.0 | 179.3 |
| 23 | 88.4 | 96.3 | 92.3 | 95.3 | 91.1 | 96.1 | 92.3 | 96.3 |
| 24 | 192.5 | 178.3 | 186.2 | 180.0 | 187.8 | 178.6 | 186.2 | 181.0 |
| 25 | 6.0 | 6.0 | 6.0 | 6.0 | 6.0 | 6.0 | 6.0 | 5.9 |
| 1′ | | 41.7 | 41.7 | 45.2 | 45.2 | 49.4 | 49.5 | 41.3 |
| 2′ | | 38.4 | 38.4 | 35.6$^{e}$ | 35.6$^{c}$ | 35.6 | 35.8 | 38.4 |
| 3′ | | 27.0$^{a}$ | 27.0$^{a}$ | 140.2$^{a}$ | 140.2 | 17.2 | 17.0 | 27.0$^{a}$ |
| 4′ | | 22.8 | 22.8 | 129.6 | 129.6 | 28.0 | 28.0 | 22.8 |
| 5′ | | 22.8 | 22.8 | 129.8 | 129.8 | 11.5 | 11.5 | 22.8 |
| 6′ | | | | 127.6 | 127.6 | | | |

$^{a-e}$ Assignments with the same superscript in the same column may be interchanged.

as *S* by analysis of the NMR data of the tetronic acid terminus (Tables 1 and 3). The configuration at C-13 was assumed to be the same as that of sarcotrine A (**5**).

*epi*-Sarcotrine C (**10**) was isolated as a yellow oil. In the FABMS, compound **10** showed the same $[M + H]^+$ ion at $m/z$ 484 as **9**. The molecular formula of **10** was established as $C_{30}H_{45}NO_4$ on the basis of HRFABMS. Both the $^{1}$H and $^{13}$C NMR spectra of **10** showed a close similarity to those of **9**. However, the NMR data of the tetronic acid terminus were close to those of the typical 21*R* isomer. As expected, a clear difference was noticed in the CD spectral data. The CD spectrum of **9** revealed Cotton effects opposite in sign to those of **10** (see Experimental Section). Hence, the structure of **10** was proposed as the 21*R* epimer of **9**. The configuration at C-13 was assumed to be the same as that of sarcotrine A (**5**).

Sarcotrine D (**11**) was isolated as a yellow oil. In the FABMS, compound **11** showed the $[M + H]^+$ ion at $m/z$ 484, indicating the same molecular mass as **5**, **6**, **9**, and **10**. The molecular formula of **11** was established as $C_{30}H_{45}NO_4$ on the basis of HRFABMS. Both the $^{1}$H and $^{13}$C NMR spectra of **11** showed a close homology with **5**. The methylene singlet at $\delta$ 3.98, which correlated to the carbon signal at $\delta_C$ 55.4, was assigned to H-4. The H-4 signal showed HMBC correlation to the methylene carbon signals at $\delta_C$ 41.3 (C-1′) and 24.3 (C-5). The H-5 signal showed long-range coupling to the signal at $\delta_C$ 55.4 (C-4) instead of the carbonyl carbon signal (Figure 2). Therefore, sarcotrine D (**11**) can be differentiated from **5** as carrying a $\beta$-substituted lactam ring instead of the α-substituted one. The $^{1}$H NMR and $^{13}$C NMR spectral data of **11** suggested that it has the *S* configuration at C-21 (Tables 1 and 3), and this was further affirmed by CD spectral data (Figure 4). The CD spectrum displayed Cotton effects the same in sign as those of **5**. The configuration at C-13 was assumed to be the same as that of sarcotrine A (**5**).

Although pyrrolosesterterpenes such as palinurines A and B had been artificially generated from a furanosesterterpene through fungal biotransformation,[8] compounds **5**−**11** are the only additional linear pyrrolosesterterpenes to be reported. Compounds **5**−**11** may be biosynthesized by the nucleophilic attack of the amino acid derivatives, which may arise from leucine, isoleucine, or phenylalanine, in the same manner as the enzyme-catalyzed biotransformation of palinurin.[8] Both of the electron-deficient oxymethine carbons of the furan ring will be vulnerable to

