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New Acetylenic Acids and Derivatives from the Edible Mushroom *Craterellus lutescens* (Cantharellaceae)

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Supporting Information

ABSTRACT: Thirteen new acetylenic acids and their derivatives, craterellynes G−Q (**1**, **2**, **4**−**10**, **12**, **13**), 9-epi-craterellyne H (**3**), and 14-*O*-ethyl-craterellyne O (**11**), were isolated from the fruiting bodies of edible mushrooms *Craterellus lutescens*. The structures of these compounds were identified by various spectroscopic and chemical means. The stereoconfigurations of **1**−**13** were elucidated by the combination of acetonide formation, *J*-based configuration analysis, and modified Mosher's method. Craterellyne I exhibited cytotoxicities against human cancer strains and inhibition of nitric oxide (NO) production, as well as weak antimicrobial activity against *Candida albicans*.

KEYWORDS: *acteylenic acids, mushroom, Craterellus lutescens, craterellynes, cytotoxicity*

INTRODUCTION

Mushrooms belonging to the family Cantharellaceae such as *Cantharellus cibarius*, *Cantharellus aureus*, and *Cantharellus cornucopioides* are well-known delicious foods.[1−7] Being low in fat and energy while high in digestible proteins and fiber makes them a useful contribution to vitamin and mineral intake. The basidiomycete *Cantharellus lutescens* can commonly be found in large colonies in some coniferous forests of Yunnan province, China, where the people use it for food as a substitute for *C. cibarius*.[8] However, constituents of this species have not been reported. Craterellynes A−F are six acetylenic acid analogues that we reported previously.[9] A continuing study of the species resulted in the isolation of 13 new acetylenic acid derivatives, namely, craterellynes G−Q (**1**, **2**, **4**−**10**, **12**, **13**), 9-epi-craterellyne H (**3**), and 14-*O*-ethyl-craterellyne O (**11**). These compounds were isolated as pairs of stereoisomers. The structures were established by extensive spectroscopic methods, and their absolute configurations were partially elucidated according to the modified Mosher method. This investigation revealed that acetylenic acid derivatives were the main chemical constituents of *C. lutescens*. In addition, all compounds were evaluated for their inhibitory activities on NO production in LPS-activated RAW264.7 macrophages, together with their cytotoxities against five human cancer cell lines, as well as their antimicrobial activities against *Candida albicans*.

MATERIALS AND METHODS

Instrumentation. Infrared spectroscopy (IR) was measured by a Bruker tensor 27 FT-IR spectrum apparatus (KBr pellets). Ultraviolet spectra (UV) were recorded with a UV-2401PC spectrophotometer. Optical rotations (OR) were detected by a Jasco P-1020 polarimeter. HRESIMS was performed with a 6200 Q-TOF MS system. NMR spectra were obtained with an Avance III 600 MHz spectrometer. Sephadex LH-20, ODS, and silica gel were used in column chromatography (CC). A Sepacore system was used for MPLC preparation, equipped with a pump manager (C-615), a pump module (C-605), as well as a fraction collector (C-660), and columns filled with 40−75 μm Chromatorex C-18 materials. An HPLC (Agilent 1260) equipped with Zorbax SB-C18 columns (5 μm, 150 mm × 9.4 mm or 150 mm × 21.2 mm) and a DAD detector was used for the preparation of sample.

Fungus Material. Fruiting bodies of *C. lutescens* were collected from Tiger Leaping Gorge in Yunnan province, China, on August 17, 2014, and identified by Li Zhenghui, South-Central University for Nationalities (SCUN). The voucher specimen (no. 20140817D) was deposited at the School of Pharmaceutical Sciences, SCUN.

Extraction and Isolation. Fruiting bodies of *C. lutescens* (5 kg) were macerated three times with 95% ethyl alcohol. The extract was evaporated under reduced pressure and dissected by water and ethyl acetate to afford an ethyl acetate extract (105 g). The extract was subjected to CC over silica gel (55 × 10 cm), eluting with a $CHCl_3$−CH_3OH gradient, to afford fractions A−G. Fraction B, obtained by $CHCl_3$−CH_3OH (50:1), was further purified by Sephadex LH-20 (CH_3OH) to afford eight subfractions (B1−B8). Compound **4** (2.3 mg) was isolated from fraction B3 on the basis of pre-HPLC (CH_3CN/H_2O, from 35:65 to 65:35, 30 min). Compounds **2** (3.2 mg) and **3** (2.5 mg) were obtained by pre-HPLC (CH_3CN/H_2O, from 25:75 to 55:45, 30 min) from fraction B5. Compound **1** (5.1 mg) was purified from fraction B7 by pre-HPLC (CH_3CN/H_2O, from 25:75 to 55:45, 30 min). Fraction E was first separated by MPLC

