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Triterpene-farnesyl hydroquinone conjugates from *Ganoderma calidophilum*

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ABSTRACT

Two new lanostane-type triterpenoids characterized with farnesyl hydroquinone moieties, ganocalidoins A (**1**) and B (**2**), were isolated from the fruiting body of *Ganoderma calidophilum*, together with two known tripterpenes (**3**-**4**). The structures of compounds **1** and **2** were determined by extensive spectroscopic data including HRESIMS, 1D and 2D NMR. Ganocalidoins A and B showed anti-oxidant capacity with IC_{50} values of 38.7 ± 2.8 and 34.2 ± 1.8 μM, respectively. The compounds did not show tyrosinase inhibition activity.

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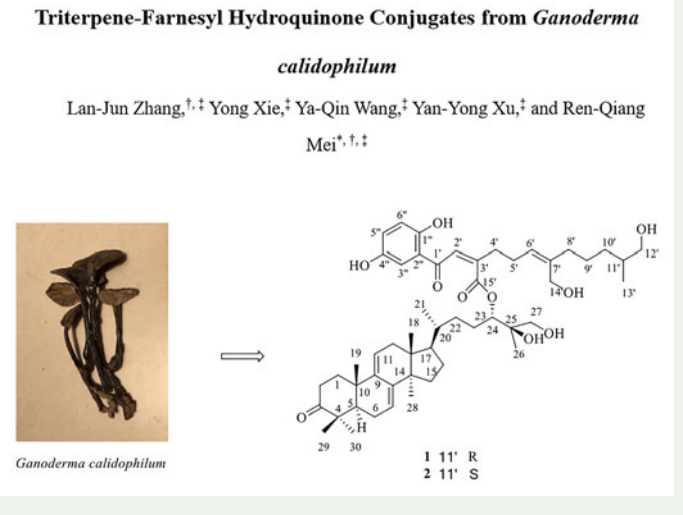

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1. Introduction

Ganoderma, genus of the Ganodermataceae family, is widely distributed in China. Many of *Ganoderma* species are used in traditional Chinese medicine as anti-aging (Zhao and Sun 1992), anti-HIV-1 protease (Dine et al. 2008), anti-cancer (Li et al. 2013; Satria et al. 2019), HMG-CoA reductase inhibition, α-Glucosidase inhibition(Wang et al. 2015), acyl CoA acyltransferase inhibition (Li et al. 2006), control blood pressure (Lee et al. 2011). Over the past decades, many chemical studies have been carried out on *Ganoderma* species, mostly focus on *Ganoderma lucidum* and *Ganoderma sinense*, distributed mainly in the middle and north of China and known as Ling zhi (*Ganoderma*) in China and other Asia countries. But few studies have been conducted on *Ganoderma calidophilum* (Huang et al. 2016, 2017), a species distributed in southern China (Zhao and Sun 1992). Previous reports have been reported triterpene-farnesyl hydroquinone conjugates from *G. sinense* (Sato et al. 2009). Subsequently four triterpene-farnesyl hydroquinone conjugates are found from *Ganoderma leucocontextum* (Wang et al. 2015). To date, only seven triterpene-farnesyl hydroquinone conjugates have been reported from the fruiting body of *Ganoderma*. In order to discover the whitening chemical constituents, two new triterpene-farnesyl hydroquinone conjugates, ganocalidoins A (**1**) and B (**2**), together with two known compounds ganodermanontriol (**3**) (Fujita et al. 1986), ganoderiol B (**4**) (Sato et al. 1986) were isolated (Supporting Information Figure S1). Moreover, DPPH free radical scavenging and tyrosinase inhibition activity of these new compounds were also reported (Figure 1).

