New Acetylenic Acids from the Marine Sponge *Stelletta* Species

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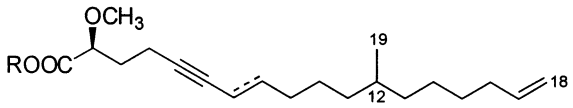
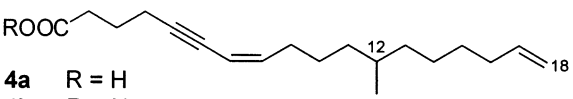
Four new acetylenic acids were isolated from the marine sponge *Stelletta* sp. by bioactivity-guided fractionation. The planar structures were established on the basis of NMR and MS analysis. The stereochemistry was defined by combined use of CD spectroscopy and a chiral anisotropic reagent, phenylglycine methyl ester (PGME). The compounds were evaluated for cytotoxicity against a small panel of five human tumor cell lines and exhibited marginal to moderate cytotoxicity.

Marine sponges of the genus *Stelletta* are reported to contain various sterols,[1−3] terpenes,[4−6] alkaloids,[7−9] and fatty acids.[10] In the course of screening for cytotoxic constituents from marine sponges, we have noticed significant activity in the crude extract of *Stelletta* sp. collected from Korean waters. In subsequent bioactivity-guided fractionation, four new acetylenic acids (**1**−**4**), including three 2-methoxy fatty acids, were isolated. The first 2-hydroxy and 2-methoxy fatty acids reported were (2*R*)-hydroxyhexadecanoic acid from the yeast *Hansenula sydowiorum*[11] and (2*R*,21*Z*)-2-methoxy-21-octacosenoic acid from the sponge *Higginsia tethyoides*.[12] Subsequently, more saturated or unsaturated 2-methoxy fatty acids with chain lengths of 14−28 carbon atoms have been reported from sponges.[13−19] To date, however, 2-methoxy fatty acids have been identified only in the phospholipids of sponges, and all of them were reported to possess the 2*R* configuration.[12,13] To the best of our knowledge, compounds **1**−**3** are the first examples of naturally occurring (2*S*)-2-methoxy fatty acids. The isolation, structure elucidation, and cytotoxicity evaluation of these compounds are described herein.



1 R = H
1a R = CH_3
2a R = H, Δ^{7Z}
2b R = Na, Δ^{7Z}
3 R = H, Δ^{7E}



4a R = H
4b R = Na

The MeOH extract of the sponge displayed toxicity to brine shrimp larvae (LD_{50}, 296 μg/mL). Guided by the brine shrimp lethality assay, the MeOH extract was further partitioned between water and CH_2Cl_2, followed by partitioning of the CH_2Cl_2 solubles between aqueous MeOH and *n*-hexane. The aqueous MeOH layer was subjected successively to reversed-phase flash column chromatography, Sephadex LH-20 column chromatography, and HPLC to afford compounds **1**−**4**.

Stellettic acid A (**1**) was isolated as a pale yellow oil. The molecular formula of **1** was established as $C_{20}H_{34}O_3$ on the basis of MS and NMR spectral analyses. In the FABMS measurement, **1** was converted in situ to its sodium salt by NaI, and the spectrum showed the $[M + H]^+$ ion of its sodium salt at *m*/*z* 345, accompanied by the $[M + Na]^+$ ion at *m*/*z* 367. The exact mass of the $[M + Na]^+$ ion (*m*/*z* 367.2227) of the sodium salt matched well with the expected molecular formula $C_{20}H_{33}O_3Na_2$ (Δ +0.2 mmu). In the ^{1}H NMR spectrum, a doublet of doublets of triplets at δ 5.82 and two doublets of doublets at δ 5.01 and 4.93 were attributed to a monosubstituted olefin. A triplet of triplets at δ 2.14 and a multiplet at δ 2.34 were attributed to α-acetylenic methylenes. The signal at δ 2.04 was assigned to allylic protons. The quintet at δ 1.48 was attributed to the H-8 protons. The doublet at δ 0.84 indicated the presence of a methyl branch on the carbon chain. The ^{1}H NMR spectrum also featured a three-proton singlet at δ 3.48 correlated with a carbon at δ 58.1, which was assigned to a methoxy group. In the HMBC spectrum, the oxymethine proton (δ 3.98, dd) was correlated with the carbonyl carbon (δ 175.1) and the methoxy carbon (δ 58.1). This indicated that the methoxy group was present at C-2. This oxymethine proton signal was correlated with methylene proton signals at δ 1.97 and 1.91, which in turn were correlated with the α-acetylenic methylene protons at δ 2.34. Thus, the triple bond was shown to be located at C-5. The position of methyl branching in **1** was clearly recognized from the FAB-CID tandem mass spectrum of the $[M + Na]^+$ ion of its sodium salt. Major fragmentations of the $[M + Na]^+$ ion were observed as odd mass ions due to the remote charge fragmentation which is characteristic of the collisional activation of an alkali-metal-cationized ion. These fragmentations involved parallel pathways of sequential CH_2 losses, except for the fragmentations occurring at the branching point, where there is significant loss of two carbon atoms. Thus, the location of the methyl branching was clear from the 28-mass gap between the fragment ion peaks at *m*/*z* 283 and 255 (Figure 1).

