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To cite this article: Hua Guo , Tao Feng , Zheng-Hui Li & Ji-Kai Liu (2012) Four new compounds from the basidiomycete *Craterellus odoratus* , Journal of Asian Natural Products Research, 14:10, 950-955, DOI: [10.1080/10286020.2012.729508](https://doi.org/10.1080/10286020.2012.729508)

To link to this article: <http://dx.doi.org/10.1080/10286020.2012.729508>



Published online: 10 Oct 2012.



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Four new compounds from the basidiomycete *Craterellus odoratus*

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(Received 17 July 2012; final version received 10 September 2012)

Four new compounds, craterellin D (**1**), 5-hydroxymethyl-2-hydroxy-4-methoxy-phenylethanone (**2**), 5-carboxyl-2-[2-(1,2-dihydroxyisopropyl)]-benzofuran (**3**), and 6 α -hydroxy-3-methoxy-4 α -methyl-2-cyclohexen-1-one (**4**), were isolated from cultures of the basidiomycete *Craterellus odoratus*, together with three known compounds craterellins A–C (**5**–**7**). Structures of **1**–**4** were elucidated on the basis of extensive spectroscopic analysis. Selected compounds were evaluated for their inhibitory activities against one isozyme of 11 β -hydroxysteroid dehydrogenase (11 β -HSD1) and cytotoxic activities on five tumor cell lines. Compound **2** was found to exhibit inhibitory activity against human 11 β -HSD1 with an IC₅₀ value of 16.4 μ g/ml.

Keywords: *Craterellus odoratus*; craterellin D; 5-hydroxymethyl-2-hydroxy-4-methoxy-phenylethanone; 5-carboxyl-2-[2-(1,2-dihydroxyisopropyl)]-benzofuran; 6 α -hydroxy-3-methoxy-4 α -methyl-2-cyclohexen-1-one

1. Introduction

The fungus *Craterellus odoratus* (Schwein.) Fr. belonging to the family Cantharellaceae, is an edible fungus with a wide distribution in most parts of China, such as Jilin, Anhui and Jiangshu Provinces. In our previous work, the fungus has been reported to possess a series of merosesquiterpenoids, while one of the merosesquiterpenoids demonstrated significant inhibitory activities against human 11 β -HSD2 [1]. In the course of searching for more biologically active compounds, we have investigated an enlarged culture on the fungus. As a result, four new compounds, craterellin D (**1**), 5-hydroxymethyl-2-hydroxy-4-methoxy-phenylethanone (**2**), 5-carboxyl-2-[2-(1,2-dihydroxyisopropyl)]-benzofuran (**3**), 6 α -hydroxy-3-methoxy-4 α -methyl-2-cyclohexen-1-one (**4**), together with three known

compounds, craterellin A (**5**) [1], craterellin B (**6**) [1], and craterellin C (**7**) [1], were obtained (Figure 1). Compounds **1** and **2** were evaluated for their inhibitory activities against one isozyme of 11 β -hydroxysteroid dehydrogenase (11 β -HSD1), while compounds **1**, **2**, and **5**–**8** were evaluated for cytotoxic activities on five human cancer cell lines. Herein, we report the isolation, structural elucidation, and bioassay results of these compounds.

2. Results and discussion

Craterellin D (**1**) was obtained as a yellow oil. Its formula was determined to be C₂₂H₃₄O₅ by HR-ESI-MS, which indicated six degrees of unsaturation. The IR spectrum showed absorption bands for OH (3424 cm⁻¹) and C=C (1633 cm⁻¹). The ¹³C NMR and DEPT spectra (Table 1)

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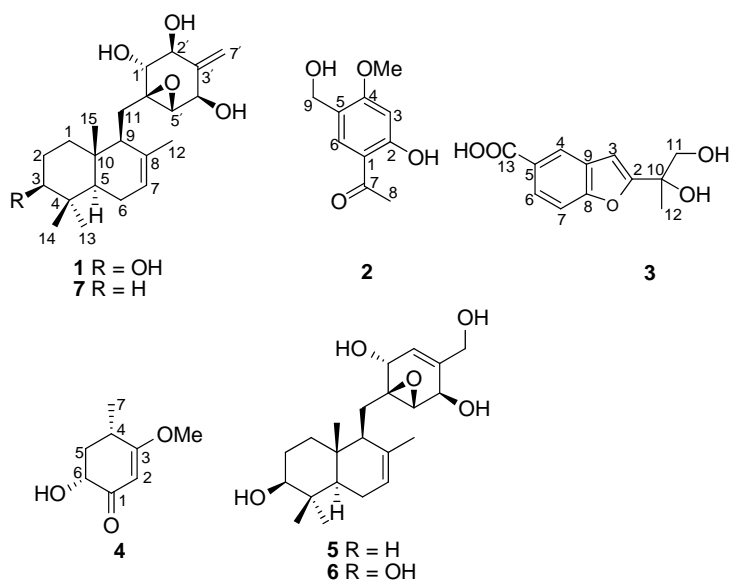


Figure 1. Structures of compounds 1–7.