2. Results and discussion

Ganocalidoin A (**1**) was obtained as a yellow, amorphous powder. The molecular formula of **1** was determined as $C_{51}H_{74}O_{10}$ by HRESIMS: *m/z* 869.5189 $[M+Na]^+$ (calcd for $C_{51}H_{74}O_{10}Na$, 869.5174). The IR spectrum showed the presence of carbonyl (1708 cm^{-1}) and aryl (1003, 1039 and 1113 cm^{-1}) groups. The UV spectrum showed the presence of a conjugated system (λ_{max} 235, 195 nm) (Sato et al. 2009). The ^1H NMR spectrum exhibited six methyl singlets, two methyl doublets. The ^{13}C NMR spectrum exhibited 51 carbon signals which were assigned by a DEPT experiment as 8 methyls, 16 methylenes (including three oxymethylenes at δ_C 68.1, 60.0 and 68.4), 12 methines (including an oxymethine at δ_C 79.1) and 15 nonprotonated carbons,

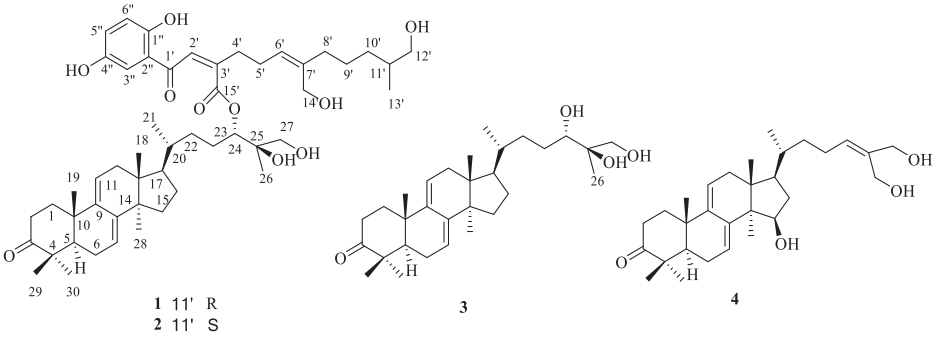

Figure 1. Molecular structures of **1–4** isolated from *Ganoderma calidophilum*.

including a quaternary carbon substituted by oxygen at δ_C 74.8, a carboxylic carbon at δ_C 168.3 and two carbonyl carbons at δ_C 199.2 and 219.2. The ^{1}H and ^{13}C NMR signals for the aliphatic part of the structure of compound **1** resembled those of triterpenes (Fujita et al. 1986). Detailed analysis of the 2D NMR spectra of **1** established that the triterpene moiety in the structure of **1** corresponded to ganodermanontriol (Fujita et al. 1986). On the basis of ^{1}H-^{1} H COSY and HSQC spectroscopic evidence, the presence of a farnesyl group in the structure of **1** was suggested, which was confirmed by HMBC correlations between the allylic methyl groups and the corresponding methylene and methine carbons (Supporting Information Figure S2). The characteristic ABX-type signals for the aromatic protons in the ^{1}H NMR spectrum indicated the presence of a 1,2,4-trisubstituted phenyl ring (Reynolds et al. 1985; Reynolds and Rodriguez 1986). The linkage sites of the triterpene, farnesyl and phenyl moieties were determined by HMBC, in which H-3′′ (δ_H 7.11) was correlated with the carbonyl C-1′ carbon of the farnesyl moiety at δ_C 199.2. The HMBC correlations of H-24 (δ_H 4.99) with C-15′ (δ_C 168.3) suggested that the ganodermanontriol unit and the 2-(2-(2,5-dihydroxyphenyl)-2-oxoethylidene)-11-hydroxy-6,10-dimethylundeca-5,9-dienoic acid unit (called oxoganomycin A unit) are conjugated through an ester linkage (C-24 to C-15′). The configurations of the olefin of **1** were determined on the basis of NOESY data, the NOE correlations of H-2′ with H-4′, and H-6′ with H-8′ assigned the Z and Z configurations for the C-2′— C-3′ and C-6′— C-7′ double bonds respectively (Supporting Information Figure S2), the configurations of C-11′ remain unassigned. Therefore, the absolute configuration of **1** was arbitrarily established as 11′*R*.