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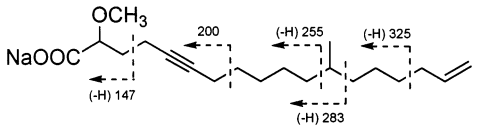

Figure 1. Key FAB-CID tandem mass fragmentations of the $[M + Na]^+$ ion of the sodium salt of **1**.

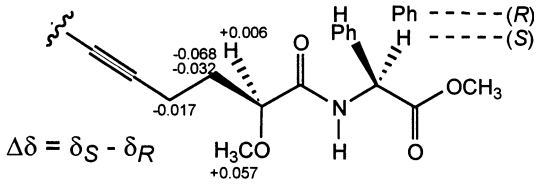

Figure 2. Conformations of the PGME amides of **1** and resulting $\Delta\delta$ values.

The similar optical rotation values and CD data of compounds **1**-**3** implied that they share the same configuration at C-2. The CD spectrum of **1** exhibited a positive Cotton effect at 214 nm ($\Delta\epsilon$ 1.32) and a weakly positive one at 266 nm ($\Delta\epsilon$ 0.19). The strong Cotton effect at 214 nm was opposite in sign to that of methyl (2*R*,21*Z*)-2-methoxy-21-octacosenoate.[12] Therefore, the methyl ester (**1a**) of **1** was prepared for comparison of its CD spectrum with that of this stereochemically defined 2-methoxy fatty acid methyl ester. The CD spectrum of **1a** also exhibited a strongly positive Cotton effect at 214 nm ($\Delta\epsilon$ 1.51) and a weakly positive one at 264 nm ($\Delta\epsilon$ 0.34). Thus, the *S* configuration at C-2 was suggested. The configuration at C-2 was further corroborated by Kusumi's method.[20] Phenylglycine methyl ester (PGME) can be used as a chiral anisotropic reagent for the determination of the absolute configuration of 2-substituted chiral carboxylic acids. The $\Delta\delta$ values ($\delta_S - \delta_R$) of the PGME amides of compound **1** (Figure 2) were reasonably comparable to those of the PGME amides of the model compound L-leucic acid acetate.[20] However, the configuration of the methyl branch remains to be determined. Thus, the structure of **1** was determined to be (2*S*)-2-methoxy-12-methyloctadec-17-en-5-ynoic acid.

(*Z*)-Stellettic acid B (**2a**) was isolated as a pale yellow oil. The molecular formula of **2a** was established as $C_{20}H_{32}O_3$ on the basis of MS and NMR spectral analyses. The FABMS spectrum of **2a** showed the $[M + H]^+$ ion of its sodium salt at *m*/*z* 343, accompanied by the $[M + Na]^+$ ion at *m*/*z* 365. The exact mass of the $[M + Na]^+$ ion (*m*/*z* 365.2070) of the sodium salt matched well with the expected molecular formula $C_{20}H_{33}O_3Na_2$ (Δ +0.1 mmu). The ^{1}H NMR spectrum of **2a** was similar to that of **1** except for the additional resonances due to extra olefinic protons. In the HMBC spectrum of **2a**, the additional olefinic proton triplets at δ 5.87 and 5.46 were correlated with the acetylenic carbons C-5 and C-6, respectively, indicating that the double bond was conjugated with the triple bond. The geometry of the double bond was deduced to be *cis* on the basis of the coupling constant of the olefinic protons (J = 11.0 Hz). The position of the methyl branch was again clear from the 28-mass gap between the fragment ions at *m*/*z* 281 and 253 in the FAB-CID tandem mass spectrum. The optical rotation and CD data of **2a** were similar in magnitude and sign to those of **1**. Accordingly, the same absolute stereochemistry was presumed for **2a**, and the structure was defined as (*Z*)-(2*S*)-2-methoxy-12-methyloctadeca-7,17-dien-5-ynoic acid.