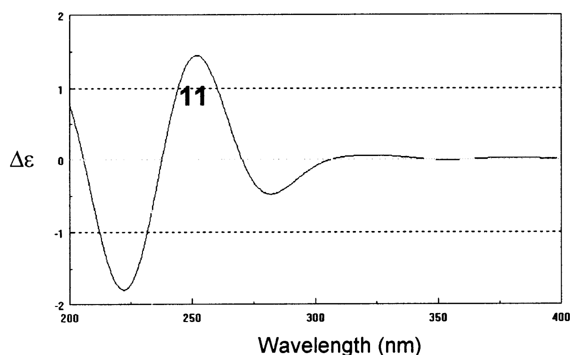


**Figure 4.** CD spectrum of compound **11**.

nucleophilic addition to give either **5** or **11**. Two trinorsesterterpene alkaloids with structure similar to sarcotrines have been recently isolated from the sponge of the genus *Sarcotragus* collected from Korean waters.[11] It is quite feasible that sarcotrines and the trinorsesterterpene alkaloids share the same biogenetic precursor, and the latter are the degradation products of the former.

Sarcotin F (**12**) was isolated as a yellow oil. The molecular formula of **12** was established as $C_{26}H_{36}O_6$ on the basis of HRFABMS. Compound **12** showed spectroscopic data suggestive of the sesterterpene tetronic acid structure with an oxidized furan ring. The presence of a 5-methoxy-2(5*H*)-furanone moiety was deduced from the characteristic NMR signals at $\delta_C$ 173.4 (C-4), 144.6 (C-2), 139.5 (C-3), 104.4 (C-1), $\delta$ 5.83 (H-1), 6.95 (H-2), and 57.0 (OMe)/3.51 (s, 3H).[12] The long-range correlation between H-5 and C-4 was observed in the HMBC spectrum (Figure 2). The $^1$H NMR and $^{13}$C NMR spectral data of the tetronic acid terminus of **12** suggested that it has an *S* configuration at C-21. The stereochemistry at C-1 could not be deduced from CD data since **12** contained two similar chromophores [2(5*H*)-furanones] in its structure.[4,13] The configuration at C-13 was assumed to be the same as that of the sarcotin A (**1**). It is known that alkylfurans could react with singlet oxygen to produce many products including an endoperoxide, to which solvent might be added to produce derivatives such as **12**.[14]

Sarcotin G (**13**) was isolated as a yellow oil. The molecular formula of **13** was established as $C_{26}H_{32}O_7$ on the basis of HRFABMS. The $^1$H NMR and $^{13}$C NMR spectral pattern appeared similar to that of ircinin-1.[2] Analysis of the spectroscopic data suggested that they share the same carbon framework as ircinin-1, but include a 5-methoxy-2(5*H*)-furanone moiety in place of one of the furan rings. The H-7 signal ($\delta$ 6.83) displayed a HMBC correlation to C-6 ($\delta_C$ 110.3) and C-9 ($\delta_C$ 172.7). Further evidence for the location of the C-9 carbonyl group was obtained from the HMBC correlation with the H-10 methylene proton signal at $\delta$ 2.22. Full assignment of the $^1$H and $^{13}$C NMR was achieved on the basis of the analysis of COSY, HMQC, and HMBC data. The stereochemistry at C-6 was proposed to be *S*, according to the negative Cotton effect at 230 nm ($\pi-\pi^*$) and the positive Cotton effect at 303 nm (n$-\pi^*$).[4] The stereochemistry at C-18 is believed to be the same as that of ircinins isolated from the same specimen.

Sarcotin H (**14**) was isolated as a yellow oil. The same molecular formula as **13** ($C_{26}H_{32}O_7$) was established on the basis of HRFABMS. The $^1$H NMR and $^{13}$C NMR spectral pattern appeared to be similar to that of ircinin-2.[2] The $^1$H NMR and $^{13}$C NMR spectral data of **14** indicated that it is the regio/geometric isomer of **13**. The absolute configurations at C-6 and C-18 were deduced to be the same as those of **13**, because **14** showed CD data and optical rotation similar to those of **13** (see Experimental Section). Sarcotins G (**13**) and H (**14**) could be depicted as further oxidized forms of ircinin-1 and -2, respectively.

Sarcotin I (**15**) was isolated as a yellow oil. A $\beta$-substituted furan unit was recognized by the broad singlets at $\delta$ 7.36, 7.23, and 6.28 in the $^1$H NMR spectrum (Table 2). Most of the $^1$H and $^{13}$C NMR data were in accordance with those of **1** and **4**.[2] However, the signals corresponding to the tetronic acid moiety were replaced by an oxymethine signal ($\delta$ 4.20, $\delta_C$ 70.7) and a carbonyl signal ($\delta_C$ 177.8). This oxymethine proton at $\delta$ 4.20 and the carboxylic carbon at $\delta_C$ 177.8 showed long-range coupling in the HMBC spectrum. The molecular formula of **15** was established as $C_{22}H_{31}O_4Na$ on the basis of HRFABMS. Thus, the structure of **15** could be defined as the Na salt of a linear trinorsesterterpene acid. An attempt to convert **15** to its MTPA ester by the modified Mosher's method was unsuccessful due to a rapid decomposition of the reactant. The absolute configuration of C-13 was assumed to be the same as **1**, and the stereochemistry of C-21 remains to be determined.

