

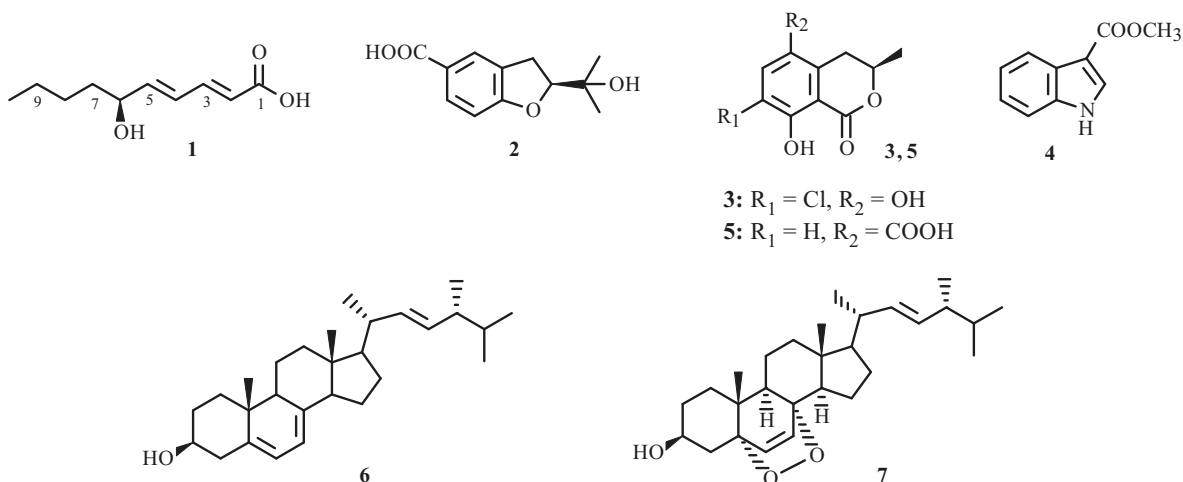
## NEW FATTY ACID FROM A GORGONIAN-DERIVED *Xylaria* sp. FUNGUS

Da-Wei Sun, Fei Cao, Min Liu, Fei-Fei Guan,  
and Chang-Yun Wang\*

A new fatty acid, (2E,4E,6S)-6-hydroxydeca-2,4-dienoic acid (**1**), along with six known compounds (**2–7**), were isolated from the gorgonian-derived fungus *Xylaria* sp. C-2, collected from the South China Sea. The structure of **1** was elucidated by 1D, 2D NMR, and MS spectrometry. The absolute configuration of **1** was determined by using the modified Mosher's method. All of the isolated compounds were evaluated for their cytotoxic and antibacterial activities.

**Keywords:** gorgonian-derived fungus, *Xylaria* sp., C<sub>10</sub> unsaturated hydroxy fatty acid, absolute configuration.

Fungi of the genus *Xylaria*, which is the largest genus of the family Xylariaceae, have been shown to be a potential source of novel secondary metabolites relevant for drug discovery. In recent years, secondary metabolites have been reported from marine-derived fungus *Xylaria* sp., such as cytochalasins with cytotoxic activity [1], sesquiterpenes with antifouling activity [2], and polyketides with enzyme-inhibitory activity [3]. In the course of our search for new bioactive secondary metabolites from gorgonian-derived fungi in the South China Sea [4–6], the chemical investigation of a gorgonian-derived fungus *Xylaria* sp. C-2 led to the isolation of a new fatty acid (**1**), together with six known compounds (**2–7**). Herein we report the isolation, structure elucidation, and bioactivity of these metabolites.

