A New Pigment from the Fruiting Bodies of the Basidiomycete *Lactarius deliciosus*

Xiao-Long Yang[a,c], Du-Qiang Luo[a,b], and Ji-Kai Liu[a]

[a] State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, China
[b] College of Life Sciences, Hebei University, Baoding 071002, China
[c] Graduate School of the Chinese Academy of Sciences, Beijing 100039, China

Reprint requests to Prof. Dr. J. K. Liu. E-mail: jkliu@mail.kib.ac.cn

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A new purple azulene pigment (**1**) was isolated from the fruiting bodies of the basidiomycete *Lactarius deliciosus* together with one known pigment (**2**). The structures of the pigments were established on the basis of spectral evidence (MS, IR, 1D- and 2D-NMR experiments).

Key words: Lactarius deliciosus, Basidiomycete, Azulene

Introduction

The fungal subdivision Basidiomycotina produces many toxic sesquiterpenes derived from the protoilludane skeleton. This skeleton is transformed and rearranged to a multitude of compounds [1]. The rich variation of structures is mirrored in a rich variation of activities as well. Currently only the minority of these compounds have been tested in biological assays. However, for some sesquiterpenes there are already interesting data on their different biological activities and some have been identified as inhibitors of enzymes crucial in some diseases [1]. Uvidin A, a new fatty acid ester of a drimane sesquiterpene from *Lactarius uvidus* showed insect antifeedant and cytotoxic activities [2]. Antiviral activities *in vitro* were reported for *N*-benzoylphenylisoserinates of *Lactarius* sesquiterpenoid alcohols [3]. Recently, a green pigment blennione and a red pigment lilacinone were isolated from *Lactarius blennius* and *Lactarius lilacinus*, respectively [4, 5].

The edible fungus *Lactarius deliciosus* is widely distributed in the Yunnan Province of China. The latex of the fruiting bodies of *L. deliciosus* is firstly carrot-colored, but slowly (minutes) darkens and eventually turns green, and these colors in *L. deliciosus* have previously been shown to be due to guaiane sesquiterpenes [6]. Moreover, the structure and antibiotic activity of lactaroviolin were reported [6 – 8]. Besides lactaroviolin, the free dihydroazulene alcohol [9], as well as its stearic acid ester [9], and lactarazulene [10] have been isolated from European specimens of *L. deliciosus*. In addition, lactarofulvene [11, 12] has been isolated from Californian specimens of *L. deliciosus*, while an azulenealdehyde [12] was isolated from Indian specimens of *L. deterrimus*, and aromatic compounds [13] were isolated from liquid cultures of *L. deliciosus*.

In the course of our continuing research on bioactive metabolites of *Lactarius* and *Russula* sp. in Yunnan Province of China [14 – 18], the chemical constituents of the fruiting bodies of *L. deliciosus* were investigated. This report describes the isolation and structure elucidation of a new pigment.

Results and Discussion

The fresh fruiting bodies of *L. deliciosus* (10 kg) were firstly extracted three times with acetone and then with $CHCl_3$/MeOH 1 : 1 twice at r. t. The combined organic phases were evaporated to afford a deep brown gum (200 g), which was partitioned against H_2O and EtOAc. The organic layer was concentrated *in vacuo* to give the residue (80 g) which was subjected to repeated column chromatography to afford **1** and **2**. On the basis of 1D- and 2D-NMR experiments (HMBC, HMQC), compound **1** was identified as 7-acetyl-4-methylazulene-1-carboxylic acid. Compound **2** was identified as 4-methyl-7-(prop-1-en-2-yl) azulene-1-carbaldehyde, by comparison of the NMR data with the data reported in the literature [6, 7].

Compound **1** was obtained as a purple powder. HR-ESI-MS of **1** indicated a molecular formula of

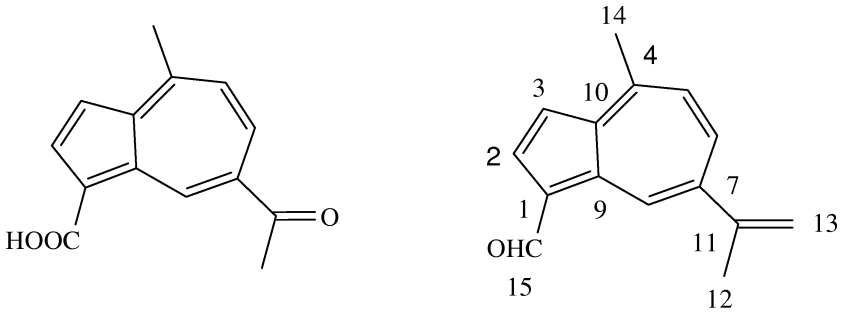

Fig. 1. Strcutures of compounds **1** and **2**.

Table 1. ^{1}H and ^{13}C NMR data (500 and 125 MHz, resp.) of **1** and **2***.