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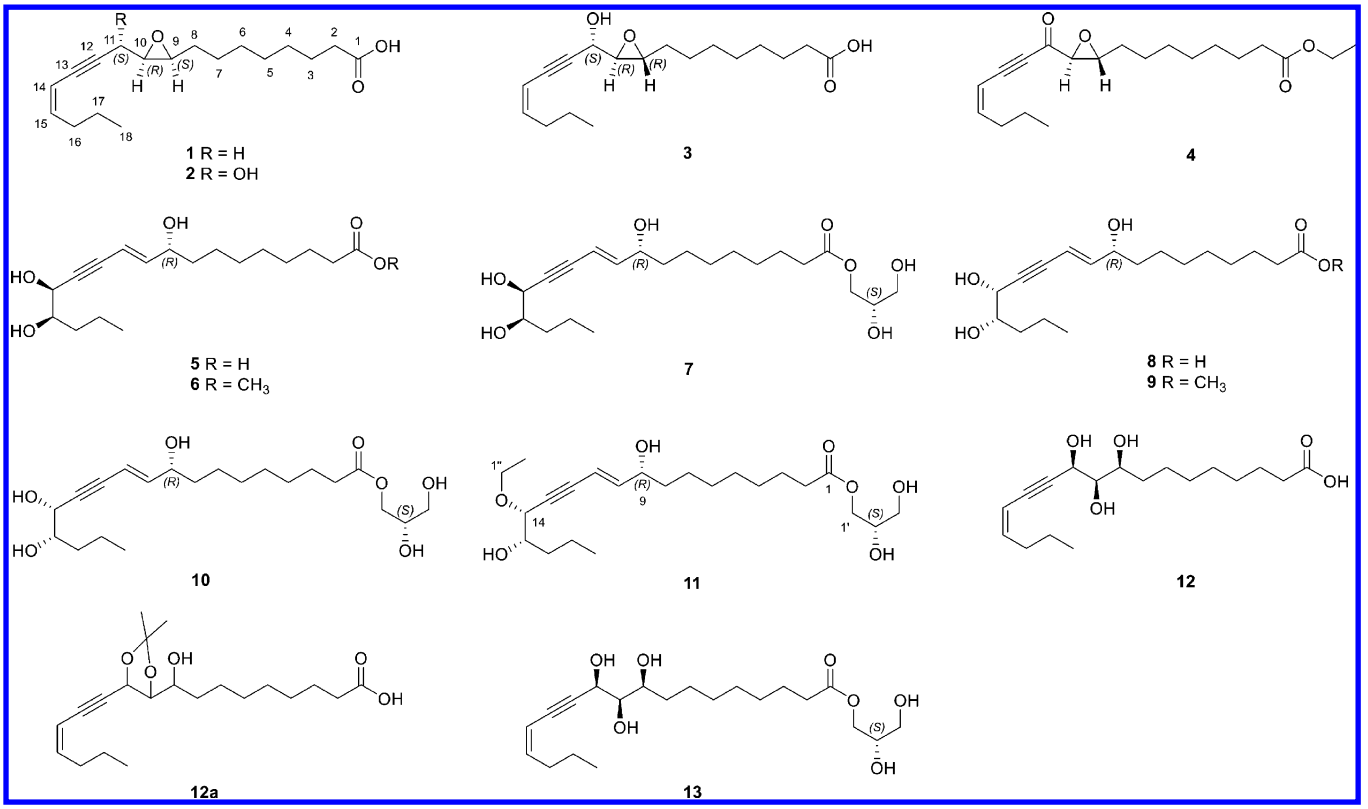

Figure 1. Structures of compounds **1**−**13**.

Table 1. ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) NMR Data of Compounds 1−4 (δ in ppm)

	1[a]		2[b]		3[a]		4[b]	
no.	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		179.0		177.7		179.0		174.0
2	2.26 (t, 7.4)	35.8	2.35 (t, 7.4)	33.7	2.26 (t, 7.3)	35.8	2.28 (t, 7.5)	34.5
3	1.60 (m)	26.4	1.63 (m)	24.8	1.60 (m)	26.4	1.62 (m)	25.0
4	1.38−1.29 (m)	30.5	1.38−1.29 (m)	29.3	1.38−1.30 (m)	30.4	1.38−1.28 (m)	29.2
5	1.38−1.29 (m)	30.4	1.38−1.29 (m)	29.2	1.38−1.30 (m)	30.3	1.38−1.28 (m)	29.2
6	1.38−1.29 (m)	30.3	1.38−1.29 (m)	29.0	1.38−1.30 (m)	30.3	1.38−1.28 (m)	29.1
7	1.51 (m)	27.5	1.50 (m)	26.7	1.46 (m)	27.1	1.47 (m)	25.9
8	1.57 (m)	28.5	1.58 (m)	28.3	1.61 (m); 1.53 (m)	32.5	1.69 (m); 1.63 (m)	31.7
9	2.99 (td, 4.2, 6.0)	58.2	3.07 (td, 4.8, 6.8)	57.6	2.96 (td, 5.7, 2.1)	57.5	3.26 (ddd, 6.5, 4.9, 1.8)	59.3
10	3.14 (ddd, 7.0, 5.8, 4.2)	56.5	3.21 (dd, 7.8, 4.8)	60.2	2.92 (dd, 6.0, 2.1)	62.2	3.34 (d, 1.8)	60.3
11	2.71 (ddd, 17.3, 5.8, 2.1); 2.47 (ddd, 17.3, 7.0, 2.1)	20.1	4.36 (d, 7.8)	62.5	4.25 (dd, 6.0, 1.6)	64.7		184.6
12		89.8		89.5		91.5		89.4
13		80.1		84.0		83.3		91.5
14	5.46 (dd, 10.7)	110.2	5.50 (d, 10.8)	108.2	5.52 (d, 10.8)	109.5	5.63 (d, 10.9)	107.1
15	5.88 (dt, 10.7, 7.4)	144.0	5.98 (dt, 10.8, 7.5)	145.6	5.98 (dt, 10.8, 7.5)	145.6	6.35 (dt, 10.9, 7.7)	153.2
16	2.28 (m)	33.2	2.28 (m)	32.5	2.30 (m)	33.3	2.38 (m)	33.2
17	1.44 (m)	23.2	1.45 (m)	22.1	1.45 (m)	23.1	1.49 (m)	22.0
18	0.94 (t, 7.4)	14.1	0.93 (t, 7.4)	13.9	0.95 (t, 7.4)	14.1	0.95 (t, 7.4)	13.8
1′							4.12 (q, 7.1)	60.4
2′							1.25 (t, 7.1)	14.4

[a]Data were measured in methanol-d_4. [b]Data were measured in $CDCl_3$.

