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A new isoprenyl phenyl ether riboside from the culture of basidiomycete *Laccaria amethystea*

Rong Liu^{a b}, Zhong-Yu Zhou^{a b}, Meng-Yuan Jiang^{a b}, Fei Wang^a & 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 Graduate University of Chinese Academy of Sciences, Beijing, 100049, China

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NOTE

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^aState Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, China; ^bGraduate University of Chinese Academy of Sciences, Beijing 100049, China

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A new isoprenyl phenyl ether riboside, 3-(3-methylbut-2-enyloxy)-4-*O*- α -D-ribofuranose benzoic acid methyl ester (**1**), was isolated from the culture of basidiomycete *Laccaria amethystea*. The structure of **1** was elucidated on the basis of extensive spectroscopic analysis.

Keywords: *Laccaria amethystea*; basidiomycete; isoprenyl phenyl ether riboside

1. Introduction

The basidiomycete *Laccaria amethystea* belonging to the family Tricholomataceae is an edible fungus with a wide distribution in most parts of China, especially in south-western China. The crude extract of this mushroom has been found to show anti-tumor activities against Ehrlich ascites carcinoma and Sarcoma-180 cell lines in mice [1]. In previous work, isoprenyl phenyl ethers have been reported to show cytotoxic effects against monkey kidney (BSC) cells [2], anti-bacterial activities against gram-positive bacteria *Staphylococcus aureus*, gram-negative bacteria *Escherichia coli* [3], and anti-fungal activity against the dermatophyte *Trichophyton mentagrophytes* [2]. So far, the secondary metabolites produced by this fungus of *L. amethystea* have not been reported. As one part of our research for naturally occurring bioactive metabolites from higher fungi in China [4–7], we have carried out a chemical investigation on the cultures of *L. amethystea*. It led to the

isolation of a new isoprenyl phenyl ether riboside, 3-(3-methylbut-2-enyloxy)-4-*O*- α -D-ribofuranose benzoic acid methyl ester (**1**) (Figure 1). In this paper, we describe the isolation and structural elucidation of the new compound **1**.

2. Results and discussion

3-(3-Methylbut-2-enyloxy)-4-*O*- α -D-ribofuranose benzoic acid methyl ester (**1**) was obtained as a colorless oil. The EI-MS showed a quasi-molecular ion peak at m/z 368. The molecular formula $C_{18}H_{24}O_8$ was established by HR-ESI-MS at m/z 391.1369 $[M + Na]^+$, indicating seven degrees of unsaturation. The IR spectrum exhibited absorptions for hydroxyl groups (3432 cm^{-1}), a carbonyl group (1718 cm^{-1}), and an aromatic moiety (1629 and 1511 cm^{-1}). The ^1H NMR spectrum (Table 1) displayed resonances for two tertiary methyls (δ_{H} 1.75, s, H-4; 1.79, s, H-5), one methoxyl (δ_{H} 3.90, s, OMe), one ABX spin system assignable to a 1,3,4-trisubstituted benzene ring at δ_{H}

*Corresponding author. Email: jkliu@mail.kib.ac.cn

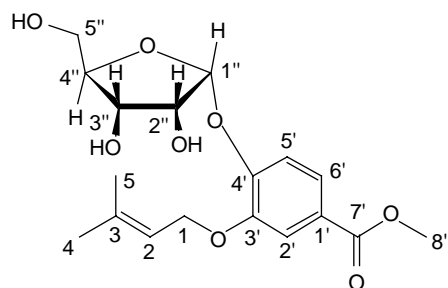


Figure 1. The structure of compound 1.

