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New acetylenic acids and derivatives from the Basidiomycete *Craterellus lutescens* (Cantharellaceae)

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ABSTRACT

Five new acetylenic acid analogues, craterellynes A–E (**1**–**5**), together with three known compounds (**6**–**8**), were isolated from the fruiting bodies of *Craterellus lutescens*. Their structures were elucidated on the basis of spectroscopic and chemical means. The absolute configurations of **1**–**6** were determined by the modified Mosher method.

1. Introduction

The genus *Craterellus* derived from higher fungi which is generally edible and widely distributed in China [1]. Previous chemical investigations on *Craterellus* sp. led to a series of acetylenic acids [2–4]. Acetylenic acids are widespread in nature and in many organisms, especially common in fungi of the group Basidiomycetes [5,6]. Over 700 naturally occurring acetylenic compounds have been established, many of them possessed a photosensitizing activity which makes them toxic to bacteria, viruses, and insects [7]. Therefore, they exhibit diverse bioactivities, including, antimicrobial, cytotoxic, *anti*-HIV activities, and enzyme inhibitions [8,9].

The basidiomycete *Craterellus lutescens* is a fungus belonging to the family Cantharellaceae and widely distributed in Yunnan, China. However, to the best of our knowledge, there is no chemical has been reported about this species regarding phytochemical as well as pharmacological research. As part of our continuing efforts to investigate bioactive metabolites from higher fungi of Yunnan province, China [10]. we researched the chemical constitutes of the fruiting bodies of *C. lutescens* and isolated five new acetylenic acid derivatives, craterellynes A–E (**1**–**5**), together with three known compounds (**6**–**8**) (Fig. 1). The absolute configurations of C-9 in compounds **1**–**6** were elucidated by the modified Mosher method.

2. Results and discussion

Compound **1** was isolated as a colorless oil, in which the molecular formula was established as $C_{19}H_{30}O_3$ by HRESIMS (*m/z* 329.2088 $[M + Na]^+$, calcd for $C_{19}H_{30}O_3$, 329.2087), requiring 5 degrees of unsaturation. The IR spectrum showed the presence of both carbonyl (1738 cm^{-1}) and hydroxy groups (3429 cm^{-1}). The ^1H NMR spectrum revealed the occurrence of four olefinic (δ_H 6.10, 5.91, 5.84, 5.57), one oxymethine (δ_H 4.17), an allylic methylene proton (δ_H 2.27), and a methyl group (δ_H 0.93). Correspondingly, the ^{13}C NMR spectrum exhibited the signals of four olefinic carbons (δ_C 145.0, 144.2, 110.4, 109.1), two acetylenic quaternary carbons (δ_C 91.5, 87.3), one oxymethine resonances (δ_C 72.6), as well as a methyl (δ_C 13.9) and a carbonyl (δ_C 174.5) group, typical of an acyclic fatty acid analogue with a terminal methyl group. A chain structure from C-18 to C-14 was readily identified by interpretation of ^1H—^1H COSY and HMBC spectra (Fig. 2). The ^1H—^1H COSY cross-peaks were observed for H-18/H-17, H-17/H-16, H-16/H-15, and H-15/H-14. The correlation from H-18 (δ_H 0.93) to C-16 (δ_C 32.5), from H-17 (δ_H 1.43) to C-15 (δ_C 144.2) and from H-16 (δ_H 2.27) to C-14 (δ_C 109.1) were demonstrated in HMBC spectra. In addition, the HMBC cross-peak between H-10 (δ_H 6.10) and C-12 (δ_C 91.5) together with the upfield shift of C-11 (δ_C 110.4) and C-14 (δ_C 109.1) revealed that one acetylene group could be placed between the two double bonds [11-13]. The location of the hydroxy group was assigned by observing COSY correlations between H-9 (δ_H 4.16) and H-10 (δ_H