(CH_3OH/H_2O from 20:80 to 100:0) to give eight fractions (E1−E8). Fractions E1 and E2 were further separated by Sephadex LH-20 (CH_3OH) to give seven subfractions (E1-1−E1-7) and nine subfractions (E2-1−E2-9), respectively. Then the subfractions E1-4, E1-5, E2-4, E2-5, and E2-6 were purified successively by pre-HPLC ($MeCN/H_2O$, from 15:85 to 35:65, 40 min) to give **6** (3.8 mg, subfraction E1-4), **5** (1.7 mg, subfraction E1-5), **8** (4.6 mg, subfraction E1-5), **7** (2.6 mg, subfraction E2-4), **10** (4.1 mg, subfraction E2-4), **13** (1.2 mg, subfraction E2-4), **11** (12.7 mg, subfraction E2-4), **9** (3.6 mg, subfraction E2-5), and **12** (5.3 mg, subfraction E2-6), respectively (structures given in Figure 1).

Craterellyne G, 1: colorless oil, $[\alpha]_D^{20}$ −7.9 (*c* 0.3, $CH_3OH/CHCl_3$ 1:1); IR (KBr) υ_{max} 3425, 2930, 2859, 1729, 1632, 1386, 1249, 1181, 733 cm^{-1}; UV ($MeOH/CHCl_3$ 1:1) λ_{max} (log ε) 237 (3.60) nm; ^{1}H

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Table 2. ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) NMR Data of Compounds 5−9 in Methanol-d_4 (δ in ppm)

	5		6		7		8		9	
no.	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		181.2		176.0		175.5		179.7		176.0
2	2.22 (t, 7.1)	37.1	2.32 (t, 7.4)	34.8	2.36 (t, 7.4)	34.9	2.25 (t, 7.2)	36.1	2.32 (t, 7.4)	34.8
3	1.60 (m)	26.9	1.61 (m)	26.0	1.62 (m)	26.0	1.60 (m)	26.5	1.61 (m)	26.0
4	1.38−1.29 (m)	30.7	1.38−1.29 (m)	30.5	1.38−1.29 (m)	30.5	1.38−1.29 (m)	30.5	1.38−1.29 (m)	30.5
5	1.38−1.29 (m)	30.6	1.38−1.29 (m)	30.3	1.38−1.29 (m)	30.3	1.38−1.29 (m)	30.4	1.38−1.29 (m)	30.3
6	1.38−1.29 (m)	30.5	1.38−1.29 (m)	30.1	1.38−1.29 (m)	30.1	1.38−1.29 (m)	30.3	1.38−1.29 (m)	30.1
7	1.50 (m); 1.34 (m)	26.4	1.49 (m); 1.34 (m)	26.4	1.49 (m); 1.34 (m)	26.4	1.50 (m); 1.34 (m)	26.4	1.49 (m); 1.34 (m)	26.4
8	1.49 (m)	38.0	1.50 (m)	38.0	1.49 (m)	38.0	1.49 (m)	38.0	1.49 (m)	38.0
9	4.07 (dt, 6.4, 6.1)	72.7	4.07 (dt, 6.2, 6.1)	72.7	4.07 (dt, 6.4, 6.1)	72.7	4.07 (dt, 6.4, 6.1)	72.7	4.07 (dt, 6.4, 6.1)	72.7
10	6.11 (dd, 15.9, 6.1)	147.6	6.11 (dd, 15.9, 6.1)	147.6	6.11 (dd, 15.9, 6.1)	147.6	6.11 (dd, 15.9, 6.1)	147.7	6.11 (dd, 15.9, 6.1)	147.7
11	5.72 (d, 15.9)	110.0	5.73 (d, 15.9)	110.0	5.73 (d, 15.9)	110.0	5.72 (d, 15.9)	109.8	5.72 (d, 15.9)	109.8
12		84.5		84.5		84.5		84.6		84.6
13		89.4		89.4		89.4		89.5		89.5
14	4.30 (d, 4.6)	67.7	4.30 (d, 4.6)	67.7	4.30 (d, 4.6)	67.6	4.23 (d, 6.4)	67.7	4.23 (d, 6.4)	67.7
15	3.57 (ddd, 9.0, 4.6, 3.2)	75.2	3.57 (ddd, 9.0, 4.6, 3.2)	75.1	3.57 (m)	75.1	3.51 (ddd, 8.8, 6.4, 2.8)	75.4	3.51 (ddd, 8.8, 6.4, 2.8)	75.4
16	1.59 (m)	35.6	1.59 (m)	35.6	1.59 (m)	35.6	1.66 (m); 1.44 (m)	35.6	1.66 (m); 1.44 (m)	35.6
17	1.58 (m); 1.40 (m)	20.0	1.58 (m); 1.40 (m)	20.0	1.58 (m); 1.40 (m)	20.0	1.58 (m); 1.42 (m)	19.9	1.58 (m); 1.42 (m)	20.0
18	0.95 (t, 7.2)	14.4	0.95 (t, 7.2)	14.4	0.96 (t, 7.2)	14.4	0.95 (t, 7.2)	14.4	0.95 (t, 7.1)	14.5
1′			3.65 (s)	52.0	4.15 (dd, 11.4, 4.3)	66.5			3.65 (s)	52.0
					4.06 (dd, 11.4, 6.3)					
2′					3.82 (m)	71.1				
3′					3.55 (dd, 5.5, 2.8)	64.0				

NMR data (Table 1); HRESIMS m/z 315.1930 $[M + Na]^+$ (calcd for $C_{18}H_{28}O_3Na$, 315.1931).