7.59 (d, $J = 1.5$ Hz, H-2'), 7.15 (d, $J = 8.3$ Hz, H-5'), and 7.64 (dd, $J = 8.3$, 1.5 Hz, H-6'), and one anomeric proton (δ_{H} 5.71, d, $J = 3.9$ Hz, H-1''). The ^{13}C NMR and DEPT spectra (Table 1) revealed 18 carbon signals, including two methyl, one methoxyl, two methylene, eight methine, and five quaternary carbons, of which the signals at δ_{C} 125.5 (s), 114.2 (d), 149.6 (s), 149.0 (s), 117.6 (d), 123.2 (d) were attributed to a benzene ring. In addition, ^{13}C NMR spectral data gave one ester carbonyl signal (δ_{C} 166.6, s, C-7'), one double bond (δ_{C} 118.6, d, C-2; 139.2, s, C-3), and one ribofuranose unit. Acid hydrolysis of **1** afforded D-ribose which was identified by comparison of its R_f and specific rotation with those of authentic sample. The small $^3J_{\text{H}1'',\text{H}2''}$ coupling constant ($J = 3.9$ Hz) deduced that the ribose possessed an α -anomeric configuration. The structure of **1** was

confirmed by careful analysis of ^1H - ^1H COSY, HSQC, and HMBC experiments.

Analysis of ^1H - ^1H COSY and HSQC spectra led to the identification of two spin systems, $\text{CH}_2(1)\text{-CH}(2)$ and $\text{CH}(5')\text{-CH}(6')$ (see numbering system for **1**). Observation of HMBC correlations from H-1 to C-2 and C-3, and from H-2 to C-4 and C-5 indicated the presence of the isoprenyl moiety, while HMBC correlation from H-1 to C-3' indicated that the isoprenyl moiety was located at the C-3' position of the benzene ring. The HMBC correlations from OMe, H-2' and H-6' to C-7' (δ_{C} 166.6) revealed that the methoxy group was situated at C-7', and the ester group was attached to C-1' (Figure 2). In addition, the HMBC correlation from H-1'' (δ_{H} 5.71, d, $J = 3.9$ Hz) to C-4' established the linkage of α -D-ribose and C-4'. Furthermore, the appearance of key HMBC correlation between H-1'' and C-4'', but no correlation between H-1'' and C-5'' suggested that the ribose should be ribofuranose rather than ribopyranose. In the ROESY spectrum, the cross-peak between H-1'' and H-3'' was observed, which further supported that the ribofuranose possessed an α -anomeric configuration. On the basis of the above evidence, compound **1** was determined as 3-(3-methylbut-2-enyloxy)-4-*O*- α -D-ribofuranose benzoic acid methyl ester (Figure 1).

Table 1. ^1H and ^{13}C NMR spectral data for compound **1** (CDCl_3 , δ in ppm, J in Hz).

Position	δ_{C}	δ_{H}	Position	δ_{C}	δ_{H}
1	65.7 (t)	4.59 (d, $J = 6.4$)	6'	123.2 (d)	7.64 (dd, $J = 8.3, 1.5$)
2	118.6 (d)	5.46 (t, $J = 6.4$)	7'	166.6 (s)	
3	139.2 (s)		8'	52.1 (q)	3.90 (s)
4	18.3 (q)	1.75 (s)	Ribose		
5	25.7 (q)	1.79 (s)	1''	102.3 (d)	5.71 (d, $J = 3.9$)
1'	125.5 (s)		2''	72.7 (d)	4.24 ^a
2'	114.2 (d)	7.59 (d, $J = 1.5$)	3''	70.9 (d)	4.10 (dd, $J = 6.1, 2.1$)
3'	149.6 (s)		4''	87.0 (d)	4.24 ^a
4'	149.0 (s)		5''	62.6 (t)	3.80 (dd, $J = 12.1, 2.9$)
5'	117.6 (d)	7.15 (d, $J = 8.3$)			3.72 (dd, $J = 12.1, 3.7$)

Note: ^a Overlapped signals.

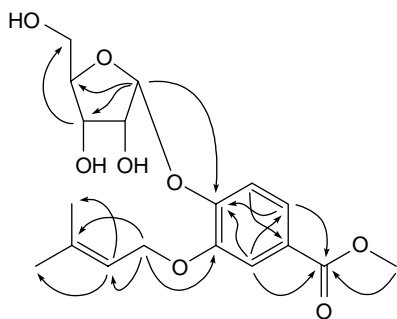


Figure 2. The key HMBC correlations of compound **1**.