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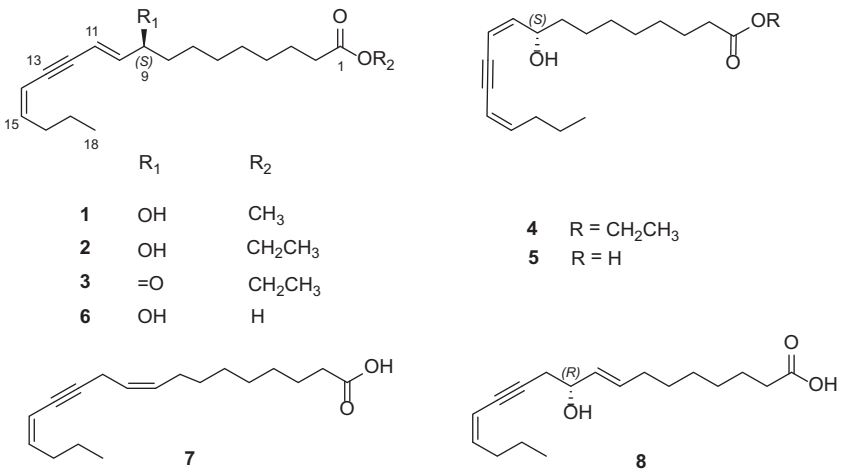

Fig. 1. Structures of compounds **1**–**8**.

6.10), as well as a HMBC correlation from H-9 (δ_H 4.16) to C-11 (δ_C 110.4). The ^{1}H and ^{13}C NMR data of **1** indicated structural features closely related to those of a known compound, (10*E*,14*Z*)-9-hydroxyoctadeca-10,14-dien-12-ynoic acid (**6**) [11,14]. However, there was an extra methoxy group at δ_H 3.66 (3H, s), which could be observed in the ^{1}H NMR spectrum of **1**. The HMBC correlation from δ_H 3.66 (3H, s) to the carbonyl group at δ_C 174.5 indicated a methyl ester in **1**. Further, the ^{1}H—^{1}H COSY cross-peaks were observed for H-2/H-3, H-3/H-4, H-7/H-8, and H-8/H-9. Configurations of two double bonds of C-10/C-11 and C-14/C-15 were determined as *E* and *Z*, respectively, on the basis of their coupling constants of 15.9 and 10.8 Hz, respectively. Therefore, the structure of **1** was determined as methyl (10*E*,14*Z*)-9-hydroxyoctadeca-10,14-dien-12-ynoate and named craterellyne A.

Compound **2** was isolated as a colorless oil with features similar to those of **1** and **6**. It possessed a molecular formula of $C_{20}H_{32}O_3$ based on HRESIMS data (*m*/*z* 343.2243 [M + Na]$^+$, calcd for $C_{20}H_{32}O_3$, 343.2244). Analysis of these NMR data together with the molecular formulas revealed that compound **2** was the ethyl ester of **6**. The methylene (δ_H 4.12) showed correlations to the methyl (δ_H 1.23) in the ^{1}H—^{1}H COSY spectra, to the carbonyl group (δ_C 174.1) in HMBC spectra. Thus, the structure of **2** was proposed as ethyl (10*E*,14*Z*)-9-hydroxyoctadeca-10,14-dien-12-ynoate and named craterellyne B. The optical rotations and NMR data of compounds **1**, **2** and **6** were similar, suggesting that they might possess the same stereoconfiguration. However, the absolute configuration of **6** has never been reported. In this work, we confirmed the absolute configuration by the modified Mosher method. From the values of $\Delta\delta$ ($\delta_S - \delta_R$) (Table S1, Supporting Information), the absolute configurations of C-9 for **1**, **2**, and **6** were assigned as *S*.

Compound **3** was isolated as a minor constituent with a molecular formula of $C_{20}H_{30}O_3$ as established by the HRESIMS data (*m*/*z* 341.2090 [M + Na]$^+$, calcd for $C_{20}H_{30}O_3$, 341.2087). The NMR data (Tables 1 and 2) demonstrated that the structure of **3** was similar to that of **2**. The major change was that the hydroxy at C-9 (δ_C 72.5) in **2** was oxygenated into a ketone group (δ_C 199.5) in **3**, which was supported by HMBC correlations (Supporting Information, Fig. S17). Therefore, the structure of **3** was determined as ethyl (10*E*,14*Z*)-9-oxooctadeca-10,14-dien-12-ynoate and named as craterellyne C.