*Craterellyne H, **2**:* colorless oil, $[\alpha]_D^{20}$ −4.1 (c 0.05, $CH_3OH/CHCl_3$ 1:1); IR (KBr) υ_{max} 3421, 2928, 2858, 1713, 1632, 1387, 1247, 1179, 1032 cm^{-1}; UV ($CH_3OH/CHCl_3$ 1:1) λ_{max} (log ε) 237 (3.17) nm; ^{1}H NMR data (Table 1); HRESIMS m/z 307.1919 $[M - H]^-$ (calcd for $C_{18}H_{28}O_4$, 307.1919).

*9-epi-Craterellyne H, **3**:* colorless oil, $[\alpha]_D^{20}$ −4.7 (c 0.2, CH_3OH); IR (KBr) υ_{max} 3421, 2930, 2859, 1730, 1613, 1385, 1247, 1179, 1032 cm^{-1}; UV (CH_3OH) λ_{max} (log ε) 226 (3.92), 199 (3.65) nm; ^{1}H NMR data (Table 1); HRESIMS m/z 331.1882 $[M + Na]^+$ (calcd for $C_{18}H_{28}O_4Na$, 331.1882).

*Craterellyne I, **4**:* colorless oil, $[\alpha]_D^{20}$ −3.3 (c 0.2, CH_3OH); IR (KBr) υ_{max} 3431, 2931, 2858, 1734, 1631, 1380, 1265, 1180, 1032 cm^{-1}; UV (MeOH) λ_{max} (log ε) 277 (3.89), 205 (3.88) nm; ^{1}H NMR data (Table 1); HRESIMS: m/z 357.2038 $[M + Na]^+$ (calculated for $C_{20}H_{30}O_4Na$, 357.2036).

*Craterellyne J, **5**:* colorless oil, $[\alpha]_D^{20}$ −11.0 (c 0.2, CH_3OH); IR (KBr) υ_{max} 3415, 2931, 2857, 1710, 1563, 1385, 1166, 1055, 959 cm^{-1}; UV (CH_3OH) λ_{max} (log ε) 228 (3.95), 196 (3.59) nm; ^{1}H NMR data (Table 2); HRESIMS m/z 325.2014 $[M - H]^-$ (calcd for $C_{18}H_{30}O_5$, 325.2020).

*Craterellyne K, **6**:* colorless oil, $[\alpha]_D^{20}$ −14.1 (c 0.2, CH_3OH); IR (KBr) υ_{max} 3416, 2931, 2859, 1736, 1591, 1439, 1384, 1170, 1032 cm^{-1}; UV (CH_3OH) λ_{max} (log ε) 228 (3.94), 197 (3.58) nm; ^{1}H NMR data (Table 2); HRESIMS m/z 363.2145 $[M + Na]^+$ (calcd for $C_{19}H_{32}O_5$, 363.2142).

*Craterellyne L, **7**:* colorless oil, $[\alpha]_D^{20}$ −5.3 (c 0.3 CH_3OH); IR (KBr) υ_{max} 3423, 2928, 2856, 1734, 1630, 1463, 1384, 1172, 1033 cm^{-1}; UV (CH_3OH) λ_{max} (log ε) 227 (4.10), 197 (3.76) nm; ^{1}H NMR data (Table 2); HRESIMS m/z 423.2357 $[M + Na]^+$ (calcd for $C_{21}H_{36}O_7$, 423.2353).

*Craterellyne M, **8**:* colorless oil, $[\alpha]_D^{20}$ −3.7 (c 0.7 CH_3OH); IR (KBr) υ_{max} 3420, 2931, 2857, 1710, 1628, 1385, 1128, 1032, 959 cm^{-1}; UV (CH_3OH) λ_{max} (log ε) 228 (3.87), 195 (3.46) nm; ^{1}H NMR data (Table 2); HRESIMS m/z 325.2016 $[M - H]^-$ (calcd for $C_{18}H_{30}O_5$, 325.2020).

*Craterellyne N, **9**:* colorless oil, $[\alpha]_D^{20}$ −7.0 (c 0.4 CH_3OH); IR (KBr) υ_{max} 3403, 2932, 2858, 1738, 1571, 1384, 1171, 1032, 959 cm^{-1}; UV (CH_3OH) λ_{max} (log ε) 227 (4.08), 197 (3.80) nm; ^{1}H NMR data (Table 2); HR-ESI-MS indicated m/z 363.2145 $[M + Na]^+$ (calculated for $C_{19}H_{32}O_5$, 363.2142).

*Craterellyne O, **10**:* colorless oil, $[\alpha]_D^{20}$ −3.2 (c 0.4 CH_3OH); IR (KBr) υ_{max} 3389, 2931, 2860, 1731, 1628, 1459, 1384, 1175, 1053 cm^{-1}; UV (CH_3OH) λ_{max} (log ε) 227 (4.15), 197 (3.84) nm; ^{1}H NMR data (Table 3); HRESIMS m/z 423.2356 $[M + Na]^+$ (calcd for $C_{21}H_{36}O_7$, 423.2353).

*14-O-ethyl-Craterellyne O, **11**:* colorless oil, $[\alpha]_D^{20}$ +3.4 (c 0.6 CH_3OH); IR (KBr) υ_{max} 3416, 2931, 2861, 1732, 1629, 1459, 1385, 1176, 1094 cm^{-1}; UV (CH_3OH) λ_{max} (log ε) 227 (4.02) nm; ^{1}H NMR data (Table 3); HRESIMS m/z 451.2672 $[M + Na]^+$ (calcd for $C_{23}H_{40}O_7$, 451.2666).