Compound **2b** was isolated as a pale yellow oil. Although compound **2b** was clearly distinct from **2a** with much shorter retention time in the reversed-phase HPLC, both of them showed the same correlation patterns in the COSY and HMBC spectra as well as the same FAB-CID tandem mass spectrum. The molecular formula of **2b** was established as $C_{20}H_{31}O_3Na$ on the basis of MS and NMR spectral analyses. The FABMS spectrum of **2b** showed the $[M + H]^+$ ion at *m*/*z* 343, accompanied by the $[M + Na]^+$ ion at *m*/*z* 365. The ^{1}H NMR spectrum of **2b** was almost identical to **2a** except for the notable upfield shift of the oxymethine proton signal (δ 3.65) compared to that of **2a** (δ 3.93). The ^{13}C NMR data of **2b** showed downfield shifts of the carbonyl carbon (δ 178.9) and oxymethine carbon (δ 82.1) compared to those of **2a** (δ 175.1 and 78.9, respectively), which are characteristic of the carboxylate form.[21] Moreover, after re-HPLC of **2b** with the acidic mobile phase (CH_3CN-H_2O-TFA, 50:50:0.01), the resultant fraction showed the same ^{1}H NMR spectrum as that of **2a**. Thus, the identity of **2b** was defined as a sodium salt of (*Z*)-stellettic acid B (**2a**).

(*E*)-Stellettic acid B (**3**) was isolated as a pale yellow oil. The molecular formula of **3** was established as $C_{20}H_{32}O_3$ on the basis of MS and NMR spectral analyses. The FABMS spectrum of **3** showed the $[M + H]^+$ ion of its sodium salt at *m*/*z* 343, accompanied by the $[M + Na]^+$ ion at *m*/*z* 365. The ^{1}H and ^{13}C NMR data were similar to those of compound **2a** except for the coupling constant of the olefinic protons (J = 16.0 Hz) and the chemical shift of the relevant allylic carbon (δ_C 33.6), which indicated *trans* geometry. The optical rotation and CD data of **3** were similar to those of **1**. Thus, the structure of **3** was determined to be (*E*)-(2*S*)-2-methoxy-12-methyloctadeca-7,17-dien-5-ynoic acid.

(*Z*)-Stellettic acid C (**4a**) was isolated as a pale yellow oil. The molecular formula of **4a** was established as $C_{20}H_{32}O_2$ on the basis of MS and NMR spectral analyses. The FABMS spectrum of **4a** showed the $[M + H]^+$ ion of its sodium salt at *m*/*z* 313, accompanied by the $[M + Na]^+$ ion at *m*/*z* 335. The exact mass of the $[M + Na]^+$ ion (*m*/*z* 335.1966) of the sodium salt matched well with the expected molecular formula $C_{20}H_{33}O_3Na_2$ (Δ +0.3 mmu). The NMR data of **4a** were similar to those of **2a** except for the replacement of the signals of the oxymethine (δ_H 3.93, δ_C 78.9) and the methoxy group (δ_H 3.31, δ_C 57.8) with those of a methylene group (δ_H 2.41, δ_C 32.6). The methylene proton signal (δ 2.41) showed correlation with the carbonyl carbon (δ 178.3) in the HMBC. Thus, the structure of **4a** was determined to be (*Z*)-12-methyloctadeca-7,17-dien-5-ynoic acid.

Same as in the case of **2a**, the sodium salt of (*Z*)-stellettic acid C was isolated and identified. Compound **4b** showed the same correlation patterns in the COSY and HMBC spectra as well as the same FAB-CID tandem mass spectrum as that of **4a**, but it had much shorter retention time in the reversed-phase HPLC. The upfield shift of the 2-methene proton signal (δ_H 2.37) of **4b** compared to that of **4a** (δ_H 2.41) indicated that it is the sodium salt of (*Z*)-stellettic acid C.

Compounds **1**−**4** showed marginal to moderate cytotoxicities against a small panel of five human tumor cell lines (Table 3).[22] Compound **4** showed a rather higher potency than the others.

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). IR spectra were measured using a JASCO FT/IR-410 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AC200 and Varian Inova 500 instruments. Chemical shifts were reported with