Compound **4** was obtained as a colorless oil with the same molecular formula as that of **2** by HRESIMS (*m*/*z* 343.2245 [M + Na]$^+$, calcd for $C_{20}H_{32}O_3$, 343.2244), suggesting that they should be isomers. Further comparison of 1D NMR data (Tables 1 and 2) displayed that compounds **4** and **2** shared the same functional group, with a key difference in the coupling constants of H-10 and H-11. The doublet of doublets of H-10 (J = 10.9, 8.4 Hz) and doublets of H-11 (J = 10.9 Hz) indicated that the double bond of C-10/C-11 should be *Z* form. The absolute configuration of C-9 in **4** was determined as *S* by the modified Mosher method (Table S1, Supporting Information). Therefore, compound **4** was determined as shown in Fig. 1 and named as craterellyne D.

Compound **5**, a colorless oil, had a molecular formula of $C_{18}H_{28}O_3$ as determined by HRESIMS (*m*/*z* 315.1930 [M + Na]$^+$, calcd for $C_{18}H_{28}O_3$, 315.1931). Its 1D NMR data (Tables 1 and 2) and optical rotations revealed a similar structure and the same configuration to those of **4**, except for the absence of the ethyl ester group. Detailed analyses of other NMR data suggested that the other parts of **5** were the same to those of **4**. Hence the structure of **5** was elucidated as shown in Fig. 1 and named as craterellyne E.

The absolute configurations of acetylenic acid derivatives with a long chain were difficult to be determined. We attempted to crystallize acetylenic acid for possible X-ray diffraction analysis by using various methods and solvents. However, none was successful. It was rationalized that a chemical derivatization of these compounds would be the only method that might aid in crystallization. However, crystallizing the chemical derivatizations was unsuccessful ultimately. Nonetheless, we successfully prepared the chiral anisotropic reagent 9-AMA [15] for the upfield shifts of 9-AMA esters were 6 to 10 times larger than those of MTPA esters [16]. Unfortunately, the synthesis of 9-AMA esters failed, since steric hindrance of the 9-AMA was larger than MTPA and our compounds had strong flexibility. Accordingly, we prepared MTPA

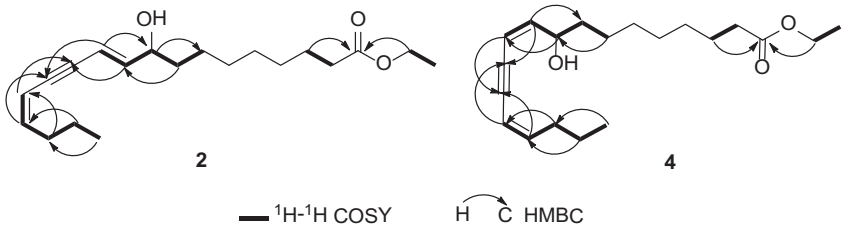

Fig. 2. Key correlations in 2D NMR spectra of compounds **2** and **4**.

Table 1
^{1}H NMR spectral data of compounds **1–6** [δ in ppm, J in Hz].