*Craterellyne P, **12**:* colorless oil, $[\alpha]_D^{20}$ −4.4 (c 0.6 CH_3OH); IR (KBr) υ_{max} 3418, 2930, 2858, 1711, 1623, 1569, 1386, 1067 cm^{-1}; UV (CH_3OH) λ_{max} (log ε) 227 (3.90), 196 (3.53) nm; ^{1}H NMR data (Table 3); HRESIMS m/z 325.2016 $[M - H]^-$ (calcd for $C_{18}H_{30}O_5$, 325.2020).

*Craterellyne Q, **13**:* colorless oil, $[\alpha]_D^{20}$ −8.8 (c 0.2 MeOH); IR (KBr) υ_{max} 3398, 2930, 2858, 1734, 1569, 1384, 1185, 1056 cm^{-1}; UV (MeOH) λ_{max} (log ε) 226 (3.71) nm; ^{1}H NMR data (Table 3); HRESIMS m/z 423.2356 $[M + Na]^+$ (calcd for $C_{21}H_{36}O_7$, 423.2353).

Test of Nitric Oxide Production Inhibitory Activity. RPMI 1640 was used as a medium of maintaining RAW264.7 macrophages cells at 37 °C, in 5% CO_2, supplemented with 10% FBS (Hyclone, Ingelheim, Germany). Then, the cells were plated and incubated in 96-well plates (2×10^5 cells/well) for 24 h. After pre-incubation, the cells were treated with 1 μg/mL LPS first and then with series dilutions of the test compounds with a maximum concentration up to 25 μM for 18 h. DMSO was used as a solvent of the compound, which could produce different concentrations with further dilution. The media were collected and assessed for NO production in each well by Griess reacting. After an incubation for 5 min, the absorbance of samples was measured at 570 nm by a microplate reader (PerkinElmer Life Sciences, Inc., Boston, MA, USA). L-NMMA (N^G-monomethyl-L-arginine, monoacetate salt) was used as a positive control (IC_{50} = 42.6 μM).

Cytotoxic Assay. The cytotoxicities of compounds against human lung cancer A-549, myeloid leukemia HL-60, breast cancer MCF-7,

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Table 3. ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) NMR Data of Compounds 10−13 in Methanol-d_4 (δ in ppm)

	10		11		12		13	
no.	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		175.5		175.5		180.5		175.5
2	2.36 (t, 7.4)	34.9	2.38 (t, 7.5)	34.9	2.24 (t, 7.5)	36.6	2.36 (t, 7.4)	34.9
3	1.63 (m)	26.0	1.64 (m)	25.9	1.60 (m)	26.7	1.62 (m)	26.0
4	1.38−1.29 (m)	30.5	1.38−1.29 (m)	30.4	1.38−1.29 (m)	30.7	1.38−1.29 (m)	30.7
5	1.38−1.29 (m)	30.3	1.38−1.29 (m)	30.3	1.38−1.29 (m)	30.5	1.38−1.29 (m)	30.4
6	1.38−1.29 (m)	30.1	1.38−1.29 (m)	30.1	1.38−1.29 (m)	30.5	1.38−1.29 (m)	30.2
7	1.49 (m); 1.34 (m)	26.4	1.51 (m); 1.35 (m)	26.4	1.60 (m)	26.7	1.60 (m)	26.4
8	1.49 (m)	38.0	1.52 (m)	37.9	1.65 (m); 1.45 (m)	33.4	1.81 (m); 1.40 (m)	34.2
9	4.07 (m)	72.7	4.09 (m)	72.6	3.75 (ddd, 9.3, 6.4, 2.5)	72.4	3.59 (dt, 8.1, 2.4)	73.2
10	6.11 (dd, 15.9, 6.1)	147.7	6.14 (dd, 15.9, 6.1)	148.0	3.46 (dd, 6.4, 4.8)	78.6	3.45 (dd, 8.1, 4.0)	78.5
11	5.72 (d, 15.9)	109.8	5.75 (d, 15.9)	109.6	4.57 (dd, 4.8, 1.7)	64.5	4.73 (dd, 4.0, 1.7)	65.7
12		84.6		86.0		92.8		92.8
13		89.5		87.5		83.4		83.4
14	4.23 (d, 6.4)	67.7	4.04 (d, 6.4)	75.7	5.52 (d, 10.8)	109.8	5.53 (d, 10.8)	110.0
15	3.51 (m)	75.4	3.61 (m)	74.0	5.94 (dt, 10.8, 7.5)	144.9	5.93 (dt, 10.8, 7.5)	144.8
16	1.66 (m); 1.44 (m)	35.6	1.67 (m); 1.48 (m)	35.8	2.32 (m)	33.3	2.32 (m)	33.4
17	1.58 (m); 1.42 (m)	20.0	1.58 (m); 1.41 (m)	19.8	1.44 (m)	23.2	1.44 (m)	23.2
18	0.95 (t, 7.1)	14.5	0.97 (t, 7.3)	14.4	0.95 (t, 7.4)	14.1	0.95 (t, 7.3)	14.1
1′	4.15 (dd, 11.4, 4.2); 4.06 (dd, 11.4, 6.2)	66.5	4.17 (dd, 11.4, 4.3); 4.08 (dd, 11.4, 6.2)	66.5			4.15 (dd, 11.4, 4.3); 4.06 (dd, 11.4, 6.3)	66.5
2′	3.82 (m)	71.1	3.84 (m)	71.1			3.82 (m)	71.1
3′		3.55 (dd, 5.5, 2.8)	64.0	3.57 (dd, 5.4, 3.5)	64.0		3.55 (dd, 5.5, 2.8)	64.1
1″				3.82 (m); 3.49 (dq, 14.0, 7.0)	65.7			
2″				1.23 (t, 7.0)	15.4			

