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New Unsaturated Lactones and a Meroterpenoid from *Ganoderma lucidum*

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Abstract

From the dried fruiting bodies of *Ganoderma lucidum*, 2 new unsaturated lactones, dayaolingzhilactones A and B (**1** and **2**), and 1 new meroterpenoid, dayaolingzhiol H (**3**), together with 10 known compounds (**4-13**), were isolated. Their chemical structures were identified by using spectroscopic data and calculated specific rotation. The inhibitory activities of compounds **1** and **2** toward acetylcholinesterase (AchE) were assessed in vitro with tacrine as the positive control. Both of them exhibited moderate AchE inhibitory activities at the concentration of 50 μM.

Keywords

lactones, *Ganoderma lucidum*, calculated specific rotation, AchE inhibitory activity

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Ganoderma lucidum (Ganodermataceae), a fungus registered in the Pharmacopoeia of China and *American Herbal Pharmacopoeia and Therapeutic Compendium*, has been used in China for thousands of years for promoting health, longevity and spiritual growth.[1] Currently, it is widely used for the treatment of bronchitis, allergies, hepatitis, hypertension, immunological disorders, neurasthenia, and diabetes.[2,3] Previous chemical studies revealed that polysaccharides and triterpenoids are the 2 main pharmacologically active constituents in *G. lucidum*.[4] In addition, a few other structural types of compounds have also been reported sporadically such as alkaloids, steroids, lactones, and fatty acids.[4] As part of our chemical studies on *Ganoderma* genus, the title material was investigated, resulting in the isolation of 2 new unsaturated lactones (**1** and **2**), a new meroterpenoid (**3**), along with 10 known compounds (**4-13**) (Figure 1). Besides, the inhibitory activities of compounds **1** and **2** toward acetylcholinesterase (AchE) were assessed. Our efforts are described below.

Compound **1**, a colorless oil, has the molecular formula $C_{15}H_{24}O_5$ based on the analysis of its HRESIMS *m/z* 283.1551 $[M-H]^-$, ^{13}C NMR, and DEPT spectra, with 4 degrees of unsaturation. The ^1H NMR data (Table 1) of **1** show 2 methyls [δ_H 1.93 (3H, s, CH_3-15); 1.78 (3H, s, CH_3-14)]. The ^{13}C NMR and DEPT spectra display 15 carbons including 2 methyls, 8 methylenes, and 5 nonprotonated carbons (1 ester carbonyl, 1 carboxylic acid, 2 sp^2 carbons, and 1 oxygenated sp^3 carbon). The architecture of **1** was constructed by 2D NMR experiments. Interpretation of 1D and 2D NMR data discloses that the structure of compound **1** resembles that of 5-hydroxy-3,4-dimethyl-5-pentyl-2(5*H*)-furanone (**4**)[5] but differs in the structure of the side chain. The ^1H-^1H COSY (Figure 2) correlations of H_2-5/H_2-6/H_2-7 and H_2-10/H_2-11/H_2-12, together with the HMBC correlations of H_2-6/C-8, H_2-11/C-9, and H_2-11, H_2-12/C-13 (δ_C 178.4), suggest the length of the side chain, and the additional carboxylic acid group is at the terminal of the chain. In addition, HMBC correlations of H_3-14/C-2, C-3, H_3-15/C-2, C-3, C-4, and H_2-5/C-4 secured the presence of an unsaturated 5-membered lactone. Thus, the planar structure of **1** has been deduced. Finally, there is 1 chiral center in the structure of **1**; its absolute configuration at C-4 in **1** was identified as 4*R* by comparing its specific rotation {$[\alpha]_D^{20}$ −11.8 (*c* 0.17, MeOH)} with the reported {$[\alpha]_D^{23}$ +5.4 (*c* 0.18, MeOH)} for 4*S* of sinularone I.[6,7] This conclusion was further supported by the calculated optical values of model