No.	**1**[b]	**2**[c]	**3**[b]	**4**[b]	**4**[d]	**5**[a]	**6**[a]	**6**[b]
2	2.29, t (7.2)	2.26, t (7.5)	2.27, t (7.5)	2.27, t (7.5)	2.29, t (7.5)	2.24, t (7.5)	2.27, t (7.2)	2.33, t (7.5)
3	1.60, m	1.61, m	1.61, m	1.61, m	1.60, m	1.60, m	1.60, m	1.61 m
4	1.32, m	1.33, m	1.33, m	1.34, m	1.34, m	1.34, m	1.35, m	1.32, m
5	1.32, m	1.33, m	1.33, m	1.34, m	1.34, m	1.34, m	1.35, m	1.32, m
6	1.32, m	1.33, m	1.33, m	1.34, m	1.34, m	1.34, m	1.35, m	1.32, m
7	1.37, m	1.36, m	1.36, m	1.41, m	1.42, m	1.42, m	1.41, m	1.36, m
8	1.53, m	1.54, m	2.53, t (7.4)	1.63, m; 1.52, m	1.61, m; 1.48, m	1.61, m; 1.48, m	1.51, m	1.54, m
9	4.16, m	4.16, m		4.67, m	4.63, m	4.63, m	4.09, m	4.17, m
10	6.10, dd (15.9, 6.3)	6.09, dd (15.9, 6.3)	6.47, d (15.9)	5.87, dd (10.9,8.4)	5.83, dd (10.9, 8.7)	5.83, dd (10.9, 8.7)	6.05, dd (15.8, 6.2)	6.10, dd (15.9, 6.3)
11	5.84, d (15.9)	5.83, d (15.9)	6.80, d (15.9)	5.70, d (10.9)	5.69, d (10.9)	5.69, d (10.9)	5.82, d (15.8)	5.85, d (15.9)
14	5.57, d (10.8, 1.1)	5.56, d (10.8, 1.6)	5.65, d (10.8)	5.60, d (10.8)	5.62, d (10.7)	5.62, d (10.7)	5.58, d (10.8)	5.57, d (10.6)
15	5.91, dt (10.8, 7.4)	5.89, dt (10.8, 7.5)	6.07, dt (10.8, 7.6)	5.94, dt (10.8, 7.5)	5.96, dt (10.7, 7.5)	5.96, dt (10.7, 7.5)	5.91, dt (10.8, 7.5)	5.91, dt (10.7, 7.5)
16	2.27, m	2.27, m	2.31, m	2.29, m	2.31, m	2.31, m	2.29, m	2.29, m
17	1.43, m	1.44, m	1.45, m	1.45, m	1.46, m	1.46, m	1.45, m	1.44, m
18	0.93, t (7.4)	0.91, t (7.4)	0.94, t (7.4)	0.94, t (7.4)	0.96, t (7.5)	0.95, t (7.4)	0.94, t (7.4)	0.93, t (7.4)
1'	3.66, s	4.12, q (7.1)	4.12, q (7.1)	4.12, q (7.1)	4.11, q (7.1)			
2'		1.23, t (7.1)	1.25, t (7.1)	1.25, t (7.1)	1.24, t (7.1)			

[a] Data were measured at 600 MHz in methanol-d_4.
[b] Data were measured at 600 MHz in $CDCl_3$.
[c] Data were measured at 400 MHz in $CDCl_3$.
[d] Data were measured at 400 MHz in methanol-d_4.

esters of compounds **1**, **2**, and **4** to determine the absolute configurations even the $\Delta\delta$ values were small.

Compounds **2** and **4** were evaluated for their cytotoxic (HL-60, SMMC-7721, A-549, MCF-7 and SW480) and DPPH radical scavenging activities, but the results were disappointing. Recently, we found that the acid derivative of compound **3** had a PPAR-γ transcriptional activity [11], which might be a promising lead compound for the treatment of type II diabetes [17]. Due to the limit amount of compound **3**, we had no chance to test its PPAR-γ transcriptional activity. Consequently, further pharmacological investigation and in vivo physiological functions of these compounds are necessary.

3. Experimental section

3.1. General experimental procedures

Optical rotations were measured on a Jasco-P-1020 polarimeter (Horiba, Kyoto, Japan). UV spectrum was recorded on a Shimadzu UV-2401PC (Shimadzu, Kyoto, Japan). IR spectra were obtained by using a Bruker Tensor 27 FT-IR spectrometer with KBr pellets. NMR spectra were acquired with instruments of Bruker Avance III 600 MHz spectrometer (Bruker Biospin GmbH, Karlsruhe, Germany). HRESIMS were recorded on an Agilent 6200 Q-TOF MS system (Agilent Technologies, Santa Clara, CA, USA). Sephadex LH-20 (Amersham Biosciences, Upssala, Sweden) and silica gel (Qingdao Haiyang Chemical Co., Ltd) were used for column chromatography (CC). Medium Pressure Liquid Chromatography (MPLC) was performed on a Büchi Sepacore System equipping with pump manager C-615, pump modules C-605 and fraction collector C-660 (Büchi Labortechnik AG, Flawil, Switzerland), and columns packed with Chromatorex C-18 (40–75 μm, Fuji Silysia Chemical Ltd., Kasugai, Japan). Preparative High Performance Liquid Chromatography (prep-HPLC) was performed on an Agilent 1260 liquid chromatography system equipped with Zorbax SB-C18 columns (5 μm, 9.4 mm × 150 mm or 21.2 mm × 150 mm) and a DAD detector.

Table 2
^{13}C NMR spectral data of compounds **1–6**.

