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Two new polyols and a new phenylpropanoid glycoside from the basidiomycete *Lactarius deliciosus*

Zhong-Yu Zhou [a,b,*], Jian-Wen Tan [a], Ji-Kai Liu [b]

[a] Key Laboratory of Plant Resources Conservation and Sustainable Utilization, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China
[b] State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, China

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ABSTRACT

Two new polyols, 3-hydroxymethyl-2-methylenepentane-1,4-diol (**1**) and 1-methylcyclohexane-1,2,4-triol (**2**), and a new phenylpropanoid glycoside, eugenyl 4″-*O*-acetyl-*β*-rutinoside (**3**), together with seven known steroids (**5**–**11**) were isolated from the fruiting bodies of the basidiomycete *Lactarius deliciosus*. The structures of these compounds were elucidated by the analysis of spectroscopic data.

1. Introduction

Lactarius deliciosus is an edible basidiomycete belonging to the family Russulaceae. When the fruiting bodies are injured, the latex is firstly carrot-colored, but then slowly (within minutes) darkens, and eventually turns green, these color changes having been reported to be due to guaiane sesquiterpenes [1]. A galaxy of guaiane sesquiterpenes, including lactaroviolin [2], delicial [1], deterrol [1], the free dihydroazulene alcohol and its stearic acid ester [3], lactarazulene [4], and lactarofulvene [5] have been isolated from specimens of *L. deliciosus* in different areas of the world. In addition, aromatic compounds from liquid cultures of *L. deliciosus* have been reported [6]. Our previous investigations of this fungus have reported 5 guaiane sesquiterpenes from the specimens collected from Kunming of Yunnan province [7,8]. An extensive review related to occurrence, chemistry, total synthesis and some biological aspects of characteristic constituents in *Lactarius* species was reported by Daniewski and Vidari [9]. In this paper, we report the isolation and structure elucidation of two new polyols (**1** and **2**) and a new phenylpropanoid glycoside (**3**) from Tibet specimens of *L. deliciosus*.

2. Experimental procedure

2.1. General

Optical rotations were measured on a Horiba SEPA-300 polarimeter. UV spectra were measured on a Shimadzu UV-2401 PC spectrophotometer. IR spectra were obtained on a Bruker Tensor 27 FT-IR spectrometer with KBr pellets. NMR spectra were recorded on a Bruker AV-400 spectrometer, and chemical shifts (δ) were expressed in ppm with reference to the solvent signals. EI-MS and FAB-MS were recorded on a VG Autospec-3000 spectrometer. HRESI-MS were acquired on an API QSTAR Pulsar 1 spectrometer. Preparative HPLC was performed on an Agilent 1100 series with a Zorbax SB-C18 (5 μm, 9.4×150 mm) column. Silica gel (200–300 mesh, Qingdao Marine Chemical Inc., Qingdao, China), RP-18 gel (40–75 μm, Fuji Silysia Chemical Ltd., Aichi, Japan) and Sephadex LH-20 (Amersham Biosciences, Uppsala, Sweden) were used for column chromatography. Fractions were

* Corresponding author at. Key Laboratory of Plant Resources Conservation and Sustainable Utilization, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China. Fax: +86 20 37082054.
E-mail address: zhouzhongyu@scbg.ac.cn (Z.-Y. Zhou).

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monitored by TLC and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in ethanol.

2.2. Fungal material

The fungus *L. deliciosus* was collected from Linzhi of Tibet, China, in July 2007, and identified by Prof. Mu Zang, Kunming Institute of Botany. The voucher specimen (HFG07092) was deposited at the Herbarium of the Kunming Institute of Botany, CAS.