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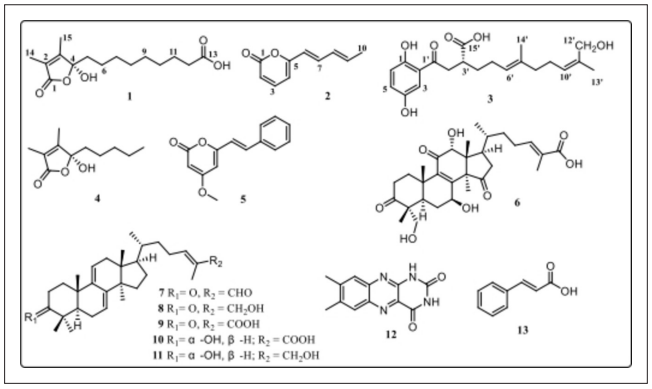

Figure 1. Chemical structures of compounds **1** to **13**.

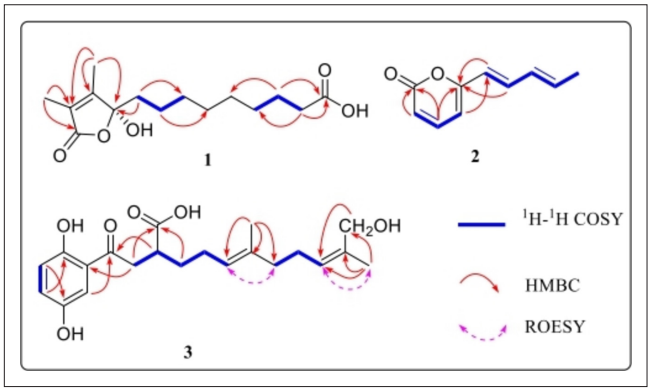

Figure 2. Key COSY, HMBC, and ROESY correlations of **1** to **3**.

compounds (–54.0 for 4*R*, +54.0 for 4*S*) at B3LYP/6-311G(d,p) level in MeOH (see Supplementary Material 1, Table S1). Thus, the structure of **1**, named dayaolingzhilactone A, was identified.

Compound **2** possesses a molecular formula of $C_{10}H_{10}O_2$ based on its HRESIMS, ^{13}C NMR, and DEPT data, with 6 degrees of unsaturation. The ^{1}H NMR spectrum of **2** (Table 1) shows signals assignable to 1 methyl [δ_H 1.86 (3H, d, J = 6.7 Hz, CH_3-10)] and 7 olefinic/aromatic protons [δ_H 7.48 (1H, dd, J = 9.2, 6.9 Hz, H-3), 7.05 (1H, dd, J = 15.3, 10.8 Hz, H-7), 6.29 (1H, dd, J = 16.2, 10.8 Hz, H-8), 6.26 (1H, d, J = 6.9 Hz, H-4), 6.17 (1H, d, J = 9.2 Hz, H-2), 6.14 (1H, overlap, H-6), 6.11 (1H, overlap, H-9)]. The ^{13}C NMR and DEPT

Table 1. ^{1}H and ^{13}C NMR Data of **1** to **3** (δ in ppm, J in Hz).

	1[a]			**2**[b]			**3**[b]	
No.	δ_H (J in Hz)	δ_C	No.	δ_H (J in Hz)	δ_C	No.	δ_H (J in Hz)	δ_C
1		174.6	1		164.3	1		156.5
2		125.7	2	6.17 (d, 9.2)	114.0	2		120.4
3		160.4	3	7.48 (dd, 9.2, 6.9)	146.4	3	7.26 (d, 2.9)	115.4
4		109.2	4	6.26 (d, 6.9)	105.9	4		150.6
5	1.95 (m); 1.73 (m)	36.9	5		161.5	5	7.00 (dd, 8.9, 2.9)	125.8
6	1.24 (m); 1.15 (m)	24.1	6	6.14 overlap	121.3	6	6.78 (d, 8.9)	119.7
7	1.31 (overlap)	30.5	7	7.05 (dd, 15.3, 10.8)	137.0	1′		205.8
8	1.31 (overlap)	30.4	8	6.29 (dd, 16.2, 10.8)	132.0	2′	3.43 (dd, 17.8, 9.3) 3.10 (dd, 17.8, 4.4)	41.4
9	1.31 (overlap)	30.3	9	6.11 overlap	137.7	3′	2.96 (m)	41.3
10	1.31 (overlap)	30.2	10	1.86 (d, 6.7)	18.7	4′	1.74 (m); 1.62 (m)	33.2
11	1.58 (m)	26.3				5′	2.10 (m)	26.5
12	2.26 (t, 7.2)	35.4				6′	5.16 (t, 7.2)	124.9
13		178.4				7′		136.8
14	1.78 (s)	8.2				8′	2.01 (m)	41.0
15	1.93 (s)	10.8				9′	2.16 (m)	27.1
16						10′	5.26 (t, 7.3)	128.5
17						11′		135.8
18						12′	4.05 (s)	61.4
19						13′	1.74 (s)	21.5
20						14′	1.62 (s)	16.1
						15′		179.5