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

position	**1**[b]	**2a**[c]	**2b**[d]	**3**[b]	**4a**[d]	**4b**[d]
2	3.98 (dd, 8.5, 4.5)	3.93 (dd, 8.5, 4.5)	3.65 (dd, 8.5, 4.5)	3.98 (dd, 8.5, 4.5)	2.41 (m)	2.37 (m)
3	1.97 (m)	1.95 (m)	1.95 (m)	2.04 (m)	1.81 (quint, 7.0)	1.81 (quint, 7.0)
	1.91 (m)	1.91 (m)	1.83 (m)	1.96 (m)		
4	2.34 (m)	2.48 (m)	2.44 (m)	2.47 (m)	2.41 (m)	2.37 (m)
7	2.14 (tt, 7.0, 2.0)	5.46 (dt, 11.0, 1.5)	5.41 (dt, 11.0, 1.5)	5.45 (dt, 16.0, 1.5)	5.46 (dt, 11.0, 1.5)	5.46 (dt, 11.0, 1.5)
8	1.48 (q, 7.0)	5.87 (m)	5.81 (m)	6.08 (dt, 16.0, 7.0)	5.87 (dt, 11.0, 7.0)	5.87 (dt, 11.0, 7.0)
9	1.26−1.40 (m)	2.26 (q, 7.0)	2.25 (q, 7.0)	2.06 (m)	2.25 (q, 7.0)	2.24 (q, 7.0)
10	1.26−1.40 (m)	1.29−1.38 (m)	1.26−1.40 (m)	1.26−1.40 (m)	1.29−1.38 (m)	1.29−1.38 (m)
11	1.26−1.40 (m)	1.29−1.38 (m)	1.26−1.40 (m)	1.26−1.40 (m)	1.29−1.38 (m)	1.29−1.38 (m)
	1.10 (m)	1.14 (m)	1.10 (m)	1.10 (m)	1.14 (m)	1.14 (m)
12	1.37 (m)	1.37 (m)	1.37 (m)	1.37 (m)	1.37 (m)	1.37 (m)
13	1.26−1.40 (m)	1.29−1.38 (m)	1.26−1.40 (m)	1.26−1.40 (m)	1.29−1.38 (m)	1.29−1.38 (m)
	1.10 (m)	1.14 (m)	1.10 (m)	1.10 (m)	1.14 (m)	1.14 (m)
14	1.26−1.40 (m)	1.29−1.38 (m)	1.26−1.40 (m)	1.26−1.40 (m)	1.29−1.38 (m)	1.29−1.38 (m)
15	1.26−1.40 (m)	1.29−1.38 (m)	1.26−1.40 (m)	1.26−1.40 (m)	1.29−1.38 (m)	1.29−1.38 (m)
16	2.04 (q, 7.0)	2.04 (q, 7.0)[e]	2.04 (q, 7.0)	2.06 (m)	2.04 (q, 7.0)	2.04 (q, 7.0)
17	5.82 (ddt, 17.0, 11.5, 7.0)	5.82 (m)	5.82 (m)	5.82 (ddt, 17.0, 11.5, 7.0)	5.82 (ddt, 17.0, 11.5, 7.0)	5.82 (ddt, 17.0, 11.5, 7.0)
18	5.01 (dd, 17.0, 2.0)	5.01 (dd, 17.0, 2.0)	5.01 (dd, 17.0, 2.0)	5.01 (dd, 17.0, 2.0)	5.01 (dd, 17.0, 2.0)	5.01 (dd, 17.0, 2.0)
	4.93 (dd, 11.5, 2.0)	4.94 (dd, 11.5, 2.0)	4.94 (dd, 11.5, 2.0)	4.94 (dd, 11.5, 2.0)	4.94 (dd, 11.5, 2.0)	4.94 (dd, 11.5, 2.0)
19	0.84 (d, 6.5)	0.86 (d, 6.5)	0.85 (d, 6.5)	0.85 (d, 6.5)	0.86 (d, 6.5)	0.86 (d, 6.5)
OCH_3	3.48 (s)	3.31 (s)	3.34 (s)	3.48 (s)		

[a] Multiplicities and coupling constants (in Hz) are in parentheses. [b] Measured in $CDCl_3$. [c] Measured in acetone-d_6. [d] Mesured in CD_3OD. [e] Signals were overlapped with the solvent peak.

Table 2. ^{13}C NMR Data of Compounds **1**−**4** (50 MHz)[a]

position	**1**[b]	**2a**[c]	**2b**[d]	**3**[b]	**4a**[d]	**4b**[d]
1	175.1	175.1	178.9	175.1	178.3	178.5
2	78.6	78.9	82.1	78.9	32.6	34.6
3	32.2	32.2	31.5	31.5	24.1	24.7
4	14.8	15.6	15.8	15.6	18.6	18.6
5	82.7[e]	93.5[e]	94.1[e]	86.8[e]	93.3[e]	93.3[e]
6	78.3[e]	77.9[e]	77.9[e]	80.7[e]	77.6[e]	77.6[e]
7	18.7	110.4	109.5	109.5	109.4	109.4
8	29.2[f]	143.5	142.7	144.4	142.7	142.7
9	29.6[f]	30.6	30.6	33.6	29.8	29.8
10	26.7[g]	27.1	26.7	26.7	26.7	26.7
11	37.5	37.6	37.1	36.8	37.1	37.1
12	32.7	33.3	32.9	32.6	32.9	32.9
13	37.5	37.3	36.9	36.8	36.9	36.9
14	27.1[g]	27.2	26.8	26.8	26.8	26.8
15	29.8[f]	29.5	29.5	29.2	29.5	29.5
16	34.2	34.4	34.1	33.6	34.1	34.1
17	139.2	139.8	139.3	139.3	139.3	139.3
18	114.7	114.6	114.4	114.4	114.4	114.4
19	19.8	19.9	19.8	19.2	19.2	19.2
OCH_3	58.1	57.8	57.8	58.8		

[a] Signals were assigned by ^{1}H−^{1}H COSY, HMQC, and HMBC experiments. [b] Measured in $CDCl_3$. [c] Measured in acetone-d_6. [d] Measured in CD_3OD. [e−g] Assignments with the same superscript in the same column may be interchanged.

