

A New Pericarboxyl Lignan from *Amauroderma rude*Miao Dong¹, Zuhong Ma², Qiaofen Yang², Qiuyue Hu², Yanqing Ye^{2,*}
and Min Zhou^{1,*}¹Key Laboratory of Chemistry in Ethnic Medicinal Resources, State Ethnic Affairs Commission &
Ministry of Education, Yunnan Minzu University, Kunming 650031, P.R. China²School of Chemistry and Environment, Yunnan Minzu University, Kunming 650031, P.R. China

(Received October 24, 2018; Revised November 29, 2018; Accepted November 30, 2018)

Abstract: A new pericarboxyl lignan (**1**), named amaurogignan 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: Pericarboxyl lignan; amaurogignan A; *Amauroderma rude*. © 2018 ACG Publications. All rights reserved.

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 frondosa* 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 pericarboxyl 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.

* Corresponding author: E-Mail: zhouminyun@163.com

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). SiO₂ (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% H₂SO₄ in EtOH. All solvents including petroleum ether (60–90 °C) 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/H₂O (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/H₂O 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 mm, D.S., 5 μm) prep. HPLC (68% MeOH/H₂O), 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-H₂O) to yield **1** (22.0 mg).

Amaurodignan A (1): yellow oil; UV λ_{max} 230, 280 and 330 nm; ¹H NMR (CD₃OD) and ¹³C NMR (CD₃OD) see Table 1; HR-ESIMS *m/z* 329.0669 [M-H]⁻ (calculated for C₁₇H₁₄O₇, 329.0667).

4-Methoxymatairesinol 4'-β-D-Glucoside (2): White oily liquid; ¹H NMR (400 MHz, CD₃OD): 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-OCH₃-4), 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'). ¹³C NMR (400 MHz, CD₃OD): 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 (OCH₃-4), 56.5 (OCH₃-3/3').

Lappaol F (3): ¹H NMR (400 MHz, CD₃OD): 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).

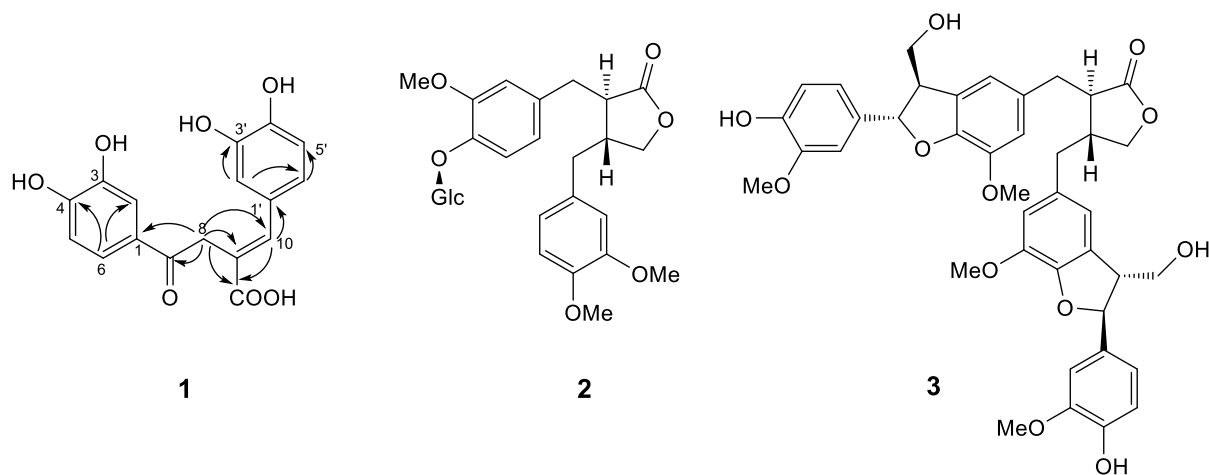


Figure 1. Key HMBC correlations of compound **1** and the structures of compounds **1-3**

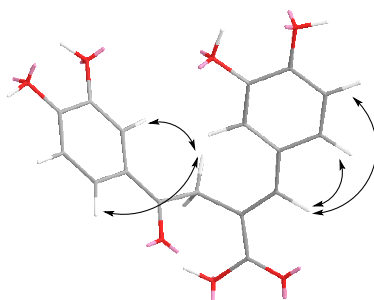


Figure 2. Key ROESY correlations (arrows) for compound **1**

3. Results and Discussion

Compound **1**, yellow oil, showed a molecular formula of $\text{C}_{17}\text{H}_{14}\text{O}_7$, as deduced from HRESI (-) MS at m/z 329.0669 ($[\text{M}-\text{H}]^-$, calcd 329.0667). Its ^1H 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 olefinic, six aromatics, one ketone carbonyl and a carboxyl), seven methines (including one olefinic 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 pericarboxyl 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. ^1H 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, <i>br s</i>)	114.7
3		150.9
4		145.0
5	6.75 (1H, <i>d</i> , $J = 8.2$)	114.5
6	7.41 (1H, <i>d</i> , $J = 8.2$)	121.9
7		197.3
8	4.10 (2H, <i>br s</i>)	37.5
9		124.5
10	7.74 (1H, <i>s</i>)	142.1
11		170.2
1'		127.0
2'	6.63 (1H, <i>d</i> , $J = 8.2$)	115.0
3'		144.9
4'		146.4
5'	6.58 (1H, <i>dd</i> , $J = 8.2, 1.5$)	121.5
6'	6.70 (1H, <i>d</i> , $J = 1.5$)	115.9

Acknowledgments

Financial support of the National Natural Science Foundation of China (No. 31600282 and 31560099).