[a]Recorded at 400 (100) MHz in methanol-d_4.
[b]Recorded at 600 (150) MHz in methanol-d_4.

spectra display 10 carbons which are classified into 1 methyl, 7 sp^2 methines, and 2 nonprotonated carbons (1 ester carbonyl and 1 oxygenated sp^2 carbon), accounting for 5 degrees of unsaturation. The ^{1}H-^{1}H COSY (Figure 2) gives the correlations of H-2/H-3/H-4 and H-6/H-7/H-8/H-9/H_3-10. The remaining 1 degree of unsaturation could be attributed to the presence of a ring formed by the oxygen bridge between C-1 (δ_C 164.3) and C-5 (δ_C 161.5), which was further certified by the HMBC correlations of H-2, H-3/C-1 and H-4, H-6/C-5. The couple constants of $J_{6,7}$ value (15.3 Hz) and $J_{8,9}$ value (16.2 Hz) suggest the configurations of the double bonds $\Delta^{6(7)}$ and $\Delta^{8(9)}$ are both of *trans*-orientation. Thus, the structure of **2** was determined (Figure 1).

The molecular formula of compound **3** was determined to be $C_{21}H_{28}O_6$ (8 degrees of unsaturation) based on its HRESIMS, ^{13}C NMR, and DEPT data. The ^{1}H NMR spectrum of **3** (Table 1) contains resonances for a typical ABX aromatic spin system [δ_H 7.26 (1H, d, J = 2.9 Hz, H-3), 7.00 (1H, dd, J = 8.9, 2.9 Hz, H-5), 6.78 (1H, d, J = 8.9 Hz, H-6)] and 2 olefinic protons [δ_H 5.26 (1H, t, J = 7.3 Hz, H-10′), 5.16 (1H, t, J = 7.2 Hz, H-6′)], suggesting the presence of a 1,2,4-trisubstituted aromatic ring and 2 double bonds in **3**. The ^{13}C NMR and DEPT spectra contain 21 carbons, attributed to 2 methyls, 6 methylenes (including 1 oxygenated), 6 methines, and 7 nonprotonated carbons (1 ketone carbonyl, 1 ester carbonyl, 2 sp^2 carbons, and 3 aliphatic carbons including 2 oxygenated). The ^{1}H-^{1}H COSY correlations of H_2-2′/H-3′/H_2-4′/H_2-5′/H-6′ and H_2-8′/H_2-9′/H-10′, together with the HMBC correlations of H_2-12′, H_3-13′/C-10′, C-11′, H_3-14′/C-6′, C-7′, C-8′, H-3′/C-1′ (δ_C 205.8), and H_2-2′, H_2-4′/C-15′ (δ_C 179.7), reveal the structure of the side chain from C-1′ to C-15′ in **3** as shown (Figure 1), and the HMBC correlations of H_2-2′/C-2 and H-3/C-1′ suggest the side chain is attached to C-2. For the geometry of the double bonds, the ROESY correlations of H-6′/H_2-8′ and H-10′/H_3-13′ indicate that the configurations of the $\Delta^{6'(7')}$and $\Delta^{10'(11')}$double bonds are both *E*-form. Thus, the planar structure of **3** was deduced. Compound **3** was proved to be optically pure by chiral HPLC analysis, and its absolute configuration was determined as 3′*R* by comparing its optical rotation (OR) {$[\alpha]_D^{20}$ +17.2 (*c* 0.06, MeOH)} with {$[\alpha]_D^{25}$ +10.5 (*c* 0.07, MeOH)} of applanatumol S.[6,7]