2.3. Extraction and isolation

Dry fruiting bodies (1.75 kg) were extracted three times with chloroform and methanol (1:1, v/v), and the solvent was evaporated *in vacuo* to give a crude extract (120 g), which was suspended in H_2O and then successively extracted three times with ethyl acetate. The organic layer gave 45 g of EtOAc extract, which was applied on silica gel column chromatography eluted with a $CHCl_3$–MeOH gradient (100:0–0:100 gradient system) to afford fractions A–G. Fraction B eluted with $CHCl_3$–MeOH (95:5, v/v) was separated by silica gel (pure $CHCl_3$) and Sephadex LH-20 ($CHCl_3$–MeOH, 1:1, v/v) column chromatography to obtain **8** (5.4 mg). Fraction C eluted with $CHCl_3$–MeOH (90:10, v/v) was subjected to silica gel column chromatography eluting with petroleum ether–acetone (10:1–2:1) to give subfractions C1 and C2. After repeated silica gel and Sephadex LH-20 ($CHCl_3$–MeOH, 1:1, v/v) column chromatography, a mixture (3.8 mg) of **5** and **6** from C1, **10** (16.1 mg) and **11** (5.0 mg) from C2, **7** (3.2 mg) and **9** (4.5 mg) from fraction E eluted with $CHCl_3$–MeOH (70:30, v/v) were afforded, respectively. Fraction G eluted with $CHCl_3$–MeOH (50:50, v/v) was divided into subfractions G1 and G2 by passage over silica gel column chromatography, eluted with chloroform–acetone (30:1–5:1). Subfraction G1 was subjected to preparative HPLC (MeCN–H_2O, 5%–25%) to provide **3** (45.0 mg). **1** (8.8 mg) and **2** (7.6 mg) were obtained from subfraction G2 by repeated reverse phase silica gel (MeOH–H_2O, 10%–30%) and Sephadex LH-20 ($CHCl_3$–MeOH, 1:1, v/v) column chromatography.

3-Hydroxymethyl-2-methylenepentane-1,4-diol (**1**) (Fig. 1): colorless oil, $[\alpha]^{26}_D = -29.0$ ($c = 0.23$, CH_3OH); IR (KBr) ν_{max}: 3423, 2933, 1638, 1107 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; EI-MS *m/z* (%): 146 $[M]^+$; HRESIMS (positive) *m/z* 147.1019 (calculated for $C_7H_{15}O_3$, 147.1021).

1-Methylcyclohexane-1,2,4-triol (**2**) (Fig. 1): Colorless oil, $[\alpha]^{26}_D = -54.0$ ($c = 0.19$, CH_3OH); IR (KBr) ν_{max}: 3445, 2953 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; EI-MS *m/z* (%): 146 $[M]^+$; HRESIMS (positive) *m/z* 147.1023 (calculated for $C_7H_{15}O_3$, 147.1021).

Eugenyl 4″-*O*-acetyl-*β*-rutinoside (**3**) (Fig. 1): amorphous powder, $[\alpha]^{26}_D = -63.3$ ($c = 0.40$, CH_3OH); IR (KBr) ν_{max}: 3432, 2925, 1728, 1638, 1264, 1041 cm^{-1}; UV (MeOH) λ_{max} (logε) nm: 204 (4.12), 224 (3.50), 278 (3.13); ^1H and ^{13}C NMR, see Table 1; negative FABMS *m/z* 513 $[M-H]^-$, 1027 $[2M-H]^-$; HRESIMS (negative) *m/z* 513.1967 (calculated for $C_{24}H_{33}O_{12}$, 513.1972).

3. Results and discussion

Compound **1** was obtained as colorless oil and was assigned a molecular formula of $C_7H_{14}O_3$ by positive HRESIMS (*m/z* $[M+H]^+$ 147.1019, calculated for $C_7H_{15}O_3$: 147.1021), which required for one degree of unsaturation in the molecule. The IR spectrum showed absorptions at 3423 cm^{-1}, revealing the presence of hydroxyl groups. The ^{13}C and DEPT NMR spectra (Table 1) exhibited 7 carbons, including one terminal double bond (δ_C 114.4, t, C-6; 148.3, s, C-2), two methines (δ_C 54.5, d, C-3; 67.8, d, C-4), two oxymethylenes (δ_C 65.7, t, C-1; 63.5, t, C-7) and one methyl (δ 21.5). The presence of the terminal double bond was also confirmed from the ^1H NMR spectrum, which showed signals at δ_H 5.23 (br. s, H-6a), 5.02 (br. s, H-6b). Since the terminal double bond accounted for one degree of unsaturation, **1** was acyclic. The HMBC correlations observed from two olefinic protons to one oxymethylene (C-1) and one methine (C-3), from the oxymethine signal at δ 3.97 to carbon resonances at δ 148.3 (C-2), 54.5 (C-3), 63.5 (C-7), and 21.5 (C-5), from the oxymethylene protons at δ 3.73 and 3.67 to carbon signals at δ 148.3 (C-2), 54.5 (C-3), and 67.8