Sarcotin J (**16**) was characterized as the methyl ester of sarcotin I (**15**). The FABMS of **16** showed the $[M + Na]^+$ ion at *m/z* 397. The $^1$H and $^{13}$C NMR spectral data of **16** were very similar to those of **15**, except for an extra methoxyl signal ($\delta$ 3.68, s; $\delta_C$ 52.3). The long-range couplings of the carbonyl carbon ($\delta_C$ 176.2) with the oxymethine proton and methoxyl protons were observed. Sarcotins I (**15**) and J (**16**) had a unique $C_{22}$ skeleton, which might be a degradation product of the relevant sesterterpenes.[15] Only a few $C_{22}$ trinorsesterterpene derivatives have been described to date.[11,15]

Sarcotins K (**17**) and L (**18**) were isolated as an inseparable mixture. The FABMS of **17** and **18** showed a single $[M + Na]^+$ ion at *m/z* 323. The molecular formulas of **17** and **18** were established as $C_{20}H_{28}O_2$ on the basis of HRFABMS. The carbon skeletons of **17** and **18** were easily recognized as linear bisfuranoditerpenes by analysis of the $^1$H and $^{13}$C NMR spectra. The $^1$H NMR spectrum revealed signals attributable to three $\alpha$ protons ($\delta$ 7.42, 7.33, and 7.15) and two $\beta$ protons ($\delta$ 6.33, 5.95) of the furan ring, suggesting the existence of both a $\beta$-substituted furan ring and an $\alpha$-disubstituted furan ring. This speculation was further substantiated by $^{13}$C NMR data that showed eight furano-carbon signals. Most of the $^1$H and $^{13}$C NMR signals appeared as isomeric pairs, except those corresponding to the bisfuran moiety. On comparison of the NMR data with those of ircinin-1 and ircinin-2,[2] and by the aid of a COSY experiment, two sets of individual data corresponding to each isomer could be delineated. Part of the NMR data of each of **17** and **18** corresponded well with those of ircinin-1 and ircinin-2, respectively. A vinylic proton ($\delta$ 5.15, t, $J =$ 6.6 Hz) and a vinylic methyl ($\delta$ 1.57) corresponding to structure **17** suggested the presence of a trisubstituted double bond. In addition, the $^1$H NMR spectrum showed six distinct methylenes; a singlet at $\delta$ 3.73 (s, H-5), a triplet at $\delta$ 2.37 (H-10, t, $J = 7.5$ Hz), and four multiplets at $\delta$ 2.20, 1.94, 1.37, and 1.28 (H-11, 15, 16, and 17, respectively). The COSY spectrum showed correlations between the methylene singlet at $\delta$ 3.73 and the two $\beta$ protons ($\delta$ 6.33 and 5.95) and the $\alpha$-proton ($\delta$ 7.33) of the furan rings. The residual partial structure of **17** can be easily depicted by analysis of its COSY spectral data. On the basis of these data, the gross structure of **17** was defined as a bisfuranoditerpene. The geometry of the trisubstituted double bond was assigned as *E* according to the upfield-shifted vinylic methyl signal ($\delta$ 1.57 and $\delta_C$16.0).

With the exception of the groups in the vicinity of the trisubstituted olefin, the $^1$H and $^{13}$C NMR data of **18** appeared to be very similar to those of **17**. The COSY spectrum of **18** showed that the H-10 methylene proton signal at $\delta$ 2.34 was coupled to the methylene signal at $\delta$ 1.62, which was further coupled to the allylic methylene signal at $\delta$ 2.07. This allylic methylene signal showed long-range coupling with the olefinic proton signal at $\delta$ 5.13, suggesting that the double bond was located at C-13. The geometry of the double bond was assigned as *Z* according to the downfield-shifted vinylic methyl signals ($\delta$ 1.68 and $\delta_C$ 23.6).[16] To the best of our knowledge, compounds **17** and **18** are the first linear bisfuranoditerpenes to be described.