Table 1. ^1H and ^{13}C NMR spectroscopic data of **1** at 400/100 MHz, respectively, in acetone- d_6 .

Position	δ_{H} ($J = \text{Hz}$)	δ_{C}
1	1.79, m 1.07, m	37.6, CH_2
2	1.59, m	27.7, CH_2
3	3.20, dd (7.7, 7.6)	78.2, CH
4		38.9, qC
5	1.19, m	49.9, CH
6	1.94, m	23.7, CH_2
7	5.36, brs	121.6, CH
8		135.8, qC
9	1.48, m	46.9, CH
10		36.1, qC
11	2.07, m 1.90, m	25.4, CH_2
12	1.65, s	21.6, CH_3
13	0.95, s	28.0, CH_3
14	0.83, s	15.1, CH_3
15	0.77, s	13.8, CH_3
1'	3.52, d (7.6)	76.2, CH
2'	3.85, d (7.6)	74.6, CH
3'		146.6, qC
4'	4.56, s	69.4, CH
5'	3.13, s	60.8, CH
6'		60.5, qC
7'	5.25, brs 5.21, brs	107.3, CH_2

showed 22 carbon signals attributed to four methyls, five methylenes, eight methines, and five quaternary carbons. These data revealed that compound **1** was a merosessquiterpenoid closely related to craterellin C (**7**) [1] except for an additional hydroxy substitution. The ^1H – ^1H COSY spectrum afforded a fragment of H-1/H-2/H-3, while the HMBC correlations of H-3 at δ_{H} 3.20 (1H, dd, $J = 7.7, 7.6$ Hz) with C-4 at δ_{C} 38.9, C-13 at δ_{C} 28.0, and C-14 at δ_{C} 15.1 were observed (Figure 2). These 2D NMR correlations established that the additional hydroxy group should be placed at C-3. Besides, the coupling constant of H-3 ($J = 7.7, 7.6$ Hz) indicated the β orientation of OH-3, which was supported further by the ROESY data analysis as shown in Figure 1. Analysis of other 2D NMR data (HSQC, HMBC, ^1H – ^1H COSY, and ROESY) indicated the other parts were the same to those of **7**. Therefore, the structure of **1** was established as shown in Figure 1, and has been named as craterellin D.

Compound **2**, white powder, had the molecular formula $\text{C}_{10}\text{H}_{12}\text{O}_4$, as derived

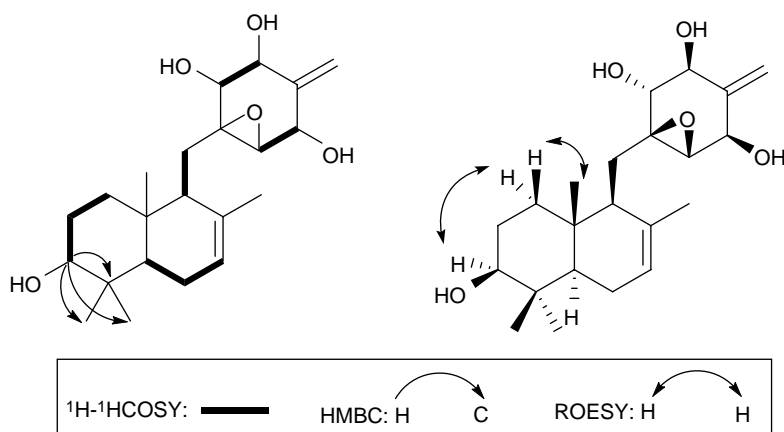


Figure 2. Key ^1H – ^1H COSY, HMBC, and ROESY correlations of **1**.

from HR-ESI-MS (m/z 196.0730; calcd 196.0736), corresponding to five degrees of unsaturation. The IR spectrum showed absorption bands of OH stretching at 3432 cm^{-1} and $\text{C}=\text{C}$ stretching at 1635 cm^{-1} . The ^{13}C NMR and DEPT spectra showed the signals of one OMe group, one oxygenated CH_2 , one CO group, and one methyl group, together

with six aromatic C-atoms (Table 2). On the basis of these data, compound **2** was readily identified as a phenylethanone derivative with substitutions of one methoxy group, one hydroxy group, and one hydroxymethyl. Positions of these substitutions were located by the ROESY data analysis in part. Due to the internal hydrogen bond between OH and the CO

Table 2. ^1H and ^{13}C NMR spectroscopic data of **2–4** at 500/100 MHz, respectively.