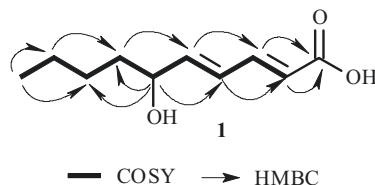


Compound **1** was obtained as a light yellowish, amorphous solid. Its molecular formula was determined as C<sub>10</sub>H<sub>16</sub>O<sub>3</sub> by extensive analysis of its 1D and 2D NMR data (Table 1) and HR-ESI-MS, which required three degrees of unsaturation. The IR absorptions at 3420 and 1697 cm<sup>-1</sup> indicated the presence of hydroxy (OH) and ester carbonyl (C=O) groups, respectively. The <sup>13</sup>C and DEPT NMR spectra displayed one methyl (δ 14.3), three methylenes (δ 23.3–37.7), five methines, including four olefinic carbons (δ 121.4–147.7) and one oxygenated methylene (δ 71.6), and an ester carbonyl carbon (δ 167.9) (Table 1).

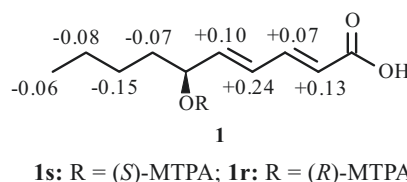
Key Laboratory of Marine Drugs, The Ministry of Education of China, School of Medicine and Pharmacy, Ocean University of China, 266003, Qingdao, P. R. China, fax: 86 532 82031536, e-mail: changyun@ouc.edu.cn. Published in *Khimiya Prirodnikh Soedinenii*, No. 2, March–April, 2017, pp. 195–198. Original article submitted October 23, 2015.

TABLE 1.  $^1\text{H}$  (600 MHz) and  $^{13}\text{C}$  (150 MHz) NMR Data of Compound **1** (acetone- $d_6$ ,  $\delta$ , ppm, J/Hz)

C atom	$\delta_{\text{C}}$	$\delta_{\text{H}}$	C atom	$\delta_{\text{C}}$	$\delta_{\text{H}}$
1	167.9	–	6	71.6	4.20 (1H, dt, $J = 12.3, 5.5$ )
2	121.4	5.87 (1H, d, $J = 15.3$ )	7	37.7	1.54 (1H, m); 1.51 (1H, m)
3	145.5	7.27 (1H, dd, $J = 15.3, 11.1$ )	8	28.3	1.37 (1H, m); 1.35 (1H, m)
4	127.2	6.44 (1H, dd, $J = 15.3, 11.1$ )	9	23.3	1.34 (1H, m); 1.30 (1H, m)
5	147.7	6.23 (1H, dd, $J = 15.3, 5.5$ )	10	14.3	0.89 (3H, t, $J = 7.2$ )

Fig. 1. The  $^1\text{H}$ - $^1\text{H}$  COSY and selective HMBC correlations of compound **1**.

The  $^1\text{H}$ - $^1\text{H}$  COSY spectrum of **1** revealed one spin system starting from H-2 ( $\delta$  5.87) and ending at H<sub>3</sub>-10 ( $\delta$  0.89) (Fig. 1). An  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ -unsaturated carbonyl moiety was established by HMBC as H-2 ( $\delta$  5.87) and H-4 ( $\delta$  6.44), which show long-range correlations with C-1 ( $\delta$  167.9) and C-2 ( $\delta$  121.4), respectively (Fig. 1). The HMBC correlations from H-6 ( $\delta$  4.20) to C-4 ( $\delta$  127.2) and C-8 ( $\delta$  28.3) indicated that the hydroxy was located at C-6. The *E* geometries of the C-2/C-3 and C-4/C-5 double bonds were assigned on the basis of the larger coupling constants between H-2 and H-3 ( $J = 15.3$  Hz), and between H-4 and H-5 ( $J = 15.3$  Hz). The UV spectrum of **1** exhibited an absorption band at  $\lambda_{\text{max}}$  255 nm, indicating a conjugated diene in **1**. The absolute configuration of C-6 in **1** was established using the modified Mosher's method; each mono-(*S*)- and (*R*)-MTPA ester was separately prepared and subjected to  $^1\text{H}$  NMR data analysis. The absolute configuration at C-6 was readily assigned as *S* based on the  $\Delta\delta_{\text{H}(\text{S-R})}$  values (Fig. 2) following the MTPA rules [7]. Thus, the structure of **1** was conclusively determined to be (2*E*,4*E*,6*S*)-6-hydroxydeca-2,4-dienoic acid.