Table 1
^1H and ^{13}C NMR (400 and 100 MHz, respectively) data for **1**–**3** in CD_3OD.

No.	1		2		3		No.	3	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C		δ_H	δ_C
1	4.05 (d, 4.2)	65.7 t		71.2 s		136.6 s	3′	3.46 (m)[a]	77.8 d
2		148.3 s	3.28 (overlapped)	74.3 d	6.83 (d, 1.8)	114.2 d	4′	3.38 (m)[a]	71.4 d
3	2.25 (m)	54.5 d	1.92 (m)	39.9 t		150.9 s	5′	3.51 (m)[a]	76.7 d
			1.64 (m)						
4	3.97 (m)	67.8 d	3.56 (m)	69.6 d		146.1 s	6′	3.97 (dd, 11.0, 1.8)	67.8 t
								3.64 (dd, 11.0, 6.1)	
5	1.18 (d, 6.4)	21.5 q	1.62 (m)	31.1 t	7.05 (d, 8.2)	118.4 d	1″	4.73 (br. s)	101.9 d
			1.59 (m)						
6	5.23 (br. s)	114.4 t	1.69 (m)	35.4 t	6.73 (dd, 8.2, 1.8)	122.0 d	2″	3.85 (m)[a]	72.2 d
	5.02 (br. s)		1.32 (m)						
7	3.73 (dd, 10.7, 6.4)	63.5 t	1.20 (s)	26.6 q	3.33 (d, 6.7)	40.8 t	3″	3.83 (m)[a]	70.3 d
	3.67 (dd, 10.7, 7.3)								
8					5.95 (ddt, 17.0, 9.8, 6.7)	139.0 d	4″	4.91 (m)[a]	75.6 d
9					5.06 (dd, 17.0, 1.6)	115.9 t	5″	3.80 (m)[a]	67.6 d
					5.02 (d, 9.8, 1.6)				
10					3.85 (s)	56.7 q	6″	1.04 (d, 6.3)	17.7 q
1′					4.82 (d, 7.6)	103.0 d	7″		172.5 s
2′					3.48 (m)[a]	74.9 d	8″	2.08 (s)	21.1 q

[a] Assignments are based on extensive 2D NMR experiments.

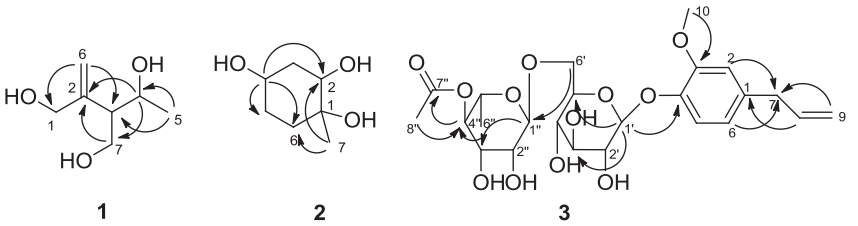

Fig. 1. Structures and key HMBC correlations of compounds **1**–**3**.

(C-4), implied the presence of a $CH_2=C(CH_2O)CH(CH_2O)CH(O)CH_3$ moiety. The above assignment, combined with the molecular formula led to the final structure determination of **1** as 3-hydroxymethyl-2-methylenepentane-1,4-diol.