3. Experimental

3.1 General experimental procedures

Optical rotation was measured on a Horbia SEPA-300 polarimeter. UV spectra were recorded on a Shimadzu UV-210 spectrometer. IR spectra were obtained on a Bruker Tensor 27 instrument with KBr pellets. Both 1D and 2D NMR experiments were performed on Bruker AM-400 and DRX-500 spectrometers in CDCl_3 with TMS as an internal standard. EI-MS was taken on a VG Auto Spec-3000 spectrometer. ESI-MS and HR-ESI-MS were recorded with an API QSTAR Pulsar 1 spectrometer. Preparative HPLC was performed using an Agilent 1100 liquid chromatograph equipped with a Zorbax SB-C₁₈, 9.4 mm \times 15 cm column. Silica gel (200–300 mesh; Qingdao Marine Chemical, Inc., Qingdao, China) and Sephadex LH-20 (Amersham Biosciences, Uppsala, Sweden) were used for column chromatography. Fractions were monitored by TLC, and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in ethanol.

3.2 Fungal material

The fungus *L. amethystea* was isolated from the tissue culture of its fruiting bodies collected at Gaoligong Mountains, Yunnan Province, China, in July 2006, and identified by Prof. Mu Zang, Kunming

Institute of Botany, Chinese Academy of Sciences (CAS). The voucher specimen has been deposited in the Herbarium of the Kunming Institute of Botany.

3.3 Cultivation

The culture medium consisted of potato (peeled off) (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 deionized H_2O . The fungus was grown in reagent bottles (500 ml; media of 300 ml). The pH was adjusted to 6.5 before autoclaving, and the fermentation was carried out on a shaker at 25°C and 150 rpm for 25 days.

3.4 Extraction and isolation

The whole culture broth of *L. amethystea* (20 liters) was filtered, and the filtrate was extracted three times with EtOAc. The organic layer was concentrated *in vacuo* to give a crude extract (4.8 g), and the residue was subjected to column chromatography over silica gel (200–300 mesh) eluting with CHCl_3 –MeOH (100:0–0:100, v/v) to afford fractions A–G. Fraction C (206 mg) was subjected to Sephadex LH-20 column chromatography using CHCl_3 –MeOH (1:1, v/v) as the eluent to produce two subfractions C₁ and C₂. Subfraction C₂ (69 mg) was further purified by preparative HPLC using CH_3CN – H_2O (45%) as the mobile phase (flow rate 5 ml/min) to give compound **1** (11 mg).

3-(3-Methylbut-2-enyloxy)-4-*O*- α -D-ribofuranose benzoic acid methyl ester (**1**): colorless oil; R_f (petroleum ether/ acetone 2:1) 0.40; $[\alpha]_D^{25.9} + 82.4$ ($c = 0.25$, MeOH); UV λ_{max} (MeOH) nm (log ϵ): 292 (3.4), 255 (3.7), 218 (4.1); IR (KBr) ν , cm^{-1} : 3432, 2926, 1718, 1629, 1511, 1271, 1205, 1042; ^1H NMR (CDCl_3 , 500 MHz) and ^{13}C NMR (CDCl_3 , 125 MHz) spectral data: see Table 1; EI-MS m/z : (%) 368 (2) $[\text{M}]^+$, 205 (5), 168 (100), 137 (34), 133 (24), 69 (68);

HR-ESI-MS m/z : 391.1369 $[M + Na]^+$
(calcd for $C_{18}H_{24}O_8Na$, 391.1368).

3.5 Acid hydrolysis of **1**

A solution of 8 mg of **1** in 2 M HCl (3 ml) was heated in a water bath at 90°C for 6 h. After cooling, the reaction mixture was neutralized with $NaHCO_3$ and extracted with EtOAc. Then, the aqueous layer was subjected to TLC analysis. Identification of D-ribose present in the aqueous layer was carried out by comparison of its R_f shift with that of an authentic sample.

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