Sarcotin M (**19**) was isolated as a yellow oil. The molecular formula of **19** was established as $C_{26}H_{38}O_6$ on the basis of HRFABMS. Compound **19** was defined as a linear furanosesterterpene tetronic acid based on the typical $^1$H and $^{13}$C NMR signals. The presence of a tertiary alcohol moiety was deduced from $^1$H NMR ($\delta$ 1.12, 3H, s, H-9) and $^{13}$C NMR ($\delta_C$ 75.0, C-8) data. The location of the tertiary hydroxyl group at C-8 was established by the COSY experiment and the comparison of $^1$H and $^{13}$C NMR data with those of 8-hydroxy-12*E*,20*Z*-variablin.[17] The HMBC data also confirmed the location of the tertiary hydroxyl group and methoxyl functionality ($\delta_C$ 90.4, C-10). The methoxyl protons ($\delta$ 3.23, s) showed long-range correlation with C-10, and H-10 showed correlations with C-11 and -8. The assignment of *E* geometry to C-11 was secured from the $J_{11,12}$ value (15.5 Hz). The *S* configuration was assigned to C-21 on the basis of the NMR data of the relevant carbons and protons and was further affirmed by CD data. The CD spectrum of **19** displayed a pattern of Cotton effects similar to that of **1**; a positive Cotton effect at 219 nm ($\pi-\pi^*$) and a negative Cotton effect at 257 nm (n$-\pi^*$) were observed. The stereochemistry at C-8 and -9 was not determined. The configuration at C-13 was assumed to be the same as that of sarcotin A (**1**).

The isolated compounds were evaluated for cytotoxicity and showed a marginal to significant activity against a small panel of five human tumor cell lines (Table 5). Of the compounds tested, some of the derivatives with the tetronic acid function (**4**–**9**, **13**, **14**, **19**) exhibited higher potencies than the trinorsesterterpenes (**15**, **16**) and diterpenes (**17**, **18**), although the presence or absence of this moiety may not be the only determining factor, as other tetronic acid containing compounds (**10**–**12**) also had lower potencies, being comparable to **15**–**18** in activity.

**Table 4.** $^{13}$C NMR Data of Compounds **12**–**19** ($CD_3OD$, 50 MHz)

| position | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 |
|---|---|---|---|---|---|---|---|---|
| 1 | 104.4 | 144.0 | 144.0 | 144.1 | 143.9 | 144.1 | 144.1 | 143.9[a] |
| 2 | 144.6 | 113.2 | 113.2 | 111.9 | 111.9 | 112.2 | 112.2 | 111.9 |
| 3 | 139.5 | 119.0 | 119.0 | 126.4 | 126.5 | 123.0 | 123.0 | 126.4 |
| 4 | 173.4 | 142.7 | 142.7 | 140.1 | 140.1 | 140.9 | 140.9 | 140.0 |
| 5 | 25.6 | 33.9 | 33.9 | 25.2 | 25.2 | 24.8 | 24.8 | 26.3 |
| 6 | 26.6 | 110.3 | 110.3 | 29.4 | 29.5 | 155.5 | 155.5 | 24.9 |
| 7 | 40.2 | 147.3 | 147.0 | 40.3 | 40.3 | 108.3 | 108.1 | 38.6 |
| 8 | 136.0 | 139.5 | 139.9 | 136.7 | 136.7 | 127.0 | 127.1 | 75.0 |
| 9 | 16.4 | 172.7 | 172.7 | 16.5 | 16.5 | 138.7 | 138.7 | 23.4 |
| 10 | 126.5[a] | 26.8 | 25.7 | 126.4[a] | 126.2[a] | 26.1 | 25.7 | 90.4 |
| 11 | 127.0[a] | 27.0 | 27.0[a] | 126.7[a] | 126.5[a] | 29.5 | 29.4 | 126.0 |
| 12 | 139.5 | 124.0 | 32.0[b] | 139.1 | 140.1 | 125.3 | 32.2 | 143.8[a] |
| 13 | 38.0 | 137.7 | 135.9 | 38.0 | 37.9 | 136.5 | 136.3 | 38.0 |
| 14 | 21.4 | 16.0 | 23.5 | 21.3 | 21.4 | 16.0 | 23.6 | 21.3 |
| 15 | 38.2 | 40.4 | 126.6 | 38.5 | 38.5 | 40.7 | 126.2 | 38.6 |
| 16 | 27.0 | 26.8 | 26.8[a] | 26.9 | 26.9 | 26.2 | 26.3 | 27.2 |
| 17 | 130.4[b] | 38.6 | 38.6 | 129.7 | 129.8 | 37.3 | 38.1 | 130.1 |
| 18 | 130.7[b] | 31.8 | 31.8[b] | 131.9 | 131.7 | 31.4 | 31.1 | 131.0 |
| 19 | 24.4 | 21.1 | 21.1 | 24.8 | 24.0 | 20.1 | 20.0 | 24.3 |
| 20 | 35.4 | 115.2 | 115.2 | 37.9 | 37.9 | 20.1 | 20.0 | 35.5 |
| 21 | 78.9 | 144.9 | 144.9 | 70.7 | 71.0 | | | 79.2 |
| 22 | 177.8 | 165.0 | 165.0 | 177.8 | 176.2 | | | 179.2 |
| 23 | 96.5 | 98.1 | 98.1 | | | | | 95.7 |
| 24 | 178.3 | 173.7 | 174.0 | | | | | 180.7 |
| 25 | 6.0 | 6.1 | 6.1 | | | | | 6.0 |
| $OCH_3$ | 57.0 | 51.7 | 51.7 | | 52.3 | | | 56.8 |