Table 3. Cytotoxicities (ED_{50}, μg/L) of Compounds **1**−**4** against Human Solid Tumor Cells[a]

compound	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
1	>30.0	25.6	>30.0	25.2	24.7
2a	12.0	17.4	7.3	6.5	17.6
2b	>30.0	>30.0	22.3	23.1	24.5
3	>30.0	>30.0	29.5	>30.0	>30.0
4a	4.3	3.9	3.4	4.1	4.8
4b	8.4	6.3	5.1	12.3	11.5
doxorubicin	0.03	0.15	0.03	0.15	0.07

[a] 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.

reference to the respective residual solvent peaks (δ_H 3.30 and δ_C 49.0 for CD_3OD; δ_H 7.27 and δ_C 77.0 for $CDCl_3$; δ_H 2.05 and δ_C 206.0, 29.8 for acetone-d_6). FAB-CID tandem MS data were obtained using a JEOL JMS SX-102A. HPLC was performed with YMC ODS-H80 (preparative, 250 × 20 mm i.d., 4 μm, 80 Å), YMC ODS-H80 (250 × 10 mm i.d., 4 μm, 80 Å), and YMC-Pack CN (250 × 10 mm i.d., 5 μm, 120 Å) columns using a Shodex RI-71 detector. Gel filtration chromatography was performed with Sephadex LH-20 (Pharmacia Biotech AB).

Animal Material. The sponge was collected by hand using scuba (20 m depth) in October 2001, off Ullung Island, Korea. The specimen was identified as *Stelletta* sp. by Prof. C. J. Sim, Hannam University. It has a cup-shaped crater of 9 cm height, 14 × 11 cm width. The surface was rough owing to the projecting brushes of orthotriaenes. The exterior was a shade of dark gray and the interior was beige. The texture was tough like a stone. The skeleton was composed of megascleres, oxea (2200−3000 μm × 50 μm), orthotriaene (1000−1500 μm × 50 μm, sometimes abnormal dichotriaene), microscleres, large oxyaster (70−85 μm in diameter), thin oxyaster (30−40 μm in diameter), small oxyaster (15−25 μm in diameter), weakly spined strongylaster (10−14 μm in diameter), and thin strongylaster (7−10 μm in diameter). A voucher specimen (registry No. Spo. 37) was deposited at the Natural History Museum, Hannam University, Korea.

Extraction and Isolation. The frozen sponge (15 kg) was extracted with MeOH at room temperature. The MeOH extract displayed moderate toxicity against brine shrimp larvae (LD_{50}, 296 μg/mL). The MeOH extract was partitioned between water and CH_2Cl_2. The CH_2Cl_2 layer was further partitioned between aqueous MeOH and *n*-hexane to yield aqueous MeOH- (5.2 g) and *n*-hexane-soluble (19.1 g) fractions. The aqueous MeOH fraction was subjected to stepped gradient reversed-phase flash column chromatography (YMC Gel ODS-A, 60 Å 500/400 mesh) eluting with a solvent system of 50 to 0% H_2O−MeOH, to afford 22 fractions (1−22). These fractions were evaluated for activity in the brine shrimp assay, and fractions 8−16 were found active. Fractions 8 and 9 were further purified by repeated reversed-phase HPLC (YMC ODS-H80, 250 × 20 mm i.d., 4 μm, 80 Å) eluting with 30% H_2O−MeOH to yield compound **2b** (146.0 mg). Fraction 13 was further separated by Sephadex LH-20 column chromatography eluting with MeOH, to afford 18 fractions. Subfraction 13-8 was purified by reversed-phase HPLC (YMC ODS-H80, 250 × 10 mm i.d., 4 μm, 80 Å) eluting with 15% H_2O−MeOH and reversed-phase HPLC (YMC-Pack CN, 250 × 10 mm i.d., 5 μm, 120 Å) eluting with the acidic mobile phase (CH_3CN−H_2O−TFA, 50:50:0.01) to yield compounds **1** (4.5 mg) and **3** (0.9 mg). Subfraction 13-10 was purified by repeated reversed-phase HPLC (YMC-Pack CN, 250 × 10 mm i.d., 5 μm, 120 Å) eluting with 42% H_2O−MeOH and then with the acidic mobile phase (CH_3CN−H_2O−TFA, 50:50:0.01) to yield compounds **2a** (1.2 mg) and **4b** (1.5 mg). The subfraction 13-15 was purified by reversed-phase

HPLC (YMC ODS-H80, 250 × 10 mm i.d., 4 μm, 80 Å) eluting with 15% H_2O−MeOH to yield compound **4a** (15.2 mg).