	1		**2***	
Pos.	δ(C) (DEPT)	δ(H)	δ(C) (DEPT)	δ(H)
1	137.7 (s)		139.0 (s)	
2	140.6 (d)	8.35 (d, 4.1)	142.6 (d)	8.20 (d, 4.2)
3	128.3 (d)	7.59 (d, 4.1)	131.4 (d)	7.34 (d, 4.2)
4	150.3 (s)		150.1 (s)	
5	119.3 (d)	7.64 (d, 10.7)	117.2 (d)	7.56 (d, 10.8)
6	137.4 (d)	8.52 (d, 10.7)	136.7 (d)	7.99 (dd, 10.8, 2.5)
7	132.4 (s)		147.2 (s)	
8	138.7 (d)	10.47 (s)	137.3 (d)	9.89 (d, 2.5)
9	141.6 (s)		144.4 (s)	
10	130.0 (s)		127.9 (s)	
11	201.8 (s)		129.9 (s)	
12	27.4 (q)	2.83 (s)	23.0 (q)	2.30 (s)
13			116.7 (t)	5.35 (s)
				5.47 (s)
14	24.9 (q)	3.02 (s)	24.7 (q)	2.91(s)
15	170.8 (s)		187.0 (d)	10.32 (s)

1: Measured in CD_3OD, **2***: in CD_3COCD_3; coupling constants are given in Hz.

$C_{14}H_{12}O_3$ ($[M+Na]^+$ at m/z 251.0688, calcd. for $C_{14}H_{12}O_3Na$ 251.0684) with 9 degrees of unsaturation. EI-MS (70 eV): m/z (%) = 228 (90) $[M]^+$, 213 (100) $[M\text{-}Me]^+$, 211 (19) $[M\text{-}OH]^+$, 185 (17) $[M\text{-}MeCO]^+$, 183 (6) $[M\text{-}HCOO]^+$, 169 (13), 149 (7), 129 (64), 128 (63), 115 (29), 57 (60). As shown in the Table 1, the ^{1}H and ^{13}C NMR spectral data of **1** were similar to **2** [6, 7], which suggested that compound **1** possesses the same azulene substitution pattern. The distinct differences between **1** and **2** are as following: the oxo group at C-11 of **1** [$\delta_C = 201.8$ (s, C-11)] is absent in **2** [$\delta_C = 129.9$ (s, C-11)] and the carboxyl group at C-1 of **1** [$\delta_C = 170.8$ (s, C-1)] is absent in **2** [$\delta_C = 187.0$ (d, C-1)]. The HMBC spectra of **1** demonstrated the following key ^3J correlations: H-C(2)→C(15); H-C(14)→C(5), C(10); H-C(12)→C(7), C(11); which were in consistency with the azulene skeleton. The cross-peaks between H-2 and H-3, H-5 and H-6 in the ^{1}H, ^{1}H-COSY spectra were also shown. Taking all data mentioned above into account, the structure of **1** was finally established as 7-acetyl-4-methylazulene-1-carboxylic acid (Fig. 1).

Experimental Section

General

NMR spectra were recorded on Bruker AM-400 and DRX-500 spectrometers, δ in ppm, J in Hz. IR spectra were obtained with a Nexus 870 FT-IR with KBr pellets. UV spectrum was recorded on UV-210 spectrometer, $\lambda_{max}(\log\varepsilon)$ in nm. EI-MS spectra were recorded with a VG Autospec-3000 spectrometer, m/z (rel. int.). HR-ESI-MS was recorded with an API QSTAR Pulsar 1 spectrometer.

Material

Column chromatography was carried out on silica gel (200 – 300 mesh, Qingdao Marine Chemical Ltd., Qingdao, P. R. China) and Sephadex LH-20 (Amersham Biosciences, Uppsala, Sweden).

Fungal material

The fresh fruiting bodies of *L. deliciosu*s were collected at Ciba country in Yunnan province, China, in November, 2005. The voucher specimen was deposited at the herbarium of the Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and isolation

The fresh fruiting bodies of *L. deliciosus* (10 kg) were extracted three times with acetone and then with $CHCl_3$/MeOH (1 : 1, v/v) two times at r. t. The combined extracts were concentrated *in vacuo* to give a deep brown gum (200 g), which was partitioned against H_2O and EtOAc. The organic layer was concentrated *in vacuo* to give the residue (80 g). The residue was subjected to silica gel column chromatography employing a gradient elution with petroleum ether/acetone

(100 : 0, 98 : 2, 95 : 5, 90 : 10, 80 : 20, 50 : 50, 0 : 100 (v/v)) to give fourteen fractions. The fractions 11 – 12 (10 g), eluted with petroleum ether/acetone 50 : 50, was further separated by repeated CC (silica gel; chloroform/methanol 98 : 2 (v/v), petroleum ether/acetone 2 : 1 (v/v)), and purified on Sephadex LH-20 chromatography with acetone to afford **1** (3 mg) and **2** (10 mg).

7-Acetyl-4-methylazulene-1-carboxylic acid (**1**). Purple powder. – UV/vis (MeOH): $\lambda_{max}(\lg\varepsilon) = 778$ (2.56), 526 (2.89), 509 (2.89), 463 (2.87), 398 (3.52), 307 (3.97), 237 nm (3.99). – IR (KBr): $\nu = 3300 - 2500$ (br, OH), 3090, 2924, 1690, 1674, 1629, 1235, 1049 cm^{-1}. – ^1H NMR (400 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD): see Table 1. –HR-ESI-MS: 251.0688 ($C_{14}H_{12}O_3$, $[M+Na]^+$; calcd. 228.0684). –MS (EI, 70 eV): m/z (%) = 228 (90) $[M]^+$, 213 (100) $[M\text{-}Me]^+$, 211 (19) $[M\text{-}OH]^+$, 185 (17) $[M\text{-}MeCO]^+$, 183 (6) $[M\text{-}HCOO]^+$, 169 (13), 149 (7), 129 (64), 128 (63), 115 (29), 57 (60).

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