Position	2 ^a		3 ^b		4 ^a	
	δ_{H} ($J = \text{Hz}$)	δ_{C}	δ_{H} ($J = \text{Hz}$)	δ_{C}	δ_{H} ($J = \text{Hz}$)	δ_{C}
1		123.4, qC				200.0, qC
2		165.1, qC		164.2, qC	5.50, s	99.7, CH
3	6.65, s	99.4, CH	6.82, s	102.8, CH		180.3, qC
4		163.8, qC	8.20, s	122.9, CH	2.67, m	33.8, CH
5		113.6, qC		125.9, qC	2.37, m	39.4, CH_2
6	8.17, s	130.5, CH	7.85, d (8.7)	125.2, CH	1.70, m	
7		203.4, qC	7.58, d (8.7)	110.9, CH	4.31, dd (12.9, 5.2)	72.2, CH
8	2.49, s	26.2, CH_3		156.5, qC	1.06, d (6.9)	17.4, CH_3
9	5.01, s	58.9, CH_2		128.4, qC		
10				71.2, qC		
11			3.62, d (10.6)	68.2, CH_2		
			3.53, d (10.6)			
12			1.46, s	23.7, CH_3		
13				167.8, qC		
OH-2	13.4, s					
OMe-3					3.48, s	56.2, CH_3
OMe-4	3.69, s	55.7, CH_3				

^a Measured in pyridine- d_5 .

^b measured in methanol- d_4 .

groups, a downfield shift at δ_{H} 13.4 (1H, s, OH-2) was observed. Thus, the OH should be located at C-2. Then, the ROESY correlations of the aromatic proton at δ_{H} 6.65 (1H, s, H-3) with OH-2 and the methoxy suggested that the methoxy should be placed at C-4. Besides, a downfield signal at δ_{H} 8.17 (1H, s, H-6) showed ROESY correlations with H-8 at δ_{H} 2.49 (3H, s) and H-9 at δ_{H} 5.01 (2H, s), which established the hydroxymethyl to be placed at C-5. Therefore, compound **2** was determined as 5-hydroxymethyl-2-hydroxy-4-methoxy-phenylethanone, as shown in Figure 1.

Compound **3** was isolated as white powder and exhibited a molecular formula of $\text{C}_{12}\text{H}_{12}\text{O}_5$, as deduced from its HR-ESI-MS at m/z 259.0589 $[\text{M} + \text{Na}]^+$, requiring for seven degrees of unsaturation. The IR spectrum revealed the presence of OH (3427 cm^{-1}), C=O (1681 cm^{-1}), and C=C (1633 cm^{-1}). The NMR data of **3** were similar to those of 5-acetyl-2-[2-(1,2-dihydroxyisopropyl)]-benzofuran [2], except that the acetyl at C-5 was replaced by a carboxyl group (δ_{C} 167.8, s), as established by the HMBC correlations from H-4 at δ_{H} 8.20 (1H, s) and H-6 at δ_{H} 7.85 (1H, d, $J = 8.7\text{ Hz}$) to C-13 at δ_{C} 167.8. Therefore, compound **3** was characterized as 5-carboxyl-2-[2-(1,2-dihydroxyisopropyl)]-benzofuran.

Compound **4** possessed a molecular formula of $\text{C}_8\text{H}_{12}\text{O}_3$ as assigned by HR-ESI-MS (m/z 156.0785; calcd 156.0786). The IR spectrum showed absorption bands at 3396, 1684, and 1602 cm^{-1} , revealing the presence of OH, C=O, and C=C groups. The ^{13}C NMR and DEPT spectra displayed eight signals as given in Table 2. Of them, signals at δ_{C} 200.0 (s, C-1), 99.7 (d, C-2), and 180.3 (s, C-3) established an α,β -unsaturated keto moiety, as supported by the HMBC correlations from H-2 to C-1 and C-3. Meanwhile, the HMBC correlation of a methoxy at δ_{H} 3.48 (3H, s, OMe-3) to C-3 indicated the substitution of a methoxy to C-3. In the ^1H – ^1H COSY

