New Acetylenic Acids from a Sponge of the Genus *Stelletta*

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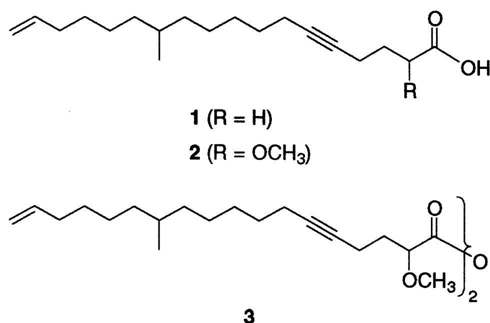
Three new acetylenic metabolites (**1**−**3**) were isolated from the sponge *Stelletta* sp. collected from Gagu-Do, Korea. On the basis of the results of combined spectroscopic analyses, the structures of these compounds were determined to be an acetylenic acid, its 2-methoxy analogue, and corresponding anhydride, respectively. The new compounds exhibited weak cytotoxicity against a human leukemia cell-line (K562).

During the course of our search for novel secondary metabolites from marine organisms, we encountered an undescribed sponge of the genus *Stelletta* (Schmidt, 1862; order Astrophorida, family Ancornidae) off the shore of Gagu-Do (Island), Korea. Although a crude extract of this specimen exhibited weak cytotoxicity (LC_{50} 490 μg/mL) against the human leukemia cell-line K562, a great abundance of this animal in several areas of Korean water prompted us to investigate chemically. Directed by the results of ^{1}H NMR analysis, the crude extracts were separated employing solvent partitioning followed by various chromatographic methods including C_{18} vacuum flash chromatography, silica column chromatography, and C_{18} HPLC to afford pure secondary metabolites. We describe herein the structure elucidation of three modified fatty acids. These compounds contained an acetylenic moiety in the middle of the molecule and a terminal double bond as common structural features.



Compound **1** was isolated as a colorless oil which analyzed for $C_{19}H_{32}O_2$ on the basis of combined HRFABMS and ^{13}C NMR spectrometry. The fatty acid nature of this compound was apparent from the characteristic features in the ^{13}C NMR data: a carbonyl carbon at δ 177.9 (C), several methylene carbons, and lack of quaternary carbons in the upfield regions. Also present were carbon signals of a terminal double bond at δ 139.2 (CH) and 114.1 (CH_2). Corresponding proton signals were observed at δ 5.81 (1H, ddt, J = 17.1, 10.3, 6.8 Hz), 4.99 (1H, ddt, J = 17.1, 2.0, 1.5 Hz), and 4.93 (1H, ddt, J = 10.3, 2.0, 1.5 Hz) in the ^{1}H NMR spectra. In addition, the ^{13}C NMR data showed two quaternary carbons at δ 81.5 (C) and 78.5 (C). Deduced from the molecular formula, four degree of unsaturation revealed that these carbon signals were derived from an acetylenic moiety.

Table 1. ^{1}H and ^{13}C NMR Assignments for **1** in $CDCl_3$.

position	δ_H	δ_C		HMBC
1		177.9	s	
2	2.49, t (7.3)	32.5	t	C-1, C-3, C-4
3	1.81, quint (7.3)	24.0	t	C-1, C-2, C-4, C-5
4	2.24, tt (7.3, 2.2)	18.2	t	C-2, C-3, C-5, C-6
5		78.5	s	
6		81.5	s	
7	2.13, tt (7.3, 2.2)	18.7	t	C-5, C-6, C-8
8	1.47, quint (7.3)	29.1	t	C-6, C-7, C-9, C-10
9	1.34, m	29.21[a]	t	
10	1.28, m	26.57[b]	t	
11	1.08, dt (7.8, 6.8)	36.86[c]	t	C-9, C-10, C-12, C-13, C-19
12	1.36, m	32.7	d	
13	1.27, m	36.91[c]	t	
14	1.31, m	26.54[b]	t	
15	1.25, m	29.27[a]	t	
16	2.04, dtt (6.8, 7.3, 1.5)	33.8	t	C-14, C-15, C-17, C-18
17	5.81, ddt (17.1, 10.3, 6.8)	139.2	d	C-15, C-16
18	4.99, ddt (17.1, 2.0, 1.5) 4.93, ddt (10.3, 2.0, 1.5)	114.1	t	C-16, C-17
19	0.84, d (6.4)	19.6	q	C-11, C-12, C-13

[a−c] Interchangeable signals.