No.	**1**[b]	**2**[c]	**3**[b]	**4**[b]	**4**[d]	**5**[a]	**6**[a]	**6**[b]
1	174.5	174.1	174.0	174.1	175.5	180.3	178.8	179.3
2	34.2	34.5	34.5	34.5	35.1	36.5	35.5	34.1
3	25.0	25.0	25.0	25.1	26.0	26.7	26.3	24.8
4	29.2	29.1	29.1	29.2	30.1	30.4	30.3	29.1
5	29.3	29.2	29.2	29.3	30.4	30.5	30.4	29.2
6	29.4	29.4	29.2	29.5	30.5	30.6	30.5	29.4
7	25.3	25.3	24.1	25.3	26.0	26.4	26.5	25.3
8	37.1	37.1	41.1	36.7	37.9	37.9	38.0	37.0
9	72.5	72.5	199.5	70.4	70.8	70.9	72.8	72.6
10	145.0	145.0	136.5	144.6	146.1	146.1	146.6	144.9
11	110.4	110.3	123.4	110.4	110.6	110.4	110.6	110.4
12	91.5	91.5	91.2	89.4	92.3	90.5	92.7	91.5
13	87.3	87.3	96.6	92.2	90.5	92.3	87.5	87.4
14	109.1	109.2	108.9	109.2	110.3	110.3	110.4	109.2
15	144.2	144.1	147.2	144.4	144.5	144.6	144.2	144.2
16	32.5	32.4	32.8	32.6	33.5	33.5	33.3	32.5
17	22.3	22.2	22.2	22.3	23.2	23.2	23.2	22.3
18	13.9	13.8	13.9	13.9	14.2	14.2	14.1	13.9
1'	51.6	60.3	60.4	60.3	61.4			
2'		14.4	14.4	14.4	14.6			

[a] Data were measured at 150 MHz in methanol-d_4,
[b] Data were measured at 150 MHz in $CDCl_3$,
[c] Data were measured at 100 MHz in $CDCl_3$,
[d] Data were measured at 100 MHz in methanol-d_4.

3.2. Fungal material

The fresh fruiting bodies of *C. lutescens* were obtained from Tiger Leaping Gorge in Yunnan province, China, in August 2014 and identified by Prof. Li Zhenghui, Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (No. 20140817C) was deposited at the herbarium of Kunming Institute of Botany, the Chinese Academy of Sciences.

3.3. Extraction and isolation

The fresh fruiting bodies of *C. lutescens* (5 kg) were macerated three times with 95% EtOH. The extraction was evaporated under reduced pressure and partitioned between EtOAc and water four times to give a crude extract (105 g). The crude extract was subject to column chromatography over silica gel (200–300 mesh, 10 × 55 cm), eluting with a $CHCl_3$-CH_3OH gradients, to afford fractions A-G. Fraction A eluted with $CHCl_3$-CH_3OH (1:0) was further purified on MPLC with a stepwise gradient of MeOH/H_2O (v/v 40:60–100:0) to afford eleven fractions (A1-A11). Each subfraction was further separated by Sephadex LH-20 (MeOH) column chromatography. Compound **8** (1.8 mg) was obtained from fraction *A*1 by prep-HPLC using a gradient eluent (MeCN-H_2O 30%–60%, 30 min). Compounds **5** (2.4 mg) and **6** (3.5 mg) were isolated from fraction A2 by prep-HPLC using a gradient eluent (MeCN-H_2O 40%–70%, 30 min). Fraction A4 was subjected on prep-HPLC (MeCN-

H_2O 50%–70%, 25 min) to afford Compound **7** (5.8 mg). A10 was separated on prep-HPLC (MeCN-H_2O 55%–75%, 25 min) to give Compound **1** (9.3 mg). A11 was purified using prep-HPLC (MeCN-H_2O 60%–80%, 25 min) to give three compounds **2** (28.5 mg), **3** (1.7 mg) and **4** (8.4 mg).

Craterellyne A (1): colorless oil, $[\alpha]_D^{20}$ −11.0 (*c* 0.009, $CHCl_3$). UV ($CHCl_3$) λ_{max} nm (log ε): 241.5 (3.75), 268.0 (3.74). IR (KBr) ν_{max} cm^{-1}: 3429, 2931, 2859, 1738, 1439, 1374, 1172. ^1H NMR data (see Table 1); ^{13}C NMR data (see Table 2); HRESIMS *m/z* 329.2088 $[M + Na]^+$ (cacld for $C_{19}H_{30}O_3$, 329.2087).