[a,b] Assignments with the same superscript in the same column may be interchanged.

**Table 5.** Cytotoxicity Data of Compounds **4**–**19**[a,b]

| compound | A549 | SK-OV-3 | SK-MEL-2 | XF498 | HCT15 |
|---|---|---|---|---|---|
| **4** | 12.3 | 9.6 | 5.6 | 9.8 | 6.5 |
| **5** | 15.1 | 5.3 | 4.1 | 5.5 | 5.0 |
| **6** | 4.3 | 4.0 | 3.4 | 3.9 | 3.8 |
| **7** | 6.3 | 6.7 | 4.3 | 5.2 | 4.9 |
| **8** | 16.8 | 13.1 | 4.8 | 10.5 | 5.4 |
| **9** | 19.0 | 6.9 | 3.8 | 5.4 | 5.3 |
| **10** | 27.1 | 26.8 | 15.9 | 25.2 | 22.3 |
| **11** | >30 | 25.9 | 13.2 | >30 | 21.6 |
| **12** | 24.1 | 15.2 | 7.6 | 20.1 | 10.5 |
| **13** | 9.1 | 10.0 | 5.1 | 7.6 | 7.3 |
| **14** | 6.7 | 6.8 | 5.9 | 6.3 | 6.1 |
| **15** | 24.8 | 23.3 | 25.7 | 25.9 | 23.7 |
| **16** | 18.1 | 10.0 | 7.8 | 24.3 | 8.7 |
| **17**, **18**[c] | >30 | 26.8 | 6.2 | 29. 6 | 23.9 |
| **19** | 9.0 | 8.4 | 9.9 | 11.3 | 10.1 |
| doxorubicin | 0.02 | 0.16 | 0.02 | 0.13 | 0.06 |

[a] Data expressed in $ED_{50}$ values ($\mu$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] Compounds were assayed in several separate batches. [c] Obtained as an inseparable mixture.

## 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). UV spectra were obtained in MeOH, using a Shimadzu mini 1240 UV–vis spectrophotometer. $^1$H and $^{13}$C NMR spectra were recorded on a Bruker AC200, DMX 600 instrument and a Varian Inova 500. Chemical shifts were reported with reference to the respective residual solvent peaks ($\delta_H$ 3.30 and $\delta_C$ 49.0 for $CD_3OD$). 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 i.d., 4 $\mu$m, 80 Å; preparative, 250 × 20 mm i.d., 4 $\mu$m, 80 Å) column using a Shodex RI-71 detector. Normal-phase HPLC was performed with an YMC Silica (semipreparative, 250 × 10 mm i.d., 5 $\mu$m, 120 Å) column using a JASCO UV-975 Intelligent UV/vis detector.