The structure of compound **1** was determined by 2D NMR experiments. A combination of the ^{1}H COSY, TOCSY, and *g*HSQC data revealed a proton spin system consisting of signals of three methylene protons at δ 2.49, 2.24, and 1.81 (Table 1). The same experiments also revealed that the remaining proton signals including the olefinic ones were sequentially connected to each other. The small coupling constant (J = 2.2 Hz) between the methylene protons at δ 2.24 and 2.13 of two spin systems suggested that these were connected to each other via an acetylenic moiety. This interpretation was confirmed by the *g*HMBC experiment in which long-range correlations were observed between the methylene protons and neighboring carbons. The location of a methyl substitutent was assigned at C-12 on the basis of tandem MS analysis (Figure 1). A literature survey showed that structurally related modified fatty acids have been isolated from a number of sponges and a hard coral.[1−6] However, compound **1** differs from other metabolites in both chain length and functional groups.

Compound **2** was assigned the molecular formula $C_{20}H_{34}O_3$ by HRFABMS and ^{13}C NMR spectrometry. The

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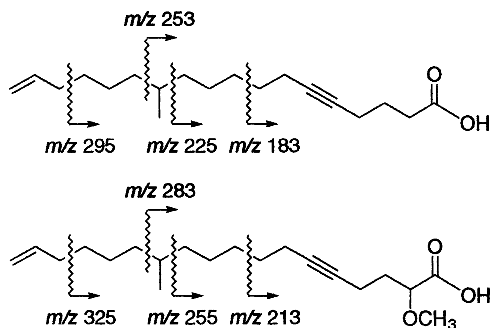

Figure 1. Tandem MS fragments of **1** and **2**.

^{13}C NMR data of this compound were highly compatible with those of **1** with the replacement of the signal of an upfield methylene with those of a methine and methyl carbon at δ 78.6 (CH) and 58.7 (CH_3) as the most noticeable difference. Corresponding changes were also observed in the ^1H NMR spectra, in which signals of additional protons appeared at δ 3.98 (1H, dd, $J = 8.3$, 3.9 Hz) and 3.49 (3H, s), implying that **2** was indeed a methoxy analogue of **1**. The *g*HMBC correlations of the former proton with the carboxylic carbon at δ 174.7 and methoxy carbon at δ 58.8 allowed the placement of the methoxy group at C-2, which was consistent with the results of the ^1H COSY and TOCSY analyses. The location of a methyl substituent (δ_C 19.7, δ_H 0.84) was secured at C-12, identical to **1**, by tandem MS analysis, in which ion clusters heavier than those of **1** by m/z 30 (−H, $+OCH_3$) were obtained.

The closely related metabolite **3** was isolated as a colorless oil. The molecular formula of this compound was analyzed for $C_{40}H_{66}O_5$, almost twice that from **2**, on the basis of HRFABMS analysis. The mass spectra also showed a prominent mass cluster at m/z 367.2221 ($C_{20}H_{33}O_3Na_2$, 367.2225, Δ −0.4 mmu), which was identical to that obtained for **2**. The NMR data including those from 2D experiments of **3** were very similar to those of **2**. However, careful examination of the ^{13}C NMR spectra revealed that the signal of the carboxylic carbon was shifted significantly: δ 174.7 for **2**, δ 168.6 for **3**. In the ^1H NMR spectra, the H-2 oxymethine proton was also shifted from δ 3.98 to 4.03 in **3**. Furthermore, **3** was converted to **2** with prolonged exposure in room temperature. These observations, coupled with the mass data, led us to conclude that **3** was a symmetric dimer of **2**. Crucial evidence was provided by the IR data, in which the carboxylic stretching band observed at 1725 cm^{-1} of **2** was replaced by those of an acid anhydride at 1745 and 1830 cm^{-1}, while the broad band of an acid at 3100−2600 cm^{-1} of **2** disappeared in **3**.[7] This interpretation was further confirmed by chemical transformation. Treatment with acetic anhydride readily converted **2** to **3**. Thus, the structure of **3** was defined as the dimeric anhydride derivative of **2**. To the best of our knowledge, this is the first example of a sponge metabolite possessing an acid anhydride functionality.

Sponge-derived acetylenic acids exhibit diverse bioactivities.[1] As a recent example, a C_{14} acetylenic acid from *Oceanapia* sp. showed significant antimicrobial activity against various bacteria and fungi.[6] In our measurement, these compounds exhibited no significant antimicrobial activity and displayed only weak cytotoxicity against the human leukemia cell-line K562 with LC_{50} values of 43.5, 51.3, and 62.5 μg/mL for **1**−**3**, respectively.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO digital polarimeter using a 5 cm cell. IR spectra were recorded on a Mattson Galaxy spectrophotometer. NMR spectra were recorded in $CDCl_3$ containing Me_4Si as internal standard on a Varian Unity 500 spectrometer. Proton and carbon NMR spectra were measured at 500 and 125 MHz, respectively. Mass spectral data were provided by the Korea Basic Science Institute, Taejeon, Korea. All solvents used were spectral grade or were distilled from glass prior to use.