Compound **2** was isolated as colorless oil and the molecular formula $C_7H_{14}O_3$ was determined by HRESIMS for the $[M+H]^+$ ion at *m/z* 147.1023 (calculated for $C_7H_{15}O_3$: 147.1021). The ^1H NMR spectrum (Table 1) exhibited one tertiary methyl (δ 1.20), and two oxymethines (δ 3.56, 3.28). The ^{13}C and DEPT NMR spectra (Table 1) displayed 7 carbons, including an oxygenated quaternary carbon (δ 71.2), two methines (δ 69.6, 74.3), three methylenes and one methyl (δ 26.6). A singlet at δ 1.20 integrating for three protons in the ^1H NMR spectrum indicated the methyl (H-7) was attached to a quaternary carbon (C-1), which was confirmed by HMBC correlations from H-7 to C-1, C-2 and C-6. The remaining carbon and carbon connections were assigned by HMBC correlations between H-4 and C-2, C-3, C-5 and C-6. The above assignment, combined with the molecular formula led to the final structure determination of **2** as 1-methylcyclohexane-1,2,4-triol.

Compound **3** was obtained as amorphous powder with a molecular formula of $C_{24}H_{34}O_{12}$ assigned by negative HRESIMS (found $[M-H]^-$ 513.1967, calculated for 513.1972) in combination with the ^{13}C and DEPT NMR analysis. The ^1H and ^{13}C NMR spectra displayed resonances for 24 carbons, including a benzene ring, a rutinosyl group, one terminal double bond, an acetyl, an oxymethyl and one methylene. The NMR signals at δ_H 6.83 (1H, d, $J=1.8$ Hz), 6.73 (1H, dd, $J=8.2$ and 1.8 Hz), and 7.05 (1H, d, $J=8.2$ Hz), and δ_C 136.6 (s), 114.2 (d), 150.9 (s), 146.1 (s), 118.4 (d), and 122.0 (d) indicated the benzene ring was 1,2,4-trisubstituted. The data of **3** were closely similar to those of eugenyl β-rutinoside (**4**) [10]. The key difference was that an additional acetyl group (δ_H 2.08, δ_C 172.5, 21.1) was present in the NMR spectra of **3**. The linkage of the acetoxyl group and C-4″ was confirmed by HMBC correlations from H-8″ to C-4″, from H-4″ to C-7″. Furthermore, the sugar units were determined by hydrolysis of **3** with 10% HCl to afford D-glucose and L-rhamnose, which were confirmed by TLC comparison with authentic samples and determination of their optical rotation values ($[\alpha]_D^{20}=+59.6$ in H_2O for glucose; $[\alpha]_D^{20}=+10.1$ in H_2O for rhamnose) [11], respectively. Proposition of the β-anomeric configuration for glucose and α-anomeric configuration for rhamnose was based on the observation of a large $^3J_{H\text{-}1',H\text{-}2'}$ coupling constant (7.6 Hz) and a broad single peak for H-1″ in the ^1H NMR spectrum, respectively. Consequently, compound **3** was determined as eugenyl 4″-*O*-acetyl-β-rutinoside.

Phenylpropanoids including flavonoids have often been considered as plant-specific secondary metabolites [12] or restricted to some bacteria [13]. Japanese research recently reported genomic evidences for the existence of a phenylpropanoid metabolic pathway in the filamentous fungus *Aspergillus oryzae*[14]. However, to the best of our knowledge, phenylpropanoids have not been found in higher fungi so far. The fruiting bodies of *L. deliciosus* grows in acidic soil of conifers and form a mycorrhizal relationship with pines and spruces [15], which suggested compound **3** may biologically come from its mycorrhiza.

The structures of the known compounds **5**–**11** isolated were identified as 3β,5α-dihydroxyergosta-7,22-dien-6β-yl linoleate [16], 3β,5α-dihydroxyergosta-7,22-dien-6β-yl oleate [17], 3β,5α,6β,9α-tetradroxyergosta-7,22-diene [18, 19], 3β-hydroxyergosta-5,8,22-trien-7-one [20], 3β,5α,9α-trihydroxyergosta-7,22-dien-6-one [21], 5α,6α-epoxyergosta-8(14),22-dien-3β,7α-diol [19], and 5α,6α-epoxyergosta-8,22-dien-3β,7α-diol [19], respectively, by comparison of their spectroscopic data with literature values.

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