Fig. 2. Values of  $\Delta\delta_{\text{H}(\text{S-R})}$  of the MTPA esters of **1**.

The structures of the known compounds **2–7** were elucidated by comparison of their  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopic data with those reported in the literature as anodendroic acid (**2**) [8], 7-chloro-5-hydroxymellein (**3**) [9], methyl indole-3-carboxylate (**4**) [10], 5-carboxymellein (**5**) [11], ergosta-5,7,22-trien-3 $\beta$ -ol (**6**) [12], and 5 $\alpha$ ,8 $\alpha$ -epidioxy-ergosta-6,22*E*-dien-3 $\beta$ -ol (**7**) [13].

Although compound **1** is a simple fatty acid, the structure of a C<sub>10</sub> unsaturated fatty acid with one hydroxyl group is uncommon in nature. A literature survey revealed that there has been only one report on (2*Z*,4*E*)-C<sub>10</sub> unsaturated hydroxy fatty acid isolated from the sea fan (gorgonian)-derived fungus *Curvularia* sp. [14]. In the present study, **1** was the first example of (2*E*,4*E*)-C<sub>10</sub> unsaturated hydroxy fatty acid from the gorgonian-derived fungus. Compounds **2–4** were isolated from the genus *Xylaria* for the first time.

The antibacterial activity was evaluated for all of the isolated compounds against a panel of pathogenic bacteria *in vitro*. Only **7** showed antibacterial activity against *Escherichia coli*, *Pseudomonas putida*, and *Kocuria rhizophila* with MIC values of 3.13, 1.56, and 6.25  $\mu\text{M}$ , respectively. Ciprofloxacin was used as standard positive control and showed antibacterial activity with MIC values of 0.39, 0.78, and 1.56  $\mu\text{M}$ , respectively. According to the results of Mondol et al. [15], the (2*E*,4*E*,9*R*)-C<sub>10</sub> unsaturated fatty acid iodomyacin D bearing a 9-OH showed antimicrobial activities against *Bacillus subtilis* and *Escherichia coli*. However, in our research, **1** which bears 6-OH displayed no antimicrobial activity. These results revealed that the substituted positions of the hydroxy groups or the absolute configurations might influence the antimicrobial activity.

The cytotoxicities were also evaluated for **1–7** against human tumor cell lines (HeLa, Hep-2, RD, and A549). Only **6** exhibited weak cytotoxicity towards the HeLa, Hep-2, and RD cell lines with IC<sub>50</sub> values of 20.1, 37.0, and 40.2  $\mu\text{M}$ , respectively.

## EXPERIMENTAL

**General.** Optical rotations were measured on a JASCO P-1020 digital polarimeter (Tokyo, Japan) at room temperature. UV spectra were recorded on a UV-2501PC spectrophotometer (Kyoto, Japan). IR spectra were determined on a Nicolet Nexus 470 spectrometer (Thermo Nicolet Corporation, USA).  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were acquired on an Agilent DD2 NMR spectrometer (500 MHz, California, USA) and a JEOL JNM-ECP NMR spectrometer (600 MHz, Tokyo, Japan). ESI-MS and HR-ESI-MS were recorded on a Q-TOF Ultima Global GAA076 LC mass spectrometer (Massachusetts, USA) in  $m/z$ . HPLC separations were performed using a Hitachi L-2000 prep-HPLC system coupled with a Hitachi L-2455 photodiode array detector (Tokyo, Japan). A Kromasil  $\text{C}_{18}$  preparative HPLC column (250  $\times$  10 mm, 5  $\mu\text{m}$ ) was used. Silica gel (200–300 mesh; Qingdao Marine Chemical Group Co., Qingdao, People's Republic of China), octadecylsilyl (ODS) silica gel (45–60 mm; Merck KGaA, Darmstadt, Germany), and Sephadex LH-20 (Amersham Biosciences Inc., Piscataway, NJ, USA) were used for column chromatography. Precoated silica gel GF254 plates (Yantai Zifu Chemical Group Co., Yantai, People's Republic of China) were used for analytical TLC analyses.