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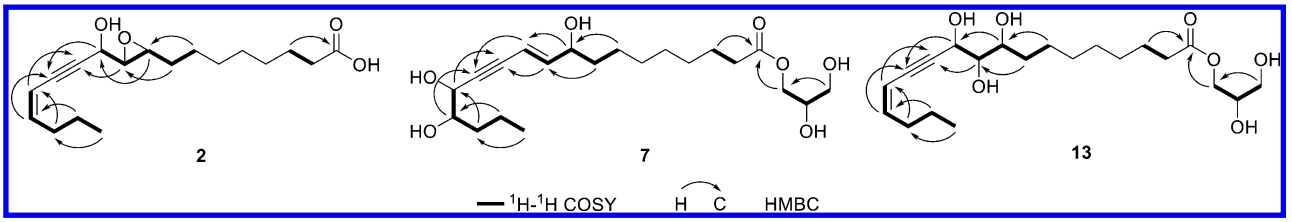

Figure 2. Key 2D NMR correlations of **2**, **7**, and **13**.

hepatocellular carcinoma SMMC-7721, and colon cancer SW480 were evaluated. DMEM or RPMI-1640, supplemented with 10% FBS (Hyclone), served as medium of maintaining cells in 5% CO_2, at 37 °C. The 3-(4,5-dimethylthiazol-2-yl)-5(3-carboxymethoxyphenyl)-2-(4-sulfopheny)-2*H*-tetrazolium (MTS) method was used for cytotoxicity assay with 96-well plates.[10] To each well was added 100 μL of cells followed by incubation for 12 h without test compounds. The test compounds were dissolved with DMSO and prepared to different concentrations (0.128, 0.64, 3.2, 16, and 80 μM), which were added to each tumor cell line for 48 h in triplicate, and the positive control was cisplatin (Sigma). The method of Reed and Muench was used as a way of calculating IC_{50} values.[11]

Antifungal Activity Assay. The antifungal activities against *Candida albicans* were evaluated using a modification of the broth microdilution method[12] in 96-well microtiter plates. Briefly, compound **4** dissolved in DMSO (concentrations of 200, 100, 50, 25, 12.5 μM) was added to each well, respectively, and then 100 μL of fungus suspension at a density of 10^5 CFU/mL was inoculated to each well and incubated at 37 °C. After an incubation of 24 h, the optical density of each well was detected at 625 nm by a microplate reader. The method of Reed and Muench was used as a way of calculating IC_{50} values.

RESULTS AND DISCUSSION

Structure Elucidation. Compound **1** was obtained as a colorless oil. Its molecular formula, $C_{18}H_{28}O_3$, was identified by HRESIMS. The ^{13}C NMR data of **1** (Table 1) showed it possessed seven aliphatic methylene carbons, a methyl carbon at δ 14.1, a carboxyl at δ 179.0, two olefinic carbons at δ 144.0 and 110.2, two acetylenic carbons at δ 89.8 and 80.1, and two oxygenated carbons at δ 58.2 and 56.5. Correspondingly, ^{1}H NMR data (Table 1) showed the occurrence of two olefinic (δ_H 5.88, 5.46), two oxymethine (δ_H 3.14, 2.99), an allylic methylene proton (δ_H 2.28) and a methyl group (δ_H 0.94). A chain structure from C-14 to C-18 was readily identified by 2D NMR data as shown in Figure 2. The HMBC signals from H-15 (δ_H 5.88) to C-13 (δ_C 80.1) and from H-14 (δ_H 5.46) to C-12 (δ_C 89.8), as well as the upfield shift of C-14 (δ_C 110.2) revealed that C-14 was connected with the acetylene group. The characteristic chemical shifts of two methine signals (δ_H 3.14, 2.99) indicated the existence of an epoxy ring. The location of the epoxy ring was assigned by observing ^{1}H−^{1}H COSY correlations for H-8/H-9, H-9/H-10, and H-10/H-11, together with HMBC correlations from H-10 (δ_H 3.14) to C-12 (δ_C 89.8) and from H-11 (δ_H 2.71, 2.47) to C-13 (δ_C 80.1). The coupling constant of H-9/H-10 (J = 4.2 Hz) and NOE data suggested the configuration of the epoxide was *cis* form.[13,14] Therefore, compound **1** was identified and named craterellyne G.

The molecular formula of craterellyne H, **2**, was established to be $C_{18}H_{28}O_4$ on the basis of HRESIMS data. The NMR data (Table 1) of **2** were in good agreement with those of **1** except that H-11 (δ_H 2.71, 2.47) was substituted by a hydroxy group (δ_C 62.5, δ_H 4.36), which was supported by a correlation from H-11 (δ_H 4.36) to C-13 (δ_C 84.0) in the HMBC spectrum. The coupling constant of H-9/H-10 (J = 4.8 Hz) and the ROESY data supported that the relative configuration of the epoxy moiety was *cis* form. The modified Mosher method was applied to determine the absolute configuration of C-11. From the values of $\Delta\delta$ ($\delta_S - \delta_R$), the absolute configuration was identified as *S* for C-11 in **2**. Thus, the absolute configuration of C-9 was elucidated as *S*, whereas C-10 was *R*.

9-Epi-craterellyne H, **3**, a colorless oil, had a molecular formula of $C_{18}H_{28}O_4$ as confirmed by HRESIMS data. Analyses of NMR data (Table 1) indicated that compounds **3** and **2** shared the same functional groups. A key difference in the coupling constant of H-10 and H-9 (J = 2.1 Hz) and ROESY data suggested that the relative configuration of the epoxy moiety should be *trans* form. In the modified Mosher's experiment, the values of $\Delta\delta$ ($\delta_S - \delta_R$) allowed the absolute configurations of C-9, C-10, and C-11 to be *R*, *R*, and *S*, respectively.