Craterellyne B (2): colorless oil, $[\alpha]_D^{20}$ −3.9 (*c* 0.003, $CHCl_3$). UV (MeOH) λ_{max} nm (log ε): 265.6 (4.04), 278.4 (3.95). IR (KBr) ν_{max} cm^{-1}: 3423, 2932, 2859, 1735, 1461, 1375, 1183. ^1H NMR data (see Table 1); ^{13}C NMR data (see Table 2); HRESIMS *m/z* 343.2243 $[M + Na]^+$ (cacld for $C_{20}H_{32}O_3$, 343.2244).

Craterellyne C (3): colorless oil, UV ($CHCl_3$) λ_{max} nm (log ε): 270.5 (3.75), 233.5 (3.65). IR (KBr) ν_{max} cm^{-1}: 3424, 2934, 2857, 2184, 1735, 1463, 1374, 1182. ^1H NMR data (see Table 1); ^{13}C NMR data (see Table 2); HRESIMS *m/z* 341.2090 $[M + Na]^+$ (cacld for $C_{20}H_{30}O_3$, 341.2087).

Craterellyne D (4): colorless oil, $[\alpha]_D^{20}$ −5.3 (*c* 0.001, $CHCl_3$). UV ($CHCl_3$) λ_{max} nm (log ε): 269.0 (3.74), 281.0 (3.64). IR (KBr) ν_{max} cm^{-1}: 3422, 2932, 2858, 1735, 1627, 1462, 1376, 1183. ^1H NMR data (see Table 1); ^{13}C NMR data (see Table 2); HRESIMS *m/z* 343.2245 $[M + Na]^+$ (cacld for $C_{20}H_{32}O_3$, 343.2244).

Craterellyne E (5): colorless oil, $[\alpha]_D^{20}$ −2.8 (*c* 0.007, MeOH). UV (MeOH) λ_{max} nm (log ε): 266.5 (3.69), 277.0 (3.59). IR (KBr) ν_{max} cm^{-1}: 3422, 2931, 2859, 1715, 1624, 1385, 1191. ^1H NMR data (see Table 1); ^{13}C NMR data (see Table 2); HRESIMS *m/z* 315.1930 $[M + Na]^+$ (cacld for $C_{18}H_{28}O_3$, 315.1931).

Craterellyne F (6): colorless oil, $[\alpha]_D^{20}$ −3.9 (*c* 0.004, MeOH + $CHCl_3$ 1:1). UV (MeOH + $CHCl_3$ 1:1) λ_{max} nm (log ε): 237.5 (3.52), 266.5 (3.47). IR (KBr) ν_{max} cm^{-1}: 3408, 2932, 2858, 2185, 1711, 1463, 1384, 1197. ^1H NMR data (see Table 1); ^{13}C NMR data (see Table 2); HRESIMS *m/z* 291.1966 $[M - H]^-$ (cacld for $C_{18}H_{28}O_3$, 291.1966).

MTPA Esters of Compounds 1, 2, and 4. A sample of **1** (3.1 mg), (*S*)-MTPA (11.7 mg), 4-(dimethylamino)pyridine (DMAP; 4.6 mg), and 1,3-dicyclohexylcarbodiimide (DCC; 27.1 mg) were dissolved in 10 mL of dry CH_2Cl_2 and stirred at room temperature for 24 h. The reaction mixture was filtered, and the concentrated filtrate was subjected on prep-HPLC (Agilent Zorbax SB-C18 columns; 5 μm; 9.4 mm × 150 mm MeCN-H_2O 55%–85%, 30 min; followed by MeCN-H_2O 85%–100%, 10 min) to afford the purified Mosher ester of **1** (4.2 mg). Other MTPA esters were prepared in the same manner for **2** and **4** and characterized by measurement of their ^1H and ^1H—^1H COSY NMR spectroscopic data in $CDCl_3$.

Bis[(*S*)-MTPA] Ester of 1: ^1H NMR ($CDCl_3$) δ 6.0157 (dd, *J* = 15.9, 7.2 Hz, H-10), 5.9567 (m, H-15), 5.8364 (d, *J* = 15.9 Hz, H-11), 5.5718 (d, *J* = 10.8 Hz, H-14), 2.2873 (m, H-2), 2.2820 (m, H-16), 1.6945 (m, H-8), 0.9377 (t, *J* = 7.4 Hz, H-18).

