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Illudin T, a new sesquiterpenoid from basidiomycete *Agrocybe salicacola*

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A new sesquiterpenoid (**1**), illudin T, was isolated from the culture of basidiomycete *Agrocybe salicacola*. The structure of the new compound was elucidated on the basis of spectral data.

Keywords: *Agrocybe salicacola*; basidiomycete; sesquiterpenoid; illudin T

1. Introduction

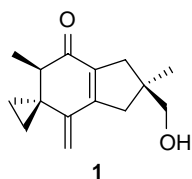
Mushrooms are an attractive delicacy due to their unique flavor, taste, and potential health benefits, and they are further extensively used as dietary supplements and nutraceuticals along with other herbal preparations for the treatment of a number of medical conditions [1]. The mushroom *Agrocybe salicacola* is an edible basidiomycete belonging to the order Agaricales [2]. The genus *Agrocybe* is reported to contain several bioactive metabolites, such as ceramide with inhibitory activity against COX-1,2 [1], indole alkaloids with free radical-scavenging ability [3], agrocybin, a peptide with anti-fungal activity [4], polysaccharides with hypoglycemic activity [5], a lectin with mitogenic activity [6], and antiproliferative and differentiating effects [7]. In our previous investigations, a novel *bis*-sesquiterpene, agrocybone, with a spirodienone structure [8] and two new aromadendrane sesquiterpenoids [9] were reported from the basidiomycete *A. salicacola*.

The illudins are a group of sesquiterpene antibiotics that have been widely reported as antibacterial and antitumor agents [10,11]. A derivative of illudin S, hydroxymethylacetylfulvene (also designated MGI 114), has been tested in Phase II human clinical trials [12]. Some basidiomycetes have been reported to produce different illudins and related compounds [13–15]. In continuation of our previous chemical investigations of *A. salicacola*, the presence of illudin-type sesquiterpenoids was discerned in the fermentation broth of the basidiomycete *A. salicacola*. One new illudin-type sesquiterpenoid, named illudin T (**1**), together with a known illudin I (**2**), was isolated. Herein, we report the isolation and structure elucidation of **1**.

2. Results and discussion

Compound **1** was isolated as white powder with $[\alpha]_D^{13.5} + 36.9$ ($c = 0.4$, CHCl_3) from the culture of *A. salicacola*. The culture broth was successively extracted with

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Figure 1. Structure of compound **1**.

EtOAc. Totally, 3.2 g of crude extract was obtained from 20 liters of culture. Next, the extract was successively purified by several chromatographic steps to yield 7 mg of **1** (Figure 1). The HR-ESI-MS showed the pseudomolecular ion at m/z 233.1538 ($[M + H]^+$), corresponding to the formula $C_{15}H_{20}O_2$. The molecular formula $C_{15}H_{20}O_2$ was further confirmed by its NMR spectral data. The IR spectrum exhibited absorptions at 3440 and 1662 cm^{-1} due to the hydroxyl and the unsaturated carbonyl groups, respectively, whereas an absorbance in the UV spectrum at 284 nm ($\log \epsilon$ 3.71) confirmed the conjugated system.

Inspection of the ^1H and ^{13}C NMR (DEPT) and HSQC experiments revealed the existence of two methyl groups, six methylene units (including one oxymethylene group and one olefinic methylene group), and one methine unit. Additionally, one ketone group and five quaternary carbons (three of them belonging to double bonds) were identified from both the ^{13}C NMR and HMBC spectra.

In the ^1H NMR spectrum (Table 1), four high-field multiplets were observed at δ 0.43 and 0.69, assigned to H-11 α and H-11 β , respectively, and δ 0.48 and 1.02, assigned to H-12 α and H-12 β , respectively. The corresponding carbons appeared at δ 18.0 and 7.9. These indicated the presence of cyclopropane moiety. Furthermore, the ^1H NMR spectrum showed resonances of the hydroxymethylene group at δ 3.48 and 3.46, one tertiary methyl at δ 1.16, one secondary methyl at δ 1.09 (d, $J = 7.2$ Hz), and its vicinal proton at δ 1.77 (q, $J = 7.2$ Hz).

Table 1. ^1H and ^{13}C NMR spectroscopic data for compounds **1** in CDCl_3 and **2** in CD_3OD .

	1		2	
	δ_{H} (J , Hz)	δ_{C}	δ_{H} (J , Hz)	δ_{C}
1		201.4		199.7
2	1.77 (1H, q, 7.2)	51.4	2.68 (1H, q, 7.20)	44.7
3		27.3		31.4
4		143.2		70.6
5		156.5		166.4
6 β	2.72 (1H, br d, 18.0)	43.0	2.68 (1H, br d, 18.4)	42.2
6 α	2.51 (1H, br d, 18.0)		2.46 (1H, br d, 18.4)	
7		42.7		42.3
8 β	2.67 (1H, br d, 17.0)	39.9	2.51 (1H, br d, 16.8)	39.7
8 α	2.34 (1H, br d, 17.0)		2.35 (1H, br d, 16.8)	
9		135.2		134.7
10	1.09 (3H, d, 7.2)	16.6	0.96 (3H, d, 6.9)	9.3
11 β	0.43 (1H, m)	18.0	0.51 (1H, m)	4.8
11 α	0.69 (1H, m)		0.55 (1H, m)	
12 β	0.48 (1H, m)	7.9	0.40 (1H, m)	2.8
12 α	1.02 (1H, m)		0.68 (1H, m)	
13	5.10 (1H, s)	111.7	1.40 (3H, s)	23.3
	4.98 (1H, s)			
14	3.48 (1H, s)	70.4	3.43 (1H, s)	70.1
	3.46 (1H, s)		3.41 (1H, s)	
15	1.16 (3H, s)	24.9	1.19 (3H, s)	24.6