Animal Material. *Stelletta* sp. (sample number 00SH-3) was collected by scuba at 15−20 m depth off the shore of Gagu-Do (Island), southwestern Korea in July 2000. The specimens were massive (147 mm × 102 mm × 43 mm) and had only one large osculum (15 mm in diameter). The color in life was dark gray outside and beige inside. Texture was very hard like stone, and the surface was very rough with protruding spicules. This sponge had oxea (1750−2150 μm × 38−60 μm), orthotriane (750−1150 μm × 24−57 μm), oxyaster (16−30 μm), and strongylaster (10−12.5 μm). A voucher specimen (registry No. Spo. 36) is currently on deposit at the Natural History Museum, Hannam University, Korea, under the curatorship of C.J.S.

Extraction and Isolation. The fresh collection was immediately frozen and kept at −25 °C until chemically investigated. The specimens were lyophilized (dry wt 1.2 kg), macerated, and repeatedly extracted with MeOH (2 L × 2) and CH_2Cl_2 (2 L × 2). The combined crude extract (98 g) was partitioned between *n*-BuOH and H_2O. The butanol layer was evaporated *in vacuo*, and the residue (25.5 g) was repartitioned between 15% aqueous MeOH and *n*-hexane. The aqueous MeOH layer (10.1 g) was separated by C_{18} reversed-phase vacuum flash chromatography using sequential mixtures of MeOH and H_2O as eluents (elution order: 50%, 40%, 30%, 20%, 10% aqueous MeOH, 100% MeOH) and finally acetone. The fraction (1.09 g) eluted with 100% MeOH was subjected to Si gel column chromatography using stepped gradient mixtures of CH_2Cl_2 and MeOH as eluents. The fraction (380 mg) eluted with 10% CH_2Cl_2 in MeOH was separated by C_{18} reversed-phase HPLC (YMC ODS-A column, 10% aqueous MeOH) to yield 9.0 mg of pure **1** as a colorless gum. The fraction (80 mg) eluted with 30% CH_2Cl_2 in MeOH was separated by reversed-phase HPLC (15% aqueous MeOH) to afford 7.0 and 4.8 mg of **2** and **3**, respectively.

12-Methyloctadeca-17-ene-5-ynoic acid (1): colorless gum; $[\alpha]^{25}_D$ −1.8° (*c* 0.05, MeOH); IR (KBr) ν_{max} 3100−2600 (br), 2925, 2865, 1710, 1640, 1435, 1240 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRFABMS m/z 337.2113 [M − H + 2Na]$^+$ (calcd for $C_{19}H_{31}O_2Na$, 337.2119, Δ −0.6 mmu).

2-Methoxy-12-methyloctadeca-17-ene-5-ynoic acid (2): colorless gum; $[\alpha]^{25}_D$ −4.6° (*c* 0.04, MeOH); IR (KBr) ν_{max} 3100−2600 (br), 2930, 2855, 1725, 1640, 1460, 1205, 1125 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.81 (1H, ddt, $J = 16.9$, 10.3, 6.8 Hz, H-17), 4.99 (1H, ddt, $J = 16.9$, 2.0, 1.5 Hz, H-18), 4.93 (1H, ddt, $J = 10.3$, 2.0, 1.3 Hz, H-18), 3.98 (1H, dd, $J = 8.3$, 3.9 Hz, H-2), 3.49 (3H, s, OMe), 2.33 (2H, tt, $J = 7.3$, 2.3 Hz, H-4), 2.14 (2H, tt, $J = 7.3$, 2.3 Hz, H-7), 2.04 (2H, br dt, $J = 6.8$, 7.3 Hz, H-16), 1.99 (1H, ddt, $J = 13.7$, 3.9, 7.3 Hz, H-3), 1.90 (1H, ddt, $J = 13.7$, 8.3, 7.3 Hz, H-3), 1.48 (2H, quint, $J = 7.3$ Hz, H-8), 1.36 (1H, m, H-12), 1.35 (2H, m, H-9), 1.32 (2H, m, H-14), 1.28 (2H, m, H-10), 1.27 (2H, m, H-13), 1.26 (2H, m, H-15), 1.08 (2H, dt, $J = 7.8$, 6.8 Hz, H-11), 0.84 (3H, d, $J = 6.4$ Hz, H-19); ^{13}C NMR ($CDCl_3$) δ 174.7 (C, C-1), 139.2 (CH, C-17), 114.1 (CH_2, C-18), 81.7 (C, C-6), 78.6 (CH, C-2), 78.0 (C, C-5), 58.8 (CH_3, OMe), 36.92 (CH_2, C-11/C-13), 36.86 (CH_2, C-11/C-13), 33.8 (CH_2, C-16), 32.7 (CH, C-12), 31.7 (CH_2, C-3), 29.26 (CH_2, C-9/C-15), 29.22 (CH_2, C-9/C-15), 29.1 (CH_2, C-8), 26.57 (CH_2, C-10/C-14), 26.54 (CH_2, C-10/C-14), 19.7 (CH_3, C-19), 18.7 (CH_2, C-7), 14.7 (CH_2, C-4); HRFABMS m/z 367.2227 [M − H + 2Na]$^+$ (calcd for $C_{20}H_{33}O_3Na_2$, 367.2225, Δ +0.1 mmu).