spectrum, a moiety of $\text{CH}_3(7)\text{-CH}(4)\text{-CH}_2(5)\text{-CH}(6)$ was detected. Furthermore, the HMBC correlations from H-4 at δ_{H} 2.67 (1H, m) to C-3 and from H-6 at δ_{H} 4.31 (1H, dd, $J = 12.9, 5.2\text{ Hz}$) to C-1 established a cyclohexanone moiety. Thus, the planar structure of **4** was determined to be 6-hydroxy-3-methoxy-4-methyl-2-cyclohexen-1-one. The relative configuration of H-4, H-6-*cis* was deduced from the ROESY correlations of H-4/H-6. Thus, compound **4** was determined as 6 α -hydroxy-3-methoxy-4 α -methyl-2-cyclohexen-1-one.

The inhibitory activities against two isozymes of 11 β -hydroxysteroid dehydrogenases (11 β -HSD1 and 11 β -HSD2) of compounds **5**–**7** have been reported [1]. In this study, compounds **1** and **2** were evaluated for their inhibitory activities against one isozyme of 11 β -hydroxysteroid dehydrogenase (11 β -HSD1) [3]. In addition, compounds **1**, **2**, and **5**–**7** were evaluated for their cytotoxicities against five human cancer cell lines (HL-60, SMMC-7721, A-549, MCF-7, and SW-480) using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) method [4–5]. The results showed that only compound **2** exhibited inhibitory activity against 11 β -HSD1 (human $\text{IC}_{50} = 16.4\text{ }\mu\text{g/ml}$).

3. Experimental

3.1 General experimental procedures

Optical rotations were measured on a Jasco-P-1020 polarimeter. UV spectra were measured on a Shimadzu UV-2401 PC spectrophotometer. IR spectra were obtained by using a Bruker Tensor 27 FT-IR spectrometer with KBr pellets. NMR spectra were acquired with instruments of Bruker DRX-500 or Bruker AV 400. ESI-MS, HR-ESI-MS, and HR-ESI-MS were measured on Bruker HCT/Esquire, API QSTAR Pulsar, and VG Autospec-3000 mass spectrometer, respectively. Preparative high pressure liquid chromatography (HPLC) was performed on an Agilent

1100 series with a Zorbax SB-C18 (5 μ m, 9.4×150 mm) column. Preparative medium pressure liquid chromatography (MPLC) was performed on a Büchi apparatus equipped with Büchi fraction collector C-660, Büchi pump module C-605, and manager C-615. Silica gel (200–300 mesh and 80–100 mesh, Qingdao Marine Chemical, Inc., Qingdao, China), RP-18 gel (40–75 μ m, Fuji Silysia Chemical Ltd, Kasugai, Aichi, Japan), and Sephadex LH-20 (Amersham Biosciences, Upssala, Sweden) were used for column chromatography (CC). Fractions were monitored by thin-layer chromatography (Qingdao Marine Chemical, Inc.) and spots visualized by heating silica gel plates immersed in vanillin–H₂SO₄ in EtOH.

3.2 Fungal material and cultivation conditions

The fungus *C. odoratus* was collected from the southern part of the Gaoligong Mountains in Yunnan Province, China, in July 2007. The fungus was identified by Prof. Mu Zang at the Kunming Institute of Botany. A voucher specimen (HFG07004) is deposited at the Herbarium of Kunming Institute of Botany. Culture medium: glucose (5%), pork peptone (0.15%), yeast (0.5%), KH₂PO₄ (0.05%), and MgSO₄ (0.05%). The initial pH was adjusted to 6.0, and the fermentation was first carried out on an erlenmeyer flask for 6 days till the mycelium biomass reached to the maximum. Later it was transferred to a fermentation tank (100 liters) at 24°C and 250 rpm for 20 days, and ventilation was settled to 1.0 vvm (vvm: air volume/culture volume/min).

3.3 Extraction and isolation

The culture broth (70 liters) was extracted three times with ethyl acetate (EtOAc) (3 \times 10 liters). The combined EtOAc extracts were evaporated *in vacuo* to give