**Fungal Material.** The fungal strain *Xylaria* sp. C-2 was isolated from a piece of fresh tissue from the inner part of an unidentified gorgonian (XS-2009-03) collected from the Xisha Islands coral reefs in the South China Sea in December 2009. The strain was deposited in the Key Laboratory of Marine Drugs, Ministry of Education of China, School of Medicine and Pharmacy, Ocean University of China, Qingdao, P. R. China, with the GenBank (NCBI) accession number KP901303.

**Extraction and Isolation.** The fungal strain was cultivated in 25 L of liquid medium (200 g/L cooked and sliced potatoes, 20 g/L glucose in artificial seawater, in 1 L Erlenmeyer flasks each containing 400 mL of culture broth) at 25.0°C without shaking for 9 weeks. The culture was filtered to separate the culture broth from the mycelia. The fermentation broth was extracted three times with an equal volume of EtOAc, and the fungal mycelia were extracted three times with  $\text{CHCl}_3$ -MeOH (1:1). The organic extracts from broth and mycelia were combined and concentrated under vacuum to afford a dry extract (25.2 g). The resulting extract was subjected to silica gel column chromatography (CC) (petroleum ether-EtOAc, gradient) to afford five fractions (Fr.1–5). Fraction 2 was isolated by silica gel CC (petroleum ether-EtOAc, 9:1) and separated by Sephadex LH-20 CC (petroleum ether- $\text{CHCl}_3$ -MeOH, 2:1:1), then further purified on semipreparative HPLC with 60% MeOH- $\text{H}_2\text{O}$  to yield **3** (5 mg) and **4** (7 mg), and with 55% MeOH- $\text{H}_2\text{O}$  to give **5** (3 mg). Fraction 3 was fractionated on silica gel CC (petroleum ether-EtOAc, 8:2) to obtain two subfractions (Subfr.3-1 and 3-2). Subfraction 3-1 was separated by Sephadex LH-20 CC (petroleum ether- $\text{CHCl}_3$ -MeOH, 2:1:1) to yield **6** (15.6 mg). Subfraction 3-2 was purified with 55% MeOH- $\text{H}_2\text{O}$  to yield **7** (14.8 mg). Fraction 4 was subjected to repeated Sephadex LH-20 CC (petroleum ether-EtOAc, 7:3) and further purified on semipreparative HPLC with 40% MeOH- $\text{H}_2\text{O}$  to afford **1** (8.0 mg) and **2** (8 mg).

**Antimicrobial Bioassay.** Antibacterial activity was evaluated by the conventional broth dilution assay [16]. Nine bacterial strains, *Bacillus subtilis*, *Tetragenococcus halophilus*, *Staphylococcus albus*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas putida*, *Nocardia brasiliensis*, *Vibrio parahaemolyticus*, and *Kocuria rhizophila* were used, and ciprofloxacin was used as a positive control.

**Cytotoxicity Test.** The cytotoxicity against four human tumor cell lines, A549 (lung carcinoma epithelium), HeLa (cervical epithelium), Hep-2 (epidermoid carcinoma-2), and RD (rhabdomyosarcoma cells), was evaluated using the SRB method [17]. Adriamycin was used as a positive control.

**Preparation of the (S)- and (R)-MTPA Ester Derivatives of 1.** To a stirred solution of **1** (1.0 mg) in pyridine (500  $\mu\text{L}$ ) was added 4-(dimethylamino)pyridine (2 mg) and (*R*)-(+)- $\alpha$ -methoxy- $\alpha$ -(trifluoromethyl) phenylacetyl chloride (MTPA-Cl, 10  $\mu\text{L}$ ). The mixture was stirred at room temperature for 12 h. The reaction mixture was then passed through a disposable pipet packed with silica gel and eluted with petroleum ether-EtOAc (3:1) to give the (*S*)-Mosher ester **1s**. Treatment of **1** (1.0 mg) with (*S*)-MTPA-Cl (10  $\mu\text{L}$ ) as described above yielded the corresponding (*R*)-Mosher ester **1r**.