Ganocalidoins B (**2**) was obtained as a yellow, amorphous powder. The molecular formula of **1** was determined as $C_{51}H_{74}O_{10}$ by HRESIMS: *m/z* 869.5185 $[M + Na]^+$ (calcd for $C_{51}H_{74}O_{10}Na$, 869.5174). The IR spectrum showed the presence of carbonyl (1705 cm^{-1}) and aryl (1003, 1041 and 1113 cm^{-1}) groups. The UV spectrum showed the presence of a conjugated system (λ_{max} 235, 196 nm). Ganocalidoins B and ganocalidoins A NMR data are very similar, ^{13}C NMR but has a slight difference at C-11′. Thus, it was reasonable to assume a different configuration at C-11′, the absolute configuration of **2** was established as 11′*S*. Thus, ganocalidoins B are identified as epimers with ganocalidoin A.

In order to discover the whitening chemical constituents, compounds **1** and **2** were tested for DPPH free radical scavenging and tyrosinase inhibition activity. Ganocalidoins A and B showed anti-oxidant capacity with IC_{50} values of 38.7 ± 2.8 and 34.2 ± 1.8 μM, respectively. Unfortunately the compounds did not show tyrosinase inhibition activity.

Two known compounds were identified as ganodermanontriol (**3**) (Fujita et al. 1986), ganoderiol B (**4**) (Sato et al. 1986) by comparison of their spectroscopic data with the literature data. See ^{1}H NMR and ^{13}C NMR data of compounds **3** and **4** in Supporting Information.

3. Experimental

3.1. General experimental procedures

Optical rotations were measured on a JASCO P-1020 digital polarimeter. UV spectra were measured using a Shimadzu UV-2401 PC spectrophotometer. IR spectra were obtained on Bruker Tensor-27 infrared spectrophotometer with KBr pellets. ESI-MS

spectra were recorded on a Bruker HTC/Esquire spectrometer, HRESIMS spectra were recorded on an API Qstar Pulsar instrument. NMR experiments were performed on Bruker AM-400, DRX-500, and Avance III 600 instruments with TMS as the internal standard. Chemical shifts (δ) were expressed in ppm with reference to the solvent signals. Column chromatography (CC) was performed on silica gel (100–200, 200–300 and 300–400 mesh, Qingdao Marine Chemical Co, China), RP-18 (40–63 μm, Merck), and Sephadex LH-20 (GE Healthcare, Sweden). Semi-preparative HPLC was run on Agilent 1100 liquid chromatograph with diode array detector (DAD), Zorbax-SB-C18 column (5 μm; 25 cm × 9.4 mm i.d.). TLC was performed on $HSGF_{254}$ (0.2 mm, Qingdao Marine Chemical Co, China) or RP-18 F_{254} (0.25 mm, Merck). Fractions were monitored by TLC and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in EtOH. DPPH and Trolox were purchased from Sigma-Aldrich; Costar 96 well plates were purchased from Corning. Mushroom tyrosinase, Kojic acid, L-Dopa were purchased from Sigma-Aldrich; Dulbecco's PBS was obtained from HyClone.

3.2. Plant material

Fruiting bodies of *Ganoderma calidophilum* were collected in Wenshan, Yunnan Province, People's Republic of China, and identified by Associate Professor. Gang Wu, Kunming Institute of Botany, Chinese Academy of Science. A voucher specimen (no. 20180310) has been deposited at the Key Laboratory of Economic Plants and Biotechnology and Yunnan Key Laboratory for Wild Plant Resources, Kunming Institute of Botany, Chinese Academy of Science (CAS).

3.3. Extraction and isolation

The powdered dry fruiting bodies of *G. calidophilum* (15 Kg) were extracted with 95% EtOH under reflux for four hours (4 × 20 L). The extract was concentrated and suspended in hot water followed by extraction with EtOAc (3 × 4 L). The EtOAc extract was concentrated (480 g) and separated by silica gel column using a gradient solvent petroleum/EtOAc (15:1-0:1) to afford fractions 1–17 (Fr. 1-17). Fr. 13 (18.6 g) was subjected to a series of silica gel CC eluting with PE/acetone (20:1 – 0:1, v/v) and then by Sephadex LH-20 (acetone), further purified by reverse phase chromatography on a C_{18} column (MeOH/H_2O, 50:50 → 100:0, v/v) to give a mixture of compounds