The known compounds were identified by comparing their spectroscopic data with the literature data. They are 5-hydroxy-3,4-dimethyl-5-pentyl-2(5*H*)-furanone (**4**),[5] 4-methoxy-6-styryl-2*H*-pyran-2-one (**5**),[8] ganoleucoin E (**6**),[9] ganoderal A (**7**),[10] ganoderol A (**8**),[11] ganoderic acid S (**9**),[11] ganoderic acid Y (**10**),[11] ganoderol B (**11**),[11] lumichrome (**12**),[12] and cinnamic acid (**13**).[13]

In this study, only the new compounds with the exception of **3** were evaluated for their inhibitory property toward AchE. It was found that both of them (50 μM) exhibit moderate inhibitory activities with the inhibition rate of 49.69% ± 0.63% and 35.45% ± 3.37%, respectively (tacrine as a positive control with the inhibition rate of 60.68% ± 2.85% at the concentration of 0.33 μM).

Experimental

General

ORs were recorded on a Jasco P-1020 digital polarimeter. UV spectra were measured on a Shimadzu UV2401PC spectrometer. CD spectra were measured on an Applied Photophysics Chirascan instrument. NMR spectra were measured on a Bruker AV 400, or 600 MHz spectrometer, with TMS as an internal standard. ESIMS and HRESIMS were measured on an Agilent 1290 UPLC/6540 Q-TOF instrument. RP-18 (40-60 μm, Daiso Co., Japan), MCI gel CHP 20P (75-150 μm, Tokyo, Japan), silica gel GF254 (80-100 mesh, Qingdao Marine Chemical Inc., China), and Sephadex LH-20 (Amersham Biosciences, Sweden) were used for column chromatography. Semipreparative HPLC was carried out using an Agilent 1200 liquid chromatograph (Agilent Technologies, Santa Clara, CA, USA). The columns used were YMC-Pack ODS-A (250 mm × 10 mm, i.d., 5 μm) and Daicel Chiralpak IC (250 mm × 10 mm, i.d., 5 μm).

Fungal Material

The fruiting bodies of *G. lucidum* were collected from Dayao County of Yunnan Province, China, in December 2015. The material was identified by Prof Zhu-Liang Yang at Kunming Institute of Botany, Chinese Academy of Sciences, and a voucher specimen (CHYX0596) has been deposited at the School of Pharmaceutical Sciences, Shenzhen University, since November 2017.

Extraction and Isolation

The dried material (4.0 kg) was soaked with 80% EtOH (20 L × 3 × 24 hours) to give a crude extract, which was extracted with EtOAc to afford an EtOAc-soluble extract (0.12 kg). The EtOAc extract was divided into 10 parts (Fr.1-Fr.10) by using an MCI gel CHP 20P column chromatography eluted with aqueous MeOH (40%-100%). Fr.5 (5.1 g) was passed through Sephadex LH-20 (MeOH) to yield 4 fractions (Fr.5.1-Fr.5.4). Fr.5.2 (0.4 g) was further separated by preparative HPLC (MeOH/H_2O, 60%) followed by semipreparative HPLC (ACN/H_2O, 40%) to give **6** (1.3 mg, t_R = 11.1 minutes, 3 mL/min). Fr.6 (3.7 g) was filtered by Sephadex LH-20 (MeOH) to give Fr.6.1 to Fr.6.6. Fr.6.3 (30.0 mg) was purified by semipreparative HPLC (ACN/H_2O, 48%) to yield **2** (1.3 mg, t_R = 14.3 minutes, 3 mL/min). Fr.6.4 (0.1 g) was first separated by preparative HPLC (MeOH/H_2O, 60%) and then purified by semipreparative HPLC (ACN/H_2O, 41%) to obtain **3** (2.6 mg, t_R = 23.7 minutes, 3 mL/min). Fr.6.5 (80.0 mg) was also first separated by preparative