**1-(S)-MTPA Ester (1s).**  $^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{OD}$ ,  $\delta$ , ppm, J/Hz): 7.27 (1H, dd, J = 15.3, 11.1, H-3), 6.45 (1H, dd, J = 15.3, 11.1, H-4), 6.15 (1H, dd, J = 15.3, 7.5, H-5), 5.95 (1H, d, J = 15.3, H-2), 5.55 (1H, dt, J = 12.0, 6.0, H-6), 3.73 (3H, s, MTPA-OCH<sub>3</sub>), 3.52 (3H, s, MTPA-OCH<sub>3</sub>), 1.67 (2H, m, H-7), 1.28 (2H, m, H-9), 1.19 (2H, m, H-8), 0.84 (3H, t, J = 7.2, H-10). ESI-MS  $m/z$  437 [M + Na]<sup>+</sup>.

**1-(R)-MTPA Ester (1r).**  $^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{OD}$ ,  $\delta$ , ppm, J/Hz): 7.20 (1H, dd, J = 15.3, 11.1, H-3), 6.21 (1H, dd, J = 15.3, 11.1, H-4), 6.05 (1H, dd, J = 15.3, 7.5, H-5), 5.82 (1H, d, J = 15.3, H-2), 5.55 (1H, dt, J = 12.0, 6.0, H-6), 3.72 (3H, s, MTPA-OCH<sub>3</sub>), 3.55 (3H, s, MTPA-OCH<sub>3</sub>), 1.74 (2H, m, H-7), 1.36 (2H, m, H-9), 1.34 (2H, m, H-8), 0.90 (3H, t, J = 7.2, H-10). ESI-MS  $m/z$  437 [M + Na]<sup>+</sup>.

**(2E,4E,6S)-6-Hydroxydeca-2,4-dienoic Acid (1).**  $\text{C}_{10}\text{H}_{16}\text{O}_3$ , light yellowish, amorphous solid.  $[\alpha]_{\text{D}}^{20} + 12.5^\circ$  (*c* 0.20,  $\text{CH}_3\text{OH}$ ). UV (MeOH,  $\lambda_{\text{max}}$ , nm) (log  $\epsilon$ ): 255 (2.89). IR (KBr,  $\nu_{\text{max}}$ ,  $\text{cm}^{-1}$ ): 3420 (br), 2959, 1697, 1249, 1001.

$^1\text{H}$  (600 MHz, acetone- $d_6$ ) and  $^{13}\text{C}$  NMR (150 MHz, acetone- $d_6$ ), see Table 1. HR-ESI-MS  $m/z$  183.1018  $[\text{M} - \text{H}]^-$ , (calcd for  $\text{C}_{10}\text{H}_{15}\text{O}_3$ , 183.1016).

**Anodendroic Acid (2).**  $\text{C}_{12}\text{H}_{14}\text{O}_4$ , white amorphous powder.  $[\alpha]_{\text{D}}^{20} +59.0^\circ$  ( $c$  0.10,  $\text{CH}_3\text{OH}$ ).  $^1\text{H}$  NMR (500 MHz,  $\text{DMSO}-d_6$ ,  $\delta$ , ppm, J/Hz): 7.74 (1H, s, H-4), 7.71 (1H, d,  $J = 8.2$ , H-6), 6.79 (1H, d,  $J = 8.2$ , H-7), 4.64 (1H, m, H-2), 3.17 (2H, m, H-3), 1.13 (3H, s, H-3'), 1.12 (3H, s, H-2'). ESI-MS  $m/z$  223  $[\text{M} + \text{H}]^+$ .