Bis[(*R*)-MTPA] Ester of 1: ^1H NMR ($CDCl_3$) δ 6.0156 (dd, *J* = 15.9, 7.2 Hz, H-10), 5.9566 (m, H-15), 5.8359 (d, *J* = 15.9 Hz, H-11), 5.5716 (d, *J* = 10.8 Hz, H-14), 2.2876 (m, H-2), 2.2819 (m, H-16), 1.6950 (m, H-8), 0.9376 (t, *J* = 7.4 Hz, H-18).

Bis[(*S*)-MTPA] Ester of 2: ^1H NMR ($CDCl_3$) δ 6.0168 (dd, *J* = 15.9, 7.0 Hz, H-10), 5.9575 (m, H-15), 5.8389 (d, *J* = 15.9 Hz, H-11), 5.5746 (d, *J* = 10.8 Hz, H-14), 2.2871 (m, H-16), 2.2608 (m, H-2), 1.6719 (m, H-8), 0.9389 (t, *J* = 7.4 Hz, H-18).

Bis[(*R*)-MTPA] Ester of 2: ^1H NMR ($CDCl_3$) δ 6.0151 (dd, *J* = 15.9, 7.0 Hz, H-10), 5.9556 (m, H-15), 5.8358 (d, *J* = 15.9 Hz, H-11), 5.5713 (d, *J* = 10.8 Hz, H-14), 2.2868 (m, H-16), 2.2611 (m, H-2), 1.6757 (m, H-8), 0.9372 (t, *J* = 7.4 Hz, H-18).

Bis[(*S*)-MTPA] Ester of 4: ^1H NMR ($CDCl_3$) δ 5.9766 (m, H-15), 5.8523 (m, H-10), 5.7195 (m, H-11), 5.6338 (d, *J* = 10.8 Hz, H-14), 2.3372 (m, H-16), 2.2717 (m, H-2), 1.9976 (m, H-8), 0.9417 (t, *J* = 7.4 Hz, H-18).

Bis[(*R*)-MTPA] Ester of 4: ^1H NMR ($CDCl_3$) δ 5.9761 (m, H-15), 5.8518 (m, H-10), 5.7190 (m, H-11), 5.6331 (d, *J* = 10.8 Hz, H-14), 2.3348 (m, H-16), 2.2733 (m, H-2), 2.0002 (m, H-8), 0.9414 (t, *J* = 7.4 Hz, H-18).

3.4. Bioassay

3.4.1. Cytotoxicity assay

The cytotoxicities against HL-60, SMMC-7721, A-549, MCF-7, SW480 cells lines of compounds **2** and **4** were tested by using the MTS (3-(4,5-dimethylthiazol-2-yl)-5(3-carboxymethoxyphenyl)-2-(4-sulfopheny)-2*H*–tetrazolium) method [18]. MTS was an analogue of MTT, which could be reduced into soluble formazan by succinate dehydrogenase in mitochondria of living cells. Moreover, the optical density value of formazan (490 nm) was proportional to the number of living cells.

3.4.2. Antioxidant assay

Free radical-scavenging activity was measured by the 1,1-diphenyl 2-picrylhydrazyl (DDPH) method [19,20] with using Trolox as standard. Changes in the absorbance of the samples were measured at 515 nm. Radical-scavenging activity was expressed as the inhibition percentage and was calculated using the following formula: radical-scavenging activity (%) = (1 − sample OD/control OD) × 100%, where OD was optical density.

Conflict of interest

The authors declare no conflict of interest for.

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Appendix A. Supplementary data.

Supplementary date of this article including MS, NMR spectra, and Mosher esters data were available online at XXX.