Stellettic acid A (1): pale yellow oil; $[\alpha]^{21}_D$ −16° (*c* 0.11, MeOH); CD (*c* 8 × 10^{-4} M, MeOH), Δ, 0 (332), +0.19 (266), +1.32 (214); IR (film) ν_{max} 2927, 2855, 2359, 1718, 1456, 1125 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; FAB-CID MS/MS *m/z* 367 $[M + 2Na - H]^+$ (100), 345 $[M + Na]^+$ (32), 325 (0.3), 311 (0.2), 297 (0.2), 283 (0.3), 255 (0.3), 242 (0.2), 227 (0.3), 213 (0.9), 169 (0.1), 117 (0.5), 89 (0.2), 46 (0.1); HRFABMS *m/z* 367.2227 ($[M + 2Na - H]^+$, calcd for $C_{20}H_{33}O_3Na_2$, 367.2225).

(*Z*)-Stellettic acid B (2a): pale yellow oil; $[\alpha]^{21}_D$ −18° (*c* 0.14, MeOH); CD (*c* 3 × 10^{-4} M, MeOH), Δ, 0 (390), +0.53 (266), +1.17 (228), +0.84 (212); IR (film) ν_{max} 3446, 2925, 2854, 2359, 1716, 1456, 1206 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; FAB-CID MS/MS *m/z* 365 $[M + 2Na - H]^+$ (100), 343 $[M + Na]^+$ (62), 323 (0.5), 309 (0.3), 295 (0.3), 281 (0.3), 253 (0.3), 239 (0.8), 225 (0.7), 211 (1.5), 165 (0.2), 117 (0.7), 89 (0.3), 46 (0.1); HRFABMS *m/z* 365.2070 ($[M + 2Na - H]^+$, calcd for $C_{20}H_{31}O_3Na_2$, 365.2069).

Compound 2b: pale yellow oil; $[\alpha]^{21}_D$ −8° (*c* 0.30, MeOH); CD (*c* 4 × 10^{-4} M, MeOH), Δ, 0 (370), +0.40 (268), +1.26 (223); IR (film) ν_{max} 3393, 2925, 2359, 1594, 1456, 1104 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; FAB-CID MS/MS *m/z* 365 $[M + Na]^+$ (100), 343 $[M + H]^+$ (10), 323 (0.4), 309 (0.2), 295 (0.3), 281 (0.3), 253 (0.3), 239 (0.9), 225 (0.6), 147 (0.4), 134 (0.4), 117 (0.7), 89 (0.3), 46 (0.2).

(*E*)-Stellettic acid B (3): pale yellow oil; $[\alpha]^{21}_D$ −6° (*c* 0.09, MeOH); CD (*c* 7 × 10^{-4} M, MeOH), Δ, 0 (322), +0.16 (266), +0.35 (222); IR (film) ν_{max} 2925, 2853, 2359, 1716, 1456, 1123 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; FAB-CID MS/MS *m/z* 365 $[M + 2Na - H]^+$ (100), 343 $[M + Na]^+$ (62), 323 (0.5), 309 (0.3), 295 (0.3), 281 (0.3), 253 (0.3), 239 (0.8), 225 (0.7), 211 (1.5), 165 (0.2), 117 (0.7), 89 (0.3), 46 (0.1).

(*Z*)-Stellettic acid C (4a): pale yellow oil; $[\alpha]^{21}_D$ +6° (*c* 0.09, MeOH); IR (film) ν_{max} 2926, 2855, 2359, 1710, 1456, 909 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; FAB-CID MS/MS *m/z* 335 $[M + 2Na - H]^+$ (100), 313 $[M + Na]^+$ (10), 293 (0.5), 279 (0.2), 265 (0.3), 251 (0.3), 223 (0.3), 209 (1.1), 196 (0.8), 117 (0.9), 104 (1.1), 89 (0.9), 59 (0.1), 46 (0.2); HRFABMS *m/z* 335.1966 ($[M + 2Na - H]^+$, calcd for $C_{19}H_{29}O_2Na_2$, 335.1963).