HPLC (MeOH/H_2O, 60%) to provide Fr.6.5.1 to Fr.6.5.7. Fr.6.5.2 (11.0 mg), Fr.6.5.4 (12.0 mg), and Fr.6.5.6 (8.0 mg) were further purified by semipreparative HPLC (ACN/H_2O, 45%, 42%, and 41%) to provide compounds **13** (8.8 mg, t_R = 19.2 minutes, 3 mL/min), **1** (2.5 mg, t_R = 9.7 minutes, 3 mL/min), and **4** (3.2 mg, t_R = 24.0 minutes, 3 mL/min), respectively. Fr.6.6 was submitted to semipreparative HPLC (MeOH/H_2O, 59%) to yield **12** (2.3 mg, t_R = 13.1 minutes, 3 mL/min). Fr.9 (36.0 g) was fractionated by Sephadex LH-20 (MeOH) to give Fr.9.1 to Fr.9.6. Fr.9.1 (50.0 mg) was then purified by Sephadex LH-20 (MeOH) followed by semipreparative HPLC (ACN/H_2O, 61%) to afford **5** (1.5 mg, t_R = 17.3 minutes, 3 mL/min). Fr.9.6 (6.4 g) was submitted to Sephadex LH-20 (MeOH) to give Fr.9.6.1 to Fr.9.6.4. Fr.9.6.2 (60.0 mg) was further separated by semipreparative HPLC (ACN/H_2O, 90%) to obtain **10** (10.5 mg, t_R = 16.3 minutes, 3 mL/min) and **11** (2.8 mg, t_R = 26.9 minutes, 3 mL/min). Fr.9.6.3 (35.0 mg) was also separated by semipreparative HPLC (ACN/H_2O, 95%) to afford **7** (10.4 mg, t_R = 20.4 minutes, 3 mL/min). Fr.9.6.4 (0.3 g) was first separated by preparative TLC ($CHCl_3$/MeOH/formic acid, 18:1:0.05) and then purified by semipreparative HPLC (ACN/H_2O, 91% and 90%) to produce compounds **8** (10.4 mg, t_R = 21.5 minutes, 3 mL/min) and **9** (8.3 mg, t_R = 21.3 minutes, 3 mL/min).

Compound 1

Colorless oil
$[\alpha]_D^{20}$: –11.8 (*c* 0.17, MeOH)
UV (MeOH) λ_{max} (log ε): 207 (3.93) nm
ESIMS *m/z*: 283 $[M–H]^-$
HRESIMS *m/z*: 283.1551 $[M–H]^-$ (calcd for $C_{15}H_{23}O_5$, 283.1551).
^1H and ^{13}C NMR: Table 1

Compound 2

White powder
UV (MeOH) λ_{max} (log ε): 355 (3.80), 253 (3.86), 204 (3.68) nm
ESIMS *m/z*: 161 $[M–H]^-$
HRESIMS *m/z*: 161.0607 $[M–H]^-$ (calcd for $C_{10}H_9O_2$, 161.0608)
^1H and ^{13}C NMR: Table 1

Compound 3

Yellow gum
$[\alpha]_D^{20}$: +17.2 (*c* 0.06, MeOH)
UV (MeOH) λ_{max} (log ε): 366 (3.62), 257 (3.88), 226 (4.22) nm
CD (MeOH): $\Delta\varepsilon_{213}$ –0.70, $\Delta\varepsilon_{232}$ +0.28
ESIMS *m/z*: 399 $[M+Na]^+$
HRESIMS *m/z* 399.1779 $[M+Na]^+$ (calcd for $C_{21}H_{28}O_6Na$, 399.1778)
^1H and ^{13}C NMR: Table 1

Computational Methods

The OR calculation was performed by a Gaussian 09 program package. The conformational search generating low-energy conformers within a 6 kcal/mol was deduced by Conflex 7 to optimize the low-energy conformations. Then, the OR calculations were conducted at the B3LYP/6-311G(d,p) level in MeOH, and the results were processed using previously described methods.[14]

Biological Evaluation

Inhibition of AchE was assayed using a microplate as previously described.[15,16] The concentration of the compounds is 50 μM, and tacrine was used as a positive control with the concentration of 0.33 μM. The assay was done in triplicate and the results are expressed as mean ± SD.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Supplemental Material

Supplemental material for this article is available online.

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