Supporting Information

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

ORCID

Miao Dong: [0000-0002-0003-9402](https://orcid.org/0000-0002-0003-9402)

Zuhong Ma: [0000-0002-1368-4902](https://orcid.org/0000-0002-1368-4902)

Qiaofen Yang: [0000-0003-3462-5079](https://orcid.org/0000-0003-3462-5079)

Qiuyue Hu: [0000-0002-9236-7963](https://orcid.org/0000-0002-9236-7963)

Yanqing Ye: [0000-0003-4244-5385](https://orcid.org/0000-0003-4244-5385)

Min Zhou: [0000-0003-1896-9832](https://orcid.org/0000-0003-1896-9832)

References

- [1] Y. Cao, S. H. Wu and Y. C. Dai (2012). Species clarification of the prize medicinal *Ganoderma* mushroom "Lingzhi", *Fungal Divers.* **56**, 49-62.
- [2] A. H. Aly, A. Debbab and P. Proksch (2011). Fifty years of drug discovery from fungi, *Fungal Divers.* **50**, 3-19.
- [3] Y. C. Dai, Z. L. Yang, B. K. Cui, C. J. Yu and L. W. Zhou (2009). Species diversity and utilization of medicinal

- mushrooms and fungi in China (Review), *Int. J. Med. Mushrooms*. **11** (3), 287-302.
- [4] D. D. De Silva, S. Rapior, F. Fons, A. H. Bahkali and K. D. Hyde (2012). Medicinal mushrooms in supportive cancer therapies: an approach to anti-cancer effects and putative mechanisms of action, *Fungal Divers.* **55**, 1–35.
- [5] Y. H. Gao and S. F. Zhou (2003). Cancer prevention and treatment by *Ganoderma*, a mushroom with medicinal properties, *Food Rev. Int.* **19** (3), 275-325.
- [6] Z. B. Lin (2009). *Lingzhi: from mystery to science*. Peking University Medical Press, Beijing.
- [7] B. G. Jung, J. A. Lee and B. J. Lee (2012). Immunoprophylactic effects of shiitake mushroom (*lentinula edodes*) against bordetella bronchiseptica in mice, *J. Microbiol.* **50** (6), 1003-1008.
- [8] C. J. Chen, R. Vijaya Krishna, C. C. Tsai, W. H. Wu, L. K. Chao, K. H. Hwang, C. M. Chien, H. Y. Chang and S. T. Chen (2010). Structure and functions of gamma-dodecalactone isolated from *Antrodia camphorata* for NK cell activation, *Bioorgan. Med. Chem.* **18** (18), 6896-6904.
- [9] C. Jiao, Y. Z. Xie, X. Yang, H. Li, X. M. Li, H. H. Pan, M. H. Cai, H. M. Zhong and B. B. Yang (2013). Anticancer activity of *amauroderma rude*, *Plos One* **8** (6), e66504.
- [10] P. M. Chan, G. Kanagasabapathy, Y. S. Tan, V. Sabaratnam and U. R. Kuppusamy (2013). *Amauroderma rugosum* (blume & t. nees) torrend: nutritional composition and antioxidant and potential anti-inflammatory properties, *Evid-Based. Compl. Alt.* **2013** (4), 304713.
- [11] S. S. Zhang, Q. Y. Ma, X. S. Zou, H. F. Dai, S. Z. Huang, Y. Luo, Z. F. Yu, H. R. Luo and Y. X. Zhao (2013). Chemical constituents from the fungus *Amauroderma amoiensis* and their *in vitro* acetylcholinesterase inhibitory activities, *Planta Med.* **79**, 87–91.
- [12] R. Tundis, G. Statti, F. Menichini and M. F. Delle (2000). Arctiin and onopordopicrin from *carduus micropterus* ssp. *Perspinosus*, *Fitoterapia* **71** (5), 600-601.
- [13] K. Umehara, M. Nakamura, T. Miyase, M. Kuroyanagi and A. Ueno (1996). Studies on differentiation inducers. vi. lignan derivatives from *arctium fructus*. (2), *Chem. Pharm. Bull.* **44** (12), 2300-2304.
- [14] M. Rischmann, R. Mues, H. Geiger, H. J. Laas and T. Eicher (1989). Isolation and synthesis of 6,7-dihydroxy-4-(3,4-dihydroxyphenyl)naphthalene-2-carboxylic acid from *pellia epiphylla*, *Phytochemistry* **28** (3), 867-869.
- [15] T. Chaudhari, S. Deo and F. Inam (2013). Natural & Synthetic 1-Phenylnaphthalene Lignans. Natural & synthetic 1-Phenylnaphthalene lignans: Immunomodulation. LAP LAMBERT Academic Publishing.
- [16] R. D. Haworth and D. Woodcock (1938). 154. the constituents of natural phenolic resins. part xi. synthesis of 2- and 3-methyl-6 : 7-dimethoxy-1-veratrylnaphthalenes, *J. Chem. Soc.* **54**, 809-813.
- [17] Z. Ali, S. Deo and F. Inam (2013). A recyclable and highly effective sulfamic acid/etoh catalytic system in synthesis and natural product chemistry of lignan compounds, *Asian J. Chem.* **25** (2), 899-904.
- [18] S. Deo, T. Chaudhari and F. Inam (2015). Cheminform abstract: microwave assisted perkin reaction for the synthesis of α -arylidine- γ -phenyl- Δ,β,γ -butenolides, *Cheminform* **45** (37), 111-117.

ACG
publications

© 2018 ACG Publications