Craterellyne I, **4**, was isolated as a colorless oil. HRESIMS showed its molecular formula was $C_{20}H_{30}O_4$. It could be observed in the NMR data (Table 1) that **4** and **3** were very similar, except that the hydroxy at C-11 (δ_C 64.7) in **3** was replaced by a ketone group (δ_C 184.6) in **4**, whereas the carboxyl group at δ 179.0 in **3** was esterified as an ethyl ester in **4**. The relative configuration of the epoxy moiety was elucidated to be the same as that of **3** by the coupling constant of H10/H-9 (J = 1.8 Hz) and ROESY data.

Craterellynes J and M, **5** and **8**, colorless oils, were obtained as a pair of diastereomers. They shared the same molecular formula $C_{18}H_{30}O_5$ as estabilised by HRESIMS. By comparison with NMR data (Table 2) of the known compound craterellyne F,[9] the acetylenic acid partial structure of C-1 to C-13 could be easily identified. The major change was that the olefinic carbons at C-14 (δ_C 110.4) and C-15 (δ_C 144.2) in craterellyne F were replaced by two oxymethine carbons (δ_C 67.7, 75.2) and (δ_C 67.7, 75.4) in **5** and **8**. A close inspection of the 2D NMR spectra (Figure 2) could further determine the location of two oxymethine carbons. The coupling constants between H-14 and H-15 in **5** and **8** were 4.6 and 6.4 Hz, respectively. In addition, H-14 and H-15 in the 14,15-*O*-isopropylidene derivatives of **5** and **8** displayed an evident ROESY correlation. Thus, the relative configurations were both *erythro* at C-14 and C-15 of **5** and **8**, whereas they were exactly opposite from each other.

Craterellynes K and N, **6** and **9**, colorless oils, were also isolated as a pair of diastereomers. HRESIMS data suggested they possessed the same molecular formula, $C_{19}H_{32}O_5$. Detailed analysis of 1D and 2D NMR data suggested that compounds **6** and **9** were methyl ester derivatives of **5** and **8**, respectively. The stereoconfigurations were also elucidated as shown in Figure 1 by coupling constants and 2D NMR data.

Craterellynes L and O, **7** and **10**, were another pair of diastereomers isolated as colorless oils. Their molecular formulas were $C_{21}H_{36}O_7$ as confirmed by the HRESIMS data.

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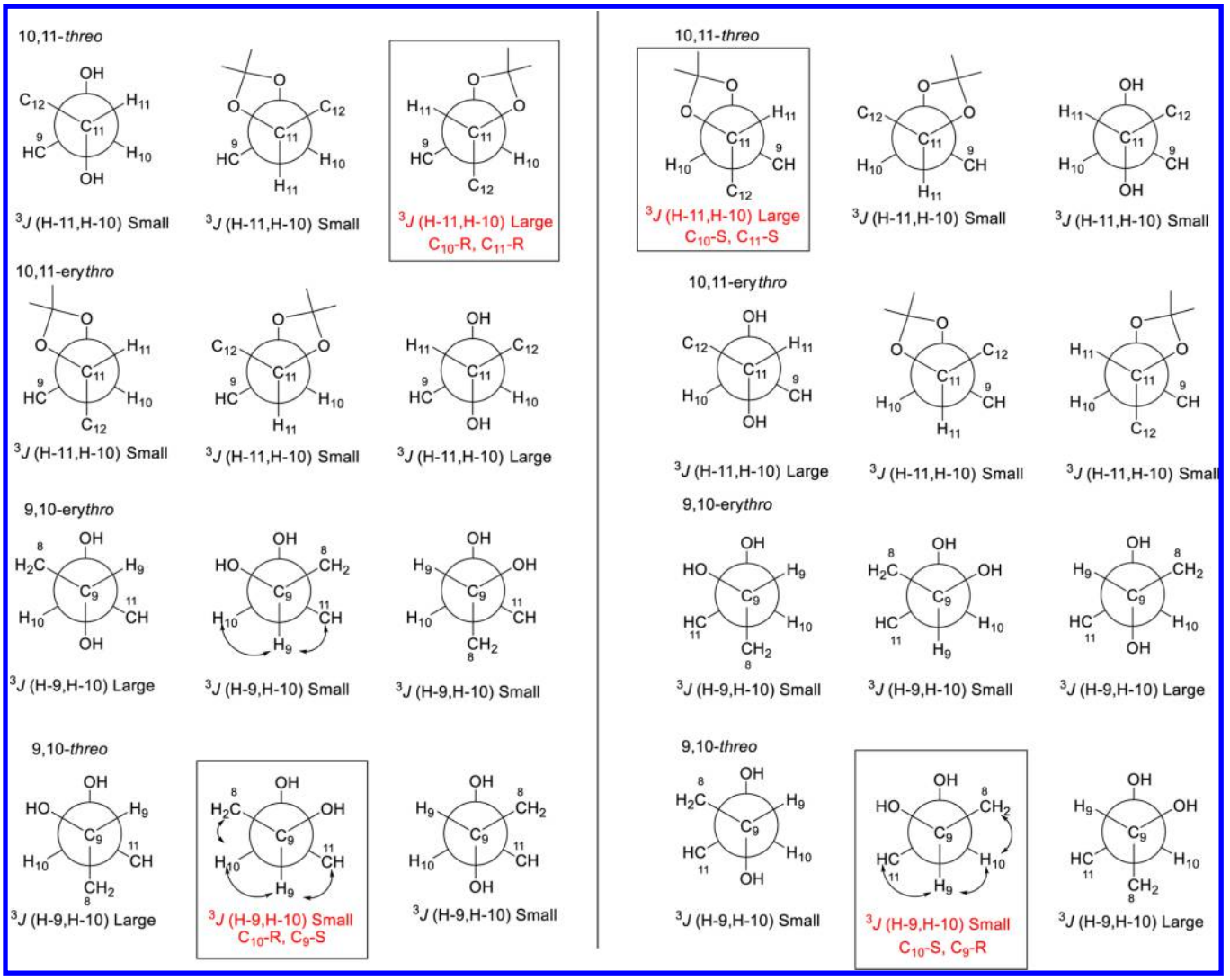

Figure 3. Stereochemistry of C-9, C-10, and C-11 in the 10,11-*O*-isopropylidene derivative of **12**. Box indicates conformation that agrees with measured data.