**Animal Material.** The sponge was collected in July 1998 (15–25 m depth), off Cheju Island, Korea. The specimen was identified as *Sarcotragus* sp. by Prof. Chung Ja Sim, Hannam University. A voucher specimen (J98J-5) of this horny sponge (registry No. Por.33) was deposited in the Natural History Museum, Hannam University, Taejon, Korea, and has been described elsewhere.[2]

**Extraction and Isolation.** The frozen 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 $\mu$g/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). These fractions were evaluated for activity in the brine

shrimp assay, and fractions Fg6−Fg9 were found active. These fractions were combined (7.4 g) and 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 10 fractions. Fractions Fg6-5−Fg6-7 (5.4 g) were combined, and the combined fraction was further separated by reversed-phase flash column chromatography (YMC Gel ODS-A, 60 Å, 500/400 mesh), eluting with 33 to 0% $H_2O$/MeCN, to afford 13 fractions. Guided by the brine shrimp assay, compound **5** (11.3 mg) was obtained by purification of fraction Fg6-5-11 by ODS HPLC. Compounds **7** (2.9 mg), **9** (6.5 mg), **10** (1.1 mg), and **11** (2.7 mg) were obtained by purification of fraction Fg6-5-10 by ODS HPLC. Compounds **6** (1.9 mg) and **8** (1.9 mg) were obtained by purification of the degradation mixture of **5** and **7**, respectively, by ODS HPLC. Compounds **12** (11.1 mg), **14** (5.4 mg), and **16** (8.6 mg) were obtained by purification of fraction Fg6-5-3, Fg6-5-2, and Fg6-5-11, respectively, by ODS HPLC. Compounds **13** (3.1 mg) and **19** (3.2 mg) were obtained by purification of fraction Fg6-5-3 by ODS HPLC. Compound **15** (44.3 mg) and the mixture of **17** and **18** (7.6 mg) were obtained by purification of fraction Fg6-5-4 by ODS HPLC. Compound **4** (1.5 mg) was obtained by purification of the degradation mixture of sarcotin A (**1**) by ODS HPLC.

***epi*-Sarcotin A (4):** light yellow oil; $[\alpha]^{25}_{D}$ +37.5° (*c* 0.04, MeOH); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 226 (4.76) nm; CD (*c* 4 × $10^{-4}$ M, MeOH), $\Delta\epsilon$, 0 (350.3), +0.24 (312.5), 0 (289.1), −0.11 (272.4), 0 (262.6), +0.09 (248.4), 0 (232.2), −0.05 (220.1), 0 (210.4); $^1$H NMR data, see Table 1; $^{13}$C NMR data, see Table 3; EIMS *m/z* 398 $[M]^+$; FABMS *m/z* 443 $[M + 2Na - H]^+$ (9), 421 $[M + Na]^+$ (3), 326 (8); HRFABMS *m/z* 421.2318 (calcd for $C_{25}H_{34}O_4Na$, 421.2355).

**Sarcotrine A (5):** colorless oil; $[\alpha]^{21}_{D}$ +36.1° (*c* 0.18, MeOH); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 240 (4.81) nm; $^1$H NMR data, see Table 1; $^{13}$C NMR data, see Table 3; CD (*c* 1 × $10^{-4}$ M, MeOH), $\Delta\epsilon$, 0 (332.7), +0.14 (313.6), 0 (298.6), −0.04 (292.0), 0 (286.4), +0.84 (263.6), 0 (244.9), −0.45 (230.7), 0 (211.4); FABMS *m/z* 484 $[M + H]^+$ (80), 482 (100), 464 (3), 370 (2), 329 (70); HRFABMS *m/z* 484.3410 (calcd for $C_{30}H_{46}NO_4$, 484.3427).

***epi*-Sarcotrine A (6):** colorless oil; $[\alpha]^{21}_{D}$ +42.8° (*c* 0.05, MeOH); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 242 (4.56) nm; $^1$H NMR data, see Table 1; $^{13}$C NMR data, see Table 3; CD (*c* 1 × $10^{-4}$ M, MeOH), $\Delta\epsilon$, 0 (310.2), +0.16 (292.0), 0 (281.0), −0.54 (265.3), 0 (250.0), +0.52 (237.8), 0 (223.3); FABMS *m/z* 506 $[M + Na]^+$ (50), 484 $[M + H]^+$ (25), 329 (10); HRFABMS *m/z* 506.3271 (calcd for $C_{30}H_{45}NO_4Na$, 506.3246).

**Sarcotrine B (7):** colorless oil; $[\alpha]^{21}_{D}$ +36.9° (*c* 0.07, MeOH); $^1$H NMR data, see Table 1; $^{13}$C NMR data, see Table 3; CD (*c* 1 × $10^{-4}$ M, MeOH), $\Delta\epsilon$, 0 (294.5), −0.05 (274.8), 0 (267.4), +0.16 (254.8), 0 (242.4), −0.08 (229.3), 0 (220.2); FAB-CID MS/MS *m/z* 518 $[M + H]^+$ (100), 404 (8), 282 (3), 200 (2); HRFABMS *m/z* 518.3240 (calcd for $C_{33}H_{44}NO_4$, 518.3271).