References

[1] N.L. Huang, Colored illustrations of macrofungi (mushrooms) of China, Acdemic press, China, 1998 31–35.
[2] V. Magnus, G. Lacan, R.T. Aplin, V. Thaller, Glycerol tridehydrocrepenynate from the basidiomycete *Craterellus cornucopioides*, Phytochemistry 28 (1989) 3047–3050.
[3] V. Magnus, G. Lacan, S. Iskric, P. Lewer, R.T. Aplin, V. Thaller, Conversion of indole-3-ethanol to fatty acid esters in *Craterellus cornucopioides*, Phytochemistry 28 (1989) 2949–2954.
[4] M.Y. Jiang, F. Wang, Z.J. Dong, Y. Zhang, H.J. Zhu, J.K. Liu, A new hydroxyl acetylenic fatty acid from the basidiomycete *Craterellus aureus* (Cantharellaceae), Acta Bot. Yunnanica 30 (2008) 614–616.
[5] D.J. Faulkner, Marine natural products, Nat. Prod. Rep. 15 (1998) 113–158.
[6] F. Bohlmann, T. Burkhardt, C. Zdero, Naturally occurring acetylenes, Acdemic press, London, 1973 1–222.
[7] J. Lam, In chemistry and biology of naturally-occurring acetylenes and related compounds (NOARC), Conference on the chemistry and biology of naturally-occurring acetylenes and related compounds, 1988.
[8] R. Negri, Polyacetylenes from terrestrial plants and fungi: recent phytochemical and biological advances, Fitoterapia 106 (2015) 92–109.
[9] A.D. Patil, W.C. Kokke, S. Cochran, T.A. Francis, T. Tomszek, J.W. Westley, Brominated polyacetylenic acids from the marine sponge *Xestospongia muta*: inhibitors of HIV protease, J. Nat. Prod. 55 (1992) 1170–1177.
[10] Y.S. Yuan, P.Q. Sun, Colored illustrations of macrofungi (mushrooms) of China, Academic press, China, 2007.
[11] S.S. Hong, H.L. Ji, W. Jeong, N. Kim, Z.J. Hui, Y.H. Bang, H.J. Lee, S.J. Lee, D.S. Jang, D. Lee, Acetylenic acid analogues from the edible mushroom Chanterelle (*Cantharellus cibarius*) and their effects on the gene expression of peroxisome proliferator-activated receptor-gamma target genes, Bioorg. Med. Chem. Lett. 22 (2012) 2347–2349.
[12] M.O. Fatope, O.A. Adoum, Y. Takeda, C_{18} acetylenic fatty acids of *Ximenia americana* with potential pesticidal activity, J. Agric. Food Chem. 48 (2000) 1872–2174.
[13] S. Matsunaga, Y. Okada, N. Fusetani, R.W.M. van Soest, An antimicrobial C_{14} acetylenic acid from a marine sponge *Oceanapia* species, J. Nat. Prod. 63 (2000) 690–691.

[14] Z. Pang, O. Sterner, Cibaric acid, a new fatty acid derivative formed enzymically in damaged fruit bodies of *Cantharellus cibarius* (Chanterelle), J. Organomet. Chem. 56 (1991) 1233–1235.
[15] F. Freire, J.M. Seco, E. Quiñoá, R. Riguera, Chiral 1,2-diols: the assignment of their absolute configuration by NMR made easy, Org. Lett. 12 (2010) 208–211.
[16] T. Kusumi, H. Takahashi, P. Xu, T. Fukushima, Y. Asakawa, T. Hashimoto, Y. Kan, Y. Inouye, New chiral anisotropic reagents, NMR tools to elucidate the absolute configurations of long-chain organic compounds, Tetrahedron Lett. 35 (1994) 4397–4400.
[17] C.S. Thakkar, R.R. Kshirsagar, V.R. Naik, A.R. Ghosh, V. Vijayakumar, A. Saklani, A.A. Kulkarni-Almeida, (E)-9-oxooctadec-10-en-12-ynoic acid from *Ixora brachiata* Roxb. Increases glucose uptake in L6 myotubes by activating the PI3K pathway, Fitoterapia 114 (2016) 26–33.
[18] T. Mosmann, Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, J. Immunol. Methods 65 (1983) 55–63.
[19] P.S. Murthy, K. Ramalakshmi, P. Srinivas, Fungitoxic activity of Indian borage (*Plectranthus amboinicus*) volatiles, Food Chem. 114 (2009) 1014–1018.
[20] M. Oktay, İ. Gülçin, Ö.İ. Küfrevioğlu, Determination of in vitro antioxidant activity of fennel (*Foeniculum vulgare*) seed extracts, Food Sci. Technol. 36 (2003) 263–271.