Compound 4b: pale yellow oil; $[\alpha]^{21}_D$ +2° (*c* 0.07, MeOH); CD (*c* 8 × 10^{-4} M, MeOH), Δ, 0 (331), +1.60 (267), +1.77 (244), +2.01 (223), +2.57 (206); IR (film) ν_{max} 2926, 2855, 2359, 1713, 1456, 1242 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; FAB-CID MS/MS *m/z* 335 $[M + 2Na - H]^+$ (100), 313 $[M + Na]^+$ (10), 293 (0.4), 279 (0.2), 265 (0.3), 251 (0.3), 223 (0.3), 209 (0.9), 195 (0.6), 117 (0.4), 104 (0.4), 87 (0.7), 59 (0.3), 46 (0.2); HRFABMS *m/z* 335.1961 ($[M + 2Na - H]^+$, calcd for $C_{19}H_{29}O_2Na_2$, 335.1963).

Preparation of the Methyl Ester of Stellettic Acid A (1a). Stellettic acid A (**1**, 1.6 mg) in Et_2O was treated with CH_2N_2 gas for 45 min, and the solvent was evaporated.[23] CH_2N_2 was generated by reaction of 1-methyl-3-nitro-1-nitrosoguanidine with 5 mol/L NaOH in a microscale generator. The reaction mixture was purified by reversed-phase HPLC (YMC ODS-H80, 250 × 20 mm i.d., 4 μm, 80 Å) eluting with 28% H_2O−MeOH to give methyl ester (**1a**, 1.1 mg).

1a: pale yellow oil; $[\alpha]^{21}_D$ −12° (*c* 0.11, MeOH); CD (*c* 3 × 10^{-4} M, MeOH), Δ, 0 (314), +0.34 (264), +1.51 (214); ^1H NMR (CD_3OD) δ 5.81 (1H, ddt, *J* = 17.0, 11.5, 7.0 Hz, H-17), 5.01 (1H, dd, *J* = 17.0, 2.0 Hz, H-18a), 4.93 (1H, dd, *J* = 11.5, 2.0 Hz, H-18b), 3.93 (1H, dd, *J* = 8.5, 4.5 Hz, H-2), 3.77 (3H, s, $COOCH_3$), 3.42 (3H, s, 2-OCH_3), 2.31 (2H, m, H-4), 2.15 (2H, tt, *J* = 7.0, 2.0 Hz, H-7), 2.04 (2H, q, *J* = 7.0 Hz, H-16), 1.97 (1H, m, H-3a), 1.91 (1H, m, H-3b), 1.48 (2H, q, *J* = 7.0 Hz, H-8), 1.37 (1H, m, H-12), 1.26−1.40 (8H, m, H-9, H-10, H-11a, H-13a, H-14, H-15), 1.10 (2H, m, H-11b, H-13b), 0.84 (3H, d, *J* = 6.5 Hz, H-19).

Preparation of (*R*)- and (*S*)-Phenylglycine Methyl Ester (PGME) Amides of 1. Compound **1** (0.7 mg) and (*S*)-PGME (1.2 mg) were dissolved in 0.5 mL of DMF and cooled to 0 °C. A 3.2 mg sample of PyBOP, 0.8 mg of HOBT, and 2 μL of *N*-methylmorpholine were added in order, and the mixture was stirred at room temperature for 1.5 h. Benzene (0.5 mL) and EtOAc (1 mL) were then added, the mixture was washed with aqueous 5% HCl, saturated $NaHCO_3$ solution, and brine, and the solvent was removed by rotary evaporation.[20] The residue was purified on Si gel in a Pasteur pipet eluting with *n*-hexane−EtOAc (2:1) and characterized by ^1H NMR. The (*R*)-PGME amide of **1** was prepared from (*R*)-PGME in a similar fashion.

(*S*)-PGME Amide of 1: pale yellow oil; ^1H NMR (CD_3OD) δ 7.374 (5H, m), 5.825 (1H, ddt, *J* = 17.0, 11.5, 7.0 Hz, H-17), 5.216 (1H, s), 5.013 (1H, dd, *J* = 17.0, 2.0 Hz, H-18a), 4.935 (1H, dd, *J* = 11.5, 2.0 Hz, H-18b), 3.861 (1H, dd, *J* = 8.5, 4.5 Hz, H-2), 3,726 (3H, s), 3.434 (3H, s, OCH_3), 2.435 (2H, m, H-4), 2.145 (2H, tt, *J* = 7.0, 2.0 Hz, H-7), 2.045 (2H, q, *J* = 7.0 Hz, H-16), 1.883 (1H, m, H-3a), 1.816 (1H, m, H-3b), 1.487 (2H, q, *J* = 7.0 Hz, H-8), 1.373 (1H, m, H-12), 1.26−1.40 (8H, m, H-9, H-10, H-11a, H-13a, H-14, H-15), 1.10 (2H, m, H-11b, H-13b), 0.84 (3H, d, *J* = 6.5 Hz, H-19).

