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A New Pericarbonyl Lignan from *Amauroderma rude*

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Abstract: A new pericarbonyl lignan (**1**), named amaurolignan A was isolated from an ethanol extract of the fruiting bodies in *Amauroderma rude* of family Ganodermataceae, together with two known lignans, 4-methoxymatairesinol 4′-*β*-D-glucoside (**2**) and lappaol F (**3**). The structures of compounds (**1-3**) were elucidated using NMR and MS spectroscopic methods.

Keywords: Pericarbonyl lignan; amaurolignan A; *Amauroderma rude*.

1. Introduction

"Lingzhi" is a mushroom that has been renowned in China for more than 2000 years because of its claimed medicinal properties and symbolic fortune, which translates as 'Ganodermataceae' in a broad sense, and in a narrow sense it represents the highly prized medicinal Ganoderma species distributed in East Asia [1]. Its medicinal properties include anti-aging, lowering blood pressure, improving immunity, and preventing and treating various cancers, chronic bronchitis, gastric ulcers, hepatitis, neurasthenia and thrombosis [2-4]. The medicinal effects of many mushrooms such as *Ganoderma lucidum*, *Lentinula edodes*, *Agaricus blazei*, *Antrodia camphorate* and *Grifola frondosaI* come from their metabolites including polysaccharides, triterpenes, lucidenic acids, adenosine, ergosterol, glucosamine and cerebrosides [5-8]. This caused us to look further into another mushroom, *Amauroderma rude* (Berk.) Torrend, which called 'Xuezhi' in China and some species in this genus have been newly recognized as medicinal fungus [9-10]. The genus *Amauroderma* contains approximately 30 species and most of the species are widespread in tropical areas and rarely studied [11].

In an attempt to look for potential active substances from the *Amauroderma rude*, the isolation and chemical identification of one new pericarbonyl lignan (**1**) and two known lignans, 4-methoxymatairesinol 4′-*β*-D-glucoside (**2**) [12] and lappaol F (**3**) [13] (Figure 1) from the ethanol extract of *A. rude* were taken. Details of the isolation and identification of compound **1** are presented herein and the known compounds **2-3** were compared of spectroscopic data with those reported.

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2. Materials and Methods

2.1. Instrumentation and Reagents

The UV data was detected by Shimadzu UV-2401A. Mass spectra were performed on a VG Autospec-3000 spectrometer under 70 eV. 1D and 2D NMR spectra were recorded on Bruker AV-400 spectrometer with TMS as the internal standard (Bruker BioSpin Group). SiO_2 (100−200 mesh, Qingdao Marine Chemical Inc., China), Lichroprep RP-18 gel (40–63 μm, Merck, Darmstadt, Germany), and MCI gel (75–150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan) were used for column chromatography. Semi-preparative HPLC was performed on an Agilent 1100 liquid chromatography with a Welch Ultimate XB-Phenyl or Ultimate XB-C18 (10 μm, 4.6 mm × 25 cm). Preparative HPLC was performed on a Shimadzu LC-8A preparative liquid chromatograph with a Shimadzu PRC-ODS (K) column. Fractions were monitored by TLC and spots were visualized by heating silica gel plates sprayed with 8% H_2SO_4 in EtOH. All solvents including petroleum ether (60-90 ºC) were distilled prior to use.

2.2 Plant Materials

The fruiting bodies of *Amauroderma rude* were collected in January 2017 from Ruili State, Yunnan Province, China, and were authenticated by Prof. Min Zhou (Key Laboratory of Chemistry in Ethnic Medicinal Resources, Yunnan Minzu University). A sample (201701A) was preserved in Yunnan Minzu University, Kunming.