***epi*-Sarcotrine B (8):** colorless oil; $[\alpha]^{21}_{D}$ +42.1° (*c* 0.04, MeOH); $^1$H NMR data, see Table 1; $^{13}$C NMR data, see Table 3; CD (*c* 1 × $10^{-4}$ M, MeOH), $\Delta\epsilon$, 0 (294.5), −0.13 (288.1), 0 (277.3), +0.18 (270.6), 0 (262.5), −0.05 (250.1), 0 (236.6), +0.46 (228.7), 0 (213.1); FABMS *m/z* 540 $[M + Na]^+$ (20), 518 $[M + H]^+$ (8), 413 (5), 329 (40); HRFABMS *m/z* 540.3085 (calcd for $C_{33}H_{43}NO_4Na$, 540.3090).

**Sarcotrine C (9):** yellow oil; $[\alpha]^{21}_{D}$ +19.1° (*c* 0.18, MeOH); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 245 (4.28) nm; $^1$H NMR data, see Table 1; $^{13}$C NMR data, see Table 3; CD (*c* 1 × $10^{-4}$ M, MeOH), $\Delta\epsilon$, 0 (284.6), −0.28 (265.1), 0 (252.1), +0.54 (239.5), 0 (230.1), −0.55 (223.2); FABMS *m/z* 506 $[M + Na]^+$ (30), 484 $[M + H]^+$ (39), 329 (38), 135 (82); HRFABMS *m/z* 506.3248 (calcd for $C_{30}H_{45}NO_4Na$, 506.3246).

***epi*-Sarcotrine C (10):** yellow oil; $[\alpha]^{21}_{D}$ +24.0° (*c* 0.06, MeOH); $^1$H NMR data, see Table 1; $^{13}$C NMR data, see Table 3; CD (*c* 1 × $10^{-4}$ M, MeOH), $\Delta\epsilon$, 0 (277.1), +0.17 (259.0), 0 (248.5), −0.10 (240.1), 0 (230.1), +0.02 (222.9), 0 (215.6), -0.03 (209.3); FABMS *m/z* 506 $[M + Na]^+$ (42), 484 $[M + H]^+$ (39), 329 (48); HRFABMS *m/z* 506.3228 (calcd for $C_{30}H_{45}NO_4Na$, 506.3246).

**Sarcotrine D (11):** yellow oil; $[\alpha]^{21}_{D}$ +65.2° (*c* 0.01, MeOH); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 239(4.34) nm; $^1$H NMR data, see Table 1; $^{13}$C NMR data, see Table 3; CD (*c* 1 × $10^{-4}$ M, MeOH), $\Delta\epsilon$, 0 (305.8), −0.50 (281.5), 0 (269.2), +1.48 (253.6), 0 (237.4), −1.76 (222.9), 0 (208.4); FABMS *m/z* 506 $[M + Na]^+$ (14), 484 $[M + H]^+$ (16), 329 (38); HRFABMS *m/z* 484.3429 (calcd for $C_{30}H_{46}NO_4$, 484.3427).

**Sarcotin F (12):** yellow oil; $[\alpha]^{21}_{D}$ +30.8° (*c* 0.36, MeOH); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 247 (4.95) nm; $^1$H NMR data, see Table 2; $^{13}$C NMR data, see Table 4; CD (*c* 1 × $10^{-4}$ M, MeOH), $\Delta\epsilon$, 0 (324.8), +0.55 (308.2), 0 (293.8), −0.54 (281.4), 0 (265.3), +0.75 (250.2), 0 (231.8), −0.52 (218.2), 0 (206.8); FABMS *m/z* 467 $[M + Na]^+$ (14), 445 $[M + H]^+$ (15), 307 (70), 136 (100); HRFABMS *m/z* 467.2402 (calcd for $C_{26}H_{36}O_6Na$, 467.2410), 445.2610 (calcd for $C_{26}H_{37}O_6$, 445.2591).

**Sarcotin G (13):** yellow oil; $[\alpha]^{21}_{D}$ +27.9° (*c* 0.09, MeOH); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 248 (4.67) nm; $^1$H NMR data, see Table 2; $^{13}$C NMR data, see Table 4; CD (*c* 1 × $10^{-4}$ M, MeOH), $\Delta\epsilon$, 0 (376.4), +1.04 (302.8), 0 (259.1), −0.2 (230.5); FABMS *m/z* 479 $[M + Na]^+$ (34), 457 $[M + H]^+$ (16), 329 (52), 307 (73), 289 (38); HRFABMS *m/z* 457.2202 (calcd for $C_{26}H_{33}O_7$, 457.2226).