a residue (30.0 g). The residue was subjected to silica gel CC with a gradient elution system of CHCl₃–MeOH (1:0 \rightarrow 0:1) to obtain 10 fractions (A–J). Fraction E was subjected to preparative MPLC with a reversed-phased C₁₈ column (MeOH–H₂O, 0:1 \rightarrow 6:4) to obtain sub-fractions E01–E10. Fraction E05 eluted with petroleum ether (PE)–acetone (3:1) was further separated by preparative HPLC (MeCN–H₂O, 40%) to give **2** (3.0 mg) and **4** (4.0 mg). Fraction G was separated by repeated silica gel CC (PE–acetone, 10:1 \rightarrow 0:1) to yield fractions G01–G14. Fraction G05 was chromatographed on a RP-18 column (MeOH–H₂O, 5:5) and then purified on Sephadex LH-20 CC (CHCl₃–MeOH, 1:1) to give **5** (15.0 mg). Compound **3** (2.0 mg) was afforded from fraction G09 by preparative HPLC (MeCN–H₂O, 3:7) and compound **1** (30.0 mg) was also obtained by preparative HPLC (MeCN–H₂O, 3:7) from the G3 fraction. Fraction G13 was separated by repeated silica gel CC (PE–acetone, 6:1 \rightarrow 0:1) to yield fractions G131–G135. Fraction G132 was chromatographed on a RP-18 column (MeOH–H₂O, 6:4) and then purified by CC with PE–acetone (2:1) to give **6** (20.0 mg) and **7** (15.0 mg).

3.3.1 Craterellin D (**1**)

Yellow oil; $[\alpha]_D^{15} + 7.0$ (*c* 3.30, MeOH); UV (MeOH) λ_{\max} (log ϵ) 202 (3.0) nm; IR (KBr) ν_{\max} 3424, 1633, and 1023 cm^{−1}; for ¹H NMR (acetone-d₆, 400 MHz) and ¹³C NMR (acetone-d₆, 100 MHz) spectroscopic data, see Table 1; ESI-MS (positive): *m/z* 401 [M + Na]⁺; HR-ESI-MS (positive): *m/z* 401.2294 [M + Na]⁺ (calcd for C₂₂H₃₄O₅Na, 401.2303).

3.3.2 5-Hydroxymethyl-2-hydroxy-4-methoxy-phenylethanone (**2**)

White powder; $[\alpha]_D^{15} - 5.1$ (*c* 0.20, MeOH); UV (MeOH) λ_{\max} (log ϵ) 320

(3.5), 273 (3.8), 228 (3.8), 216 (3.9), and 196 (3.7) nm; IR (KBr) ν_{\max} 3432 and 1635 cm^{-1} ; for ^1H NMR (pyridine- d_5 , 500 MHz) and ^{13}C NMR (pyridine- d_5 , 100 MHz) spectroscopic data, see Table 2; ESI-MS (positive): m/z 219 $[\text{M} + \text{Na}]^+$; HR-ESI-MS: m/z 196.0730 $[\text{M}]^+$ (calcd for $\text{C}_{10}\text{H}_{12}\text{O}_4$, 196.0736).

3.3.3 5-Carboxyl-2-[2-(1,2-dihydroxyisopropyl)]-benzofuran (**3**)

White powder; $[\alpha]_{\text{D}}^{15} -1.3$ (c 0.25, MeOH); UV (MeOH) λ_{\max} ($\log \epsilon$) 285 (2.7), 223 (3.9), and 197 (3.6) nm; IR (KBr) ν_{\max} 3427, 1681, and 1633 cm^{-1} ; for ^1H NMR (methanol- d_4 , 500 MHz) and ^{13}C NMR (methanol- d_4 , 100 MHz) spectroscopic data, see Table 2; ESI-MS (positive): m/z 259 $[\text{M} + \text{Na}]^+$; HR-ESI-MS (positive): m/z 259.0589 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{12}\text{H}_{12}\text{O}_5\text{Na}$, 259.0582).

3.3.4 6 α -Hydroxy-3-methoxy-4 α -methyl-2-cyclohexen-1-one (**4**)

White powder; $[\alpha]_{\text{D}}^{15} -116.3$ (c 0.10, MeOH); UV (MeOH) λ_{\max} ($\log \epsilon$) 282

(3.7), 258 (3.7), 221 (3.7), and 200 (3.7) nm; IR (KBr) ν_{\max} 3396, 1684, 1602, and 1218 cm^{-1} ; for ^1H NMR (pyridine- d_5 , 500 MHz) and ^{13}C NMR (pyridine- d_5 , 100 MHz) spectroscopic data, see Table 2; ESI-MS (positive): m/z 179 $[\text{M} + \text{Na}]^+$; HR-ESI-MS: m/z 156.0785 $[\text{M}]^+$ (calcd for $\text{C}_8\text{H}_{12}\text{O}_3$, 156.0786).

Acknowledgments

This work was financially supported by National Basic Research Program of China (973 Program, 2009CB522300) and the National Natural Science Foundation of China (Nos 30830113 and U1132607).

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