2.3. Extraction

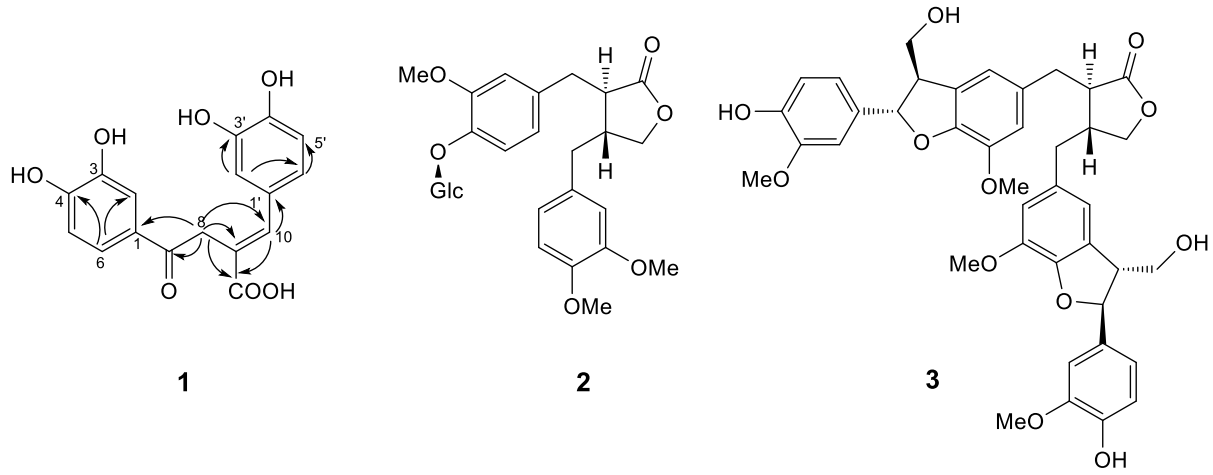
The chopped, dried fruiting bodies of *A. rude* (40 kg) was extracted with 95% ethanol solution heated under reflux (2 times/60 min), at 20 °C to give a residue (3 kg). The extract was suspended in pure water and partitioned with EtOAc. The EtOAc fraction (1.3 kg) was partitioned with a silica gel column (dichloromethane: methanol, from 1:0~80:1~20:1~8:1~5:1~2:1, each 5 L) to afford six subfractions (Fr. A-F). Fr. D (110 g) was further eluted with MCI column using a stepwise gradient of MeOH/H_2O (from 30%~55%~90%~100%, each 4 L) to afford three fractions (Fr. D1-D3). Fr. D2 (45.0 g) was loaded onto ODS (MeOH/H_2O 40%~60%~80%~100%, each 2 L) to give four fractions (Fr. D2-1~4). Fr. D2-2 (15.5 g) was separated over YMC-Pack ODS-A (20×250 mml. D.S, 5 μm) prep. HPLC (68% MeOH/H_2O), yielding **2** (25.0 mg) and **3** (30.6 mg). Fr. D2-3 (10.0 g) was further eluted with a silica gel (dichloromethane/acetone, from 80:1~20:1~10:1~8:1~2:1, each 2 L) and separated over semi-prep. HPLC (50% MeOH-H_2O) to yield **1** (22.0 mg).

*Amaurolignan A (**1**):* yellow oil; UV λ_{max} 230, 280 and 330 nm; ^{1}H NMR (CD_3OD) and ^{13}C NMR (CD_3OD) see Table 1; HR-ESIMS *m/z* 329.0669 $[M-H]^-$ (calculated for $C_{17}H_{14}O_7$, 329.0667).