2-Methoxy-12-methyloctadeca-17-ene-5-ynoic anhydride (3): colorless gum; $[\alpha]^{25}_D$ −2.7° (*c* 0.04, MeOH); IR (KBr) ν_{max} 2930, 2855, 1830, 1745, 1640, 1460, 1125 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.81 (2H, ddt, $J = 16.9$, 10.3, 6.8 Hz, H-17, H-17′), 4.99 (2H, ddt, $J = 16.9$, 2.0, 1.5 Hz, H-18, H-18′), 4.93 (2H, ddt, $J = 10.3$, 2.0, 1.5 Hz, H-18, H-18′), 4.03 (2H, dd, $J = 8.8$,

3.9 Hz, H-2, H-2′), 3.49 (6H, s, OMe), 2.35 (4H, tt, $J = 7.3$, 2.3 Hz, H-4, H-4′), 2.14 (4H, tt, $J = 7.3$, 2.3 Hz, H-7, H-7′), 2.04 (4H, br dt, $J = 6.8$, 7.3 Hz, H-16, H-16′), 1.95 (4H, m, H-3, H-3′), 1.47 (4H, quint, $J = 7.3$ Hz, H-8, H-8′), 1.36 (2H, m, H-12, H-12′), 1.35 (4H, m, H-9, H-9′), 1.32 (4H, m, H-15, H-15′), 1.28 (4H, m, H-10, H-10′), 1.27 (4H, m, H-13, H-13′), 1.26 (4H, m, H-14, H-14′), 1.08 (4H, dt, $J = 7.8$, 6.8 Hz, H-11, H-11′), 0.84 (6H, d, $J = 6.4$ Hz, H-19, H-19′); ^{13}C NMR ($CDCl_3$) δ 168.6 (C, C-1), 139.2 (CH, C-17, C-17′), 114.1 (CH_2, C-18, C-18′), 81.9 (C, C-6, C-6′), 79.1 (CH, C-2, C-2′), 77.8 (C, C-5, C-5′), 58.8 (CH_3, OMe), 36.94 (CH_2, (C-11, C-11′)/(C-13, C-13′)), 36.86 (CH_2, (C-11, C-11′)/(C-13, C-13′)), 33.8 (CH_2, C-16, C-16′), 32.7 (CH, C-12, C-12′), 31.7 (CH_2, C-3, C-3′), 29.3 ($CH_2 \times 2$, C-9, C-9′, C-15, C-15′), 29.1 (CH_2, C-8, C-8′), 26.59 (CH_2, (C-10, C-10′)/(C-14, C-14′)), 26.55 (CH_2, (C-10, C-10′)/(C-14, C-14′)), 19.6 (CH_3, C-19, C-19′), 18.7 (CH_2, C-7, C-7′), 14.9 (CH_2, C-4, C-4′); HRFABMS *m/z* 649.4808 $[M + Na]^+$ (calcd for $C_{40}H_{66}O_5$-Na, 649.4808, Δ 0 mmu).

Anhydride Derivatization of 2. Acetic anhydride (0.8 mL) was added to neat **2** (2.1 mg) in a vial (2 mL). After stirring the mixture at 50 °C for 2 h, the excess acetic anhydride was removed under vacuum. The light brown residue was dissolved with CH_2Cl_2 and filtered through a silica Cepak column. The ^{1}H and ^{13}C NMR data of the product (1.7 mg) were identical to those of natural **3**.

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