The gross structures of **7** and **10** were elucidated to be similar to those of **5** and **8**, respectively, except for an extra glycerol unit in **7** and **10**, which were supported by the 1D (Tables 2 and 3) and 2D NMR data. The correlations between H-1′ (δ_H 4.15, dd; 4.06, dd) and H-2′ (δ_H 3.82, m) and H-2′/H-3′ (δ_H 3.55, dd) could be found in ^{1}H−^{1}H COSY spectra, whereas correlations from H-1′ to C-1 (δ_C 175.5) were observed in HMBC spectra of both **7** and **10**. The monoglycerides isolated from the sponge *Stelletta* sp[15] showed the same absolute configuration as **7** and **10**. Thus, the configurations of C-2′ in **7** and **10** were presumed to be *S*.

Compound **11** possessed a molecular formula of $C_{23}H_{40}O_7$ as established by HRESIMS. The MS data, as well as analysis of NMR data (Table 3), revealed that compound **11** was an *O*-ethyl derivative of **10**. The HMBC correlations from δ_H 3.82 and 3.49 (each 1H, H-1″) to δ_C 75.7 (d, C-14) suggested that the *O*-ethyl moiety should be at C-14. Further analyses of NMR data indicated the other parts of **11** to be the same to that of **10**. Therefore, compound **11** was determined as 14-*O*-ethylcraterellyne O.

The absolute configurations of **5**−**11** at C-9 were established by applying the modified Mosher's method to the 14,15-*O*-isopropylidene derivatives of **6** and **9**. From the values of $\Delta\delta$ ($\delta_S - \delta_R$), the absolute configurations of C-9 for **5**−**11** were assigned as *R*. According to the ROESY data of 14,15-*O*-isopropylidene derivatives and NMR spectra, the relative configurations of **5**−**7** at C-14, C-15 were the same, whereas **8**−**11** were exactly opposite, respectively.

The molecular formula $C_{18}H_{30}O_5$ of **12**, namely, craterellyne P, was assigned by HRESIMS, which was the same as that of **5** and **8**. The NMR data (Table 3), as well as HMBC and ^{1}H−^{1}H COSY correlations of **12**, revealed that there were three hydroxy groups (δ_H 3.75, 3.46, 4.57) attached to C-9, C-10, and C-11 (δ_C 72.4, 78.6, and 64.5), respectively. Trying to determine the absolute configurations of C-9, C-10, and C-11, the modified Mosher's method to 9,10-*O*-isopropylidene derivative or 10,11-*O*-isopropylidene derivative of **12** was applied. However, esterification of the derivatives of **12** with MTPA was unsuccessful. Fortunately, the relative configurations could be identified according to the ROESY correlations of 10,11-*O*-isopropylidene derivative (**12a**) of **12** as well as *J*-based configuration analysis[16] (Figure 3). The coupling constants of H-10/H-11 (J = 6.5 Hz) and H-9/H-10 (J = 4.9 Hz) in **12a** indicated eight possible configurations for both C-9 and C-10 and two for both C-10 and C-11 (Figure 3). However, the correlations of H-9/H-10, H-10/H-11, and H-8/

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H-10 in the ROESY spectrum supported the configurations of C-9/C-10 and C-10/C-11 were both *threo*. Therefore, the relative configurations of C-9, C-10, and C-11 were assigned as *S**, *R**, and *R**.

Craterellyne Q, **13**, was isolated as a minor constituent, which possessed the molecular formula $C_{21}H_{36}O_7$ on the basis of HRESIMS data. Analyses of ^1H and ^{13}C NMR data (Table 3) suggested that **13** was the monoglyceride derivative of **12**. The relative configuration of **13** was the same as that of **12** upon comparison of NMR data, as well as *J*-based configuration analysis.

Biological Activity Assays. All compounds were assessed for their antimicrobial activity against *Candida albicans* and anti-NO activity. As a result, compound **4** showed activity with an MIC_{50} value of 53.5 μg/mL and an IC_{50} value of 19.8 μM. In addition, the cytotoxicity of all compounds was also evaluated, and compound **4** demonstrated a certain inhibitory activity, with IC_{50} values comparable to those of cisplatin (Table 4). However, the other compounds showed no inhibitory activity (IC_{50} > 40 μM).

Table 4. Cytotoxicities of Compound 4 (IC_{50}, μM)

compound	HL-60	A-549	SMMC-7721	MCF-7	SW480
4	11.1	39.9	18.3	21.6	17.2
cisplatin	3.1	19.0	13.0	23.6	11.2

In conclusion, we reported here the isolation of 13 new acetylenic acid derivatives from the mushroom *C. lutescens*, as well as their bioactivities. Within acetylenic acid analogues we reported previously,[9] more than 20 acetylenic acid derivatives have been reported from fruiting bodies of *C. lutescens*. The results indicated that acetylenic acid derivatives are major components in *C. lutescens*.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.7b00899.

NMR spectra and HRESIMS of compounds **1**−**13** (PDF)

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS USED

HSQC, ^1H detected heteronuclear single-quantum coherence spectroscopy; HMBC, ^1H detected heteronuclear multiple-bond coherence spectroscopy; COSY, homonuclear correlation spectroscopy; ROESY, rotating-frame Overhauser effect spectroscopy

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