**7-Chloro-5-hydroxymellein (3).**  $\text{C}_{10}\text{H}_9\text{ClO}_4$ , White amorphous powder.  $[\alpha]_{\text{D}}^{20} +15.0^\circ$  ( $c$  0.25,  $\text{CH}_3\text{OH}$ ).  $^1\text{H}$  NMR (500 MHz, acetone- $d_6$ ,  $\delta$ , ppm, J/Hz): 7.24 (1H, s, H-6), 4.82 (1H, m, H-3), 3.23 (1H, dd,  $J = 17.0, 3.2$ , H-4a), 2.70 (1H, dd,  $J = 17.0, 11.5$ , H-4b), 1.51 (3H, d,  $J = 6.3$ , H-9). ESI-MS  $m/z$  229  $[\text{M} + \text{H}]^+$ .

**Methyl Indole-3-carboxylate (4).**  $\text{C}_{10}\text{H}_9\text{NO}_2$ , white amorphous powder.  $^1\text{H}$  NMR (500 MHz, acetone- $d_6$ ,  $\delta$ , ppm, J/Hz): 8.13 (1H, s, H-2), 8.04 (1H, dd,  $J = 8.0, 2.5$ , H-4), 7.50 (1H, dd,  $J = 8.0, 2.5$ , H-7), 7.21 (2H, m, H-5, 6), 3.84 (3H, s,  $\text{OCH}_3$ ). ESI-MS  $m/z$  176  $[\text{M} + \text{H}]^+$ .

**5-Carboxymellein (5).**  $\text{C}_{11}\text{H}_{10}\text{O}_5$ , white amorphous solid.  $[\alpha]_{\text{D}}^{20} -189.0^\circ$  ( $c$  0.15,  $\text{CH}_3\text{OH}$ ).  $^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{OD}$ ,  $\delta$ , ppm, J/Hz): 8.14 (1H, d,  $J = 8.8$ , H-6), 6.93 (1H, d,  $J = 8.8$ , H-7), 4.71 (1H, m, H-3), 3.91 (1H, dd,  $J = 17.2, 3.0$ , H-4a), 3.01 (1H, dd,  $J = 17.2, 3.0$ , H-4b), 1.51 (3H, d,  $J = 6.0$ ,  $\text{CH}_3$ ). ESI-MS  $m/z$  245  $[\text{M} + \text{Na}]^+$ .

**Ergosta-5,7,22-trien-3 $\beta$ -ol (6).**  $\text{C}_{28}\text{H}_{44}\text{O}$ , white amorphous powder.  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ,  $\delta$ , ppm, J/Hz): 5.56 (1H, d,  $J = 5.6$ , H-6), 5.38 (1H, d,  $J = 5.6$ , H-7), 5.22 (1H, dd,  $J = 15.3, 7.6$ , H-23), 5.16 (1H, dd,  $J = 15.3, 7.1$ , H-22), 3.63 (1H, m, H-3), 1.03 (3H, d,  $J = 6.6$ , H-21), 0.94 (3H, s, H-19), 0.91 (3H, d,  $J = 6.8$ , H-28), 0.84 (3H, d,  $J = 6.4$ , H-27), 0.82 (3H, d,  $J = 6.5$ , H-26), 0.62 (3H, s, H-18). ESI-MS  $m/z$  397  $[\text{M} + \text{H}]^+$ .

**5 $\alpha$ ,8 $\alpha$ -Epidioxy-ergosta-6,22 $E$ -dien-3 $\beta$ -ol (7).**  $\text{C}_{28}\text{H}_{44}\text{O}_3$ , white amorphous powder.  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ,  $\delta$ , ppm, J/Hz): 6.51 (1H, d,  $J = 7.6$ , H-7), 6.24 (1H, d,  $J = 7.6$ , H-6), 5.22 (1H, dd,  $J = 15.4, 7.6$ , H-23), 5.15 (1H, dd,  $J = 15.3, 7.2$ , H-22), 3.96 (1H, m, H-3), 1.00 (3H, d,  $J = 6.6$ , H-21), 0.91 (3H, d,  $J = 6.6$ , H-28), 0.88 (3H, s, H-19), 0.84 (3H, s, H-18), 0.82 (3H, d,  $J = 6.6$ , H-27), 0.81 (3H, d,  $J = 6.6$ , H-26). ESI-MS  $m/z$  451  $[\text{M} + \text{Na}]^+$ .

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