In the ^1H NMR spectrum, the signals as two double doublets, the first at δ 2.51 and 2.72 and the second at δ 2.34 and 2.67, were observed for two isolated methylene groups. The carbons bearing these protons resonated at δ 43.0 and 39.9, respectively. The proton and carbon signals (Table 1) were assigned completely on the basis of 2D NMR spectral data (^1H - ^1H COSY, HSQC, and HMBC) and comparing the data with those of illudin I_2 [14]. The HMBC spectrum showed correlations between H-2 and C-1, C-3, C-4, C-9, C-11, and C-12; H-13 and C-3, C-4, and C-5; H-10 and C-1, C-2, and C-3; H-11 and C-2, C-3, and C-4; H-12 and C-2, C-3, and C-4; H-14 and C-6, C-7, and C-8 (Figure 2).

The relative stereochemistry of illudin T (**1**) was determined by a study of its ROESY spectrum (Figure 3). The 15-methyl group (δ 1.16) showed strong correlations with the protons at δ 2.51 (H-6 α) and 2.34 (H-8 α), whereas the 14-hydroxymethylene group showed cross-peaks with the protons at δ 2.72 (H-6 β) and 2.67 (H-8 β). The 10-methyl (δ 1.09) group also showed correlation with the proton at δ 0.48 (H-12 β).

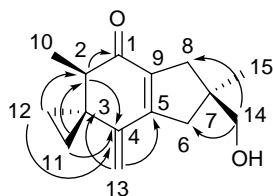


Figure 2. Selected HMBC (↷) correlations of compound **1**.

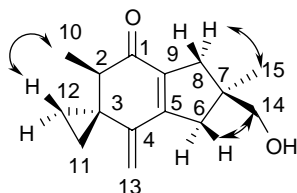


Figure 3. Selected ROESY (↷) correlations of compound **1**.

Compound **2** was purified as colorless oils. Comparison of the physicochemical properties with the reported data allowed to the identification of compound **2** as illudin I [14].

3. Experimental

3.1 General experimental procedures

Optical rotation was measured on a Horiba SEPA-300 spectropolarimeter. IR spectrum was obtained with a Bruker Tensor 27 spectrometer, with KBr pellets. 1D and 2D NMR spectra were recorded on Bruker DRX-500 spectrometer, in CD_3OD or CDCl_3 , δ in ppm, and J in Hz. ESIMS was recorded with a VG Autospec-3000 spectrometer, HR-ESI-MS was recorded with an API QSTAR Pulsar 1 spectrometer. Silica gel (200–300 mesh, Qingdao Marine Chemical, Inc., Qingdao, China) and Sephadex LH-20 (Amersham Bio-Sciences, Uppsala, Sweden) were used for column chromatography. Pre-coated silica gel GF254 plates (Qingdao Marine Chemical, Inc.) were used for TLC. Fractions were monitored by TLC, and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in ethanol.

3.2 Mushroom material and culture

The fungus *A. salicicola* was collected at the Botanic Garden of Kunming Institute of Botany, Chinese Academy of Sciences, China, in September 2008, and identified by Prof. Zang Mu, Kunming Institute of Botany. The voucher specimen has been deposited in the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences. The culture medium consisted of potato (peeled, 200 g), glucose (20 g), KH_2PO_4 (3 g), MgSO_4 (1.5 g), citric acid (0.1 g), and thiamine hydrochloride (10 mg) in 1 liter of sterilized H_2O . Reagent bottles were used as a flask (size: 500 ml; volume of media: 350 ml). The pH value was adjusted to 6.5 before autoclave.

Fermentation was carried out on a shaker at 150 rpm for 20 days at 22°C.

3.3 Extraction and isolation

The whole culture broth of *A. salicicola* (20 liters) was initially filtered, and the filtrate was extracted three times with EtOAc. The organic layer was concentrated under reduced pressure to give a crude extract (6.0 g), and this residue was subjected to column chromatography over silica gel (200–300 mesh, 3 × 45 cm), eluted with a gradient solvent system of petroleum ether–acetone, to afford fractions A–I. Fraction F (2.1 g) eluted with petroleum ether–acetone (2:1) was further purified on a silica gel column (petroleum ether–acetone, 10:1) to give **1** (7 mg). Fraction G (1.1 g), eluted with petroleum ether–acetone (1:1), was again purified by repeated reversed-phase C₁₈ (MeOH–H₂O) and Sephadex LH-20 (CHCl₃–MeOH, 1:1) column chromatography to give pure **2** (47 mg).

Compound **1**, white powder, $[\alpha]_D^{13.5} + 36.9$ ($c = 0.4$, CHCl₃), UV (CHCl₃) λ_{\max} (log ϵ) 284 (3.71) nm. IR (KBr) ν_{\max} 3440 and 1662 cm⁻¹. For ¹H and ¹³C NMR (CDCl₃) spectral data, see Table 1. HR-FAB-MS: m/z 233.1538 [M + H]⁺ (calcd for C₁₅H₂₁O₂, 233.1541).

Compound **2**, colorless oil; for ¹H and ¹³C NMR (CD₃OD) spectral data, see Table 1.

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