*4-Methoxymatairesinol 4′-β-D-Glucoside (**2**):* White oily liquid; ^{1}H NMR (400 MHz, CD_3OD): δ_H 7.07 (1H, d, *J* = 8.2 Hz, H-5′), 6.83 (1H, d, *J* = 8.6 Hz, H-5), 6.76 (1H, s, H-2), 6.66 (1H, d, *J* = 8.2 Hz, H-6), 6.61 (2H, d, *J* = 5.9 Hz, H-2′/6′), 4.19 (1H, t, *J* = 8.2 Hz, H-9′a), 3.94 (1H, t, *J* = 8.2 Hz, H-9′b), 3.87 (1H, d, *J* = 12.0 Hz, H-Glc-6a), 3.80 (1H, s, H-OCH_3-4), 3.76 (6H, s, H-3/3′), 3.70 (1H, d, *J* = 11.3 Hz, H-Glc-6b), 3.50 (2H, d, *J* = 6.7 Hz, H-Glc-2, 3), 3.42 (2H, s, H-Glc-4, 5), 2.92 (1H, dd, *J* = 13.9, 5.3 Hz, H-7a), 2.82 (1H, dd, *J* = 13.9, 7.2 Hz, H-7b), 2.68 (1H, dd, *J* = 13.2, 7.2 Hz, H-8), 2.56 (2H, d, *J* = 6.3 Hz, H-7a′/7b′), 2.49 (1H, dd, *J* = 14.6, 7.5 Hz, H-8′). ^{13}C NMR (400 MHz, CD_3OD): δ_C 132.7 (C-1), 114.8 (C-2), 149.1 (C-3), 150.4 (C-4), 113.1 (C-5), 123.0 (C-6), 35.4 (C-7), 47.6 (C-8), 134.2 (C-1′), 113.6 (C-2′), 150.6 (C-3′), 146.8 (C-4′), 117.8 (C-5′), 122.1 (C-6′), 38.9 (C-7′), 42.5 (C-8′), 72.9 (C-9′), 102.9 (Glc-1), 74.9 (Glc-2), 77.8 (Glc-3), 71.3 (Glc-4), 78.1 (Glc-5), 62.5 (Glc-6), 56.7 (OCH_3-4), 56.5 (OCH_3-3/3′).

*Lappaol F (**3**):* ^{1}H NMR (400 MHz, CD_3OD): δ_H 6.63 (1H, br s, H-2), 6.57 (2H, d, *J* = 2. 0 Hz, H-6/6'), 2.76 (1H, dd, *J* = 12.0, 8.0 Hz, H-7a), 2.92 (1H, dd, *J* = 16.0, 8.0 Hz,H-7b), 2.65 (1H, m, H-8), 6.50 (1H, br s, H-2'), 2.55 (1H, m, H-8'), 3.94 (1H, dd, *J* = 8.0, 4.0 Hz, H-9'a), 4.25 (1H, dd, *J* = 8.0, 4.0 Hz, H-9'b),

6.98 (1H, d, *J* = 2.0 Hz, H-2″), 6.74 (2H, t, *J* = 8.0 Hz, H-5″, H-5‴), 6.80 (1H, dd, *J* = 8.0, 4.0 Hz, H-6″), 5.46 (1H, t, *J* = 7.0 Hz, H-7″/7‴), 3.48 (2H, dd, *J* = 12.0, 4.0 Hz, H-8″/8‴), 7.00 (1H, d, *J* = 2.0 Hz, H-2‴), 6.82 (1H, dd, *J* = 8.0, 4.0 Hz, H-6‴). ^{13}C NMR (400 MHz, CD_3OD): δ_C 130.2 (C-1), 113.8 (C-2), 147.5 (C-3), 145.3 (C-4), 132.7 (C-5), 114.8 (C-6), 35.8 (C-7), 47.8 (C-8), 181.6 (C-9), 130.4 (C-1'), 116.0 (C-2'), 147.5 (C-3'), 145.3 (C-4'), 133.3 (C-5'), 116.1 (C-6'), 39.3 (C-7'), 42.6 (C-8'), 73.0 (C-9'), 134.3 (C-1″), 110.6 (C-2″), 148.0 (C-3″), 148.1 (C-4″), 118.3 (C-5″), 120.0 (C-6″), 89.1 (C-7″), 55.0 (C-8″), 64.6 (C-9″), 134.3 (C-1‴), 110.5 (C-2‴), 149.1 (C-3‴), 134.5 (C-4‴), 118.9 (C-5‴), 119.9 (C-6‴), 89.2 (C-7‴), 55.0 (C-8‴), 64.6 (C-9‴), 56.4, 56.4, 56.6, 56.7 (OCH_3).