**Sarcotin H (14):** yellow oil; $[\alpha]^{21}_{D}$ +35.2° (*c* 0.10, MeOH); $^1$H NMR data, see Table 2; $^{13}$C NMR data, see Table 4; CD (*c* 1 × $10^{-4}$ M, MeOH), $\Delta\epsilon$, 0 (376.4), +1.44 (277.8), 0 (231.5); FABMS *m/z* 479 $[M + Na]^+$ (42), 457 $[M + H]^+$ (8), 329 (40), 307 (30); HRFABMS *m/z* 479.2047 (calcd for $C_{26}H_{32}O_7Na$, 479.2046).

**Sarcotin I (15):** yellow oil; $[\alpha]^{21}_{D}$ +33.1° (*c* 0.14, MeOH); IR (film) $\nu_{max}$ 2928, 2360, 2330, 1726, 1023 cm$^{-1}$; $^1$H NMR data, see Table 2; $^{13}$C NMR data, see Table 4; FABMS *m/z* 405 $[M + Na]^+$ (28), 383 $[M + H]^+$ (6), 325 (20), 63 (50); HRFABMS *m/z* 405.2022 (calcd for $C_{22}H_{31}O_4Na_2$, 405.2018).

**Sarcotin J (16):** light yellow oil; $[\alpha]^{21}_{D}$ +115.4° (*c* 0.046, MeOH); $^1$H NMR data, see Table 2; $^{13}$C NMR data, see Table 4; FABMS *m/z* 397 $[M + Na]^+$ (100), 325 (15), 92 (20).

**Sarcotin K (17)** and **Sarcotin L (18)**: yellow oil; $^1$H NMR data, see Table 2; $^{13}$C NMR data, see Table 4; FABMS *m/z* 323 $[M + Na]^+$ (9), 301 $[M + H]^+$ (3), 242 (5), 199 (25), 92 (35), 63 (48); HRFABMS *m/z* 323.2005 (calcd for $C_{20}H_{28}O_2Na$, 323.1987).

**Sarcotin M (19):** yellow oil; $[\alpha]^{21}_{D}$ +26.8° (*c* 0.02, MeOH); $^1$H NMR data, see Table 2; $^{13}$C NMR data, see Table 4; CD (*c* 1 × $10^{-4}$ M, MeOH), $\Delta\epsilon$, 0 (358.4), +0.28 (315.6), 0 (293.8), −0.54 (256.9), 0 (238.2), +0.76 (218.7), 0 (206.5); FABMS *m/z* 469 $[M + Na]^+$ (85), 447 $[M + H]^+$ (9), 429 (10), 307 (40), 135 (70); HRFABMS *m/z* 469.2567 (calcd for $C_{26}H_{38}O_6Na$, 469.2566).

**Oxidative Cleavage of 1, 5, and 6.** To a biphasic solution of 6.4 mg (0.016 mmol) of **1** and 41.3 mg (0.192 mmol) of $NaIO_4$ in a mixture of 1 mL of $CCl_4$, 1 mL of $CH_3CN$, and 1.5 mL of $H_2O$ was added 21.5 mg (0.08 mmol) of $RuCl_3 \cdot xH_2O$. After vigorous stirring of the mixture for 2 h at room temperature, the solvents were removed under vacuum. The residue was dissolved in MeOH and then filtered. The filtrate was dried, extracted with $CHCl_3$, and subjected to normal-phase HPLC (YMC Silica column, hexane/$CHCl_3$ = 3:1) to give 0.9 mg of (*S*)-2-methylglutaric acid: $[\alpha]^{20}_{D}$ +22° (*c* 0.026, MeOH); $^1$H NMR ($CDCl_3$) $\delta$ 2.55 (1 H, m, H-2), 2.52 (1H, m, H-4), 2.45 (1H, m, H-4), 2.05 (1H, dtd, $J$ = 14.5, 9.5, 4.5 Hz, H-3), 1.90 (1H, ddt, $J$ = 14.5, 7.0, 4.5 Hz, H-3), 1.22 (3H, d, $J$ = 7.0 Hz, $CH_3$).[6] Oxidative cleavage of **5** (4.9 mg) and **6** (1.5 mg) was performed in the same manner to give 0.8 mg and 0.4 mg of (*S*)-2-methylglutaric acid, respectively.

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