(*R*)-PGME Amide of 1: pale yellow oil; ^1H NMR (CD_3OD) δ 7.374 (5H, m), 5.825 (1H, ddt, *J* = 17.0, 11.5, 7.0 Hz, H-17), 5.216 (1H, s), 5.013 (1H, dd, *J* = 17.0, 2.0 Hz, H-18a), 4.935 (1H, dd, *J* = 11.5, 2.0 Hz, H-18b), 3.855 (1H, dd, *J* = 8.5, 4.5 Hz, H-2), 3,726 (3H, s), 3.377 (3H, s, OCH_3), 2.452 (2H, m, H-4), 2.145 (2H, tt, *J* = 7.0, 2.0 Hz, H-7), 2.045 (2H, q, *J* = 7.0 Hz, H-16), 1.915 (1H, m, H-3a), 1.884 (1H, m, H-3b), 1.486 (2H, q, *J* = 7.0 Hz, H-8), 1.37 (1H, m, H-12), 1.26−1.40 (8H, m, H-9, H-10, H-11a, H-13a, H-14, H-15), 1.10 (2H, m, H-11b, H-13b), 0.84 (3H, d, *J* = 6.5 Hz, H-19).

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References and Notes

(1) Li, H.; Matsunaga, S.; Fusetani, N. *Experientia* **1994**, *50*, 771−773.
(2) Yan, S. J.; Su, J. Y.; Zhang, G. W.; Wang, Y. H.; Li, H. *Zhongshan Daxue Xubao, Ziran Kexueban* **2001**, *40*, 54−57.
(3) Guerriero, A.; Debitus, C.; Pietra, F. *Helv. Chim. Acta* **1991**, *74*, 487−494.
(4) Oku, N.; Matsunaga, S.; Wada, S. I.; Watabe, S.; Fusetani, N. *J. Nat. Prod.* **2000**, *63*, 205−209.
(5) McCormick, J. L.; McKee, T. C.; Cardellina, J. H.; Leid, M.; Boyd, M. R. *J. Nat. Prod.* **1996**, *59*, 1047−1050
(6) Ryu, G.; Matsunaga, S.; Fusetani, N. *J. Nat. Prod.* **1996**, *59*, 512−514.
(7) Tsukamoto, S.; Yamashita, T.; Matsunage, S.; Fusetani, N. *J. Org. Chem.* **1999**, *64*, 3794−3795.
(8) Nazawa, D.; Takikawa, H.; Mori, K. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1481−1483.
(9) Matsunaga, S.; Yamashita, T.; Tsukamoto, S.; Fusetani, N. *J. Nat. Prod.* **1999**, *62*, 1202−1204.
(10) Bergquist, P.; Lawson, M. P.; Lavis, A.; Cambie, R. C. *Biochem. Syst. Ecol.* **1984**, *12*, 63−84.
(11) Vesonder, R. F.; Stodola, F. H.; Rohwedder, W. K.; Scott, D. B. *Can. J. Chem.* **1970**, *48*, 1985−1986.
(12) Ayanoglu, E.; Kornprobst, J. M.; Aboud-Bichara, A.; Djerassi, C. *Tetrahedron Lett.* **1983**, *24*, 1111−1114.
(13) Ayanoglu, E.; Popov, S.; Kornprobst. J. M.; Aboud-Bichara, A.; Djerassi, C. *Lipids* **1983**, *18*, 830−836.
(14) Carballeira, N. M.; Negron, V.; Reyes, E. D. *Tetrahedron* **1992**, *48*, 1053−1058.
(15) Carballeira, N. M.; Alicea, J. *Lipids* **2002**, *37*, 305−308.
(16) Carballeira, N. M.; Colon, R.; Emiliano, A. *J. Nat. Prod.* **1998**, *61*, 675−676.
(17) Carballeira, N. M.; Pagan, M. *J. Nat. Prod.* **2001**, *64*, 620−623.
(18) Carballeira, N. M.; Sepulveda, J. A. *Lipids* **1992**, *27*, 72−74.
(19) Carballeira, N. M.; Shalabi, F.; Maldonado, M. E. *Lipids* **1990**, *25*, 235−237.
(20) (a) Nagai, Y.; Kusumi, T. *Tetrahedron Lett.* **1995**, *36*, 1853−1856. (b) Yabuuchi, T.; Kusumi, T. *J. Org. Chem.* **2000**, *65*, 397−404.
(21) Friebolin, H. *Basic One- and Two- Dimensional NMR Spectroscopy*; VCH Verlagesellschaft: Weinheim, 1998; p 68.
(22) It appears that these acetylenic acids constitute only part of the cytotoxic components of this sponge. More potent components are under study and will be reported elsewhere.
(23) Blank, T. H. *Aldrichim. Acta* **1983**, *16*, 3−10.

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