1 **2** **3**

Figure 1. Key HMBC correlations of compound **1** and the structures of compounds **1-3**

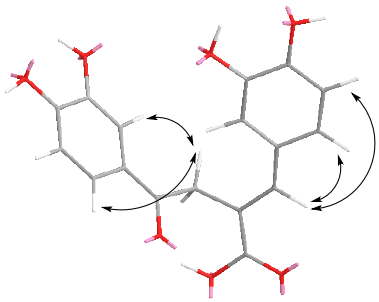


Figure 2. Key ROESY correlations (arrows) for compound **1**

3. Results and Discussion

Compound **1**, yellow oil, showed a molecular formula of $C_{17}H_{14}O_7$, as deduced from HRESI (-) MS at *m/z* 329.0669 ([M-H]$^-$, calcd 329.0667). Its ^{1}H NMR spectra (Table 1) showed feature signals for one methylene at δ_H 4.10 (br s, 2H), six aromatic proton signals at δ_H 6.58 (d, *J* = 8.2, 1H), 6.63 (d, *J* = 8.2, 1H), 6.70 (br s, 1H), 6.75 (d, *J* = 8.2, 1H), 7.38 (br s, 1H) and 7.41 (d, *J* = 8.4, 1H) assigned to two trisubstituted benzene rings, and one olefinic proton at δ_H 7.74 (s, 1H). Preliminary investigation of ^{13}C-NMR and DEPT spectra (Table 1) revealed a total of 17 carbon signals, consisting of nine quaternary carbons (including one olefenic, six aromatics, one ketone carbonyl and a carboxyl), seven methines (including one olefenic and six aromatics) and one methylene, indicating that compound **1** is a lignan. The NMR data is very closely related to *β*-(3,4-dimethoxybenzoyl)-*α*-(3,4-dimethoxybenzylidene) propionic acid [14] except for missing methoxys in **1**. This change indicated that the methoxys in *β*-(3,4-dimethoxybenzoyl)-*α*-(3,4-dimethoxybenzylidene) propionic acid was reduced to the hydroxyls in **1**, which can be observed in ^{13}C-NMR and DEPT spectrum. The key HMBC correlations from H-8 to C-1, C-7, C-9, C-10 and C-11, and from H-10 to C-1' confirmed the connection between the straight chain fragment and the two benzene rings (Figure 1). The cis-trans isomerism of double bond at $\Delta^{9(10)}$ was trans can be confirmed by the cross-peaks

of H-8/H-2/H-6 and H-10/H-5'/H-6' in ROESY spectrum (Figure 2). Thus, **1** was elucidated to be amaurolignan A.

These skeleton type of pericarbonyl lignans were rare in nature, most of them are intermediates in organic synthesis reactions, like the Perkin condensation product *α*-arylidine-*β*-benzoyl propionic acid [15-18]. To be best of our knowledge, this is the first report of natural compound with this skeleton from the fungus and the first time from Ganodermataceae.

Table 1. ^{1}H NMR and ^{13}C NMR data for compound **1** (at 400 MHz in CD_3OD, δ in ppm, *J* in Hz)

Position	H	C
1		128.9
2	7.38 (1H, *br s*)	114.7
3		150.9
4		145.0
5	6.75 (1H, *d*, *J* = 8.2)	114.5
6	7.41 (1H, *d*, *J* = 8.2)	121.9
7		197.3
8	4.10 (2H, *br s*)	37.5
9		124.5
10	7.74 (1H, *s*)	142.1
11		170.2
1'		127.0
2'	6.63 (1H, *d*, *J* = 8.2)	115.0
3'		144.9
4'		146.4
5'	6.58 (1H, *dd*, *J* = 8.2, 1.5)	121.5
6'	6.70 (1H, *d*, *J* =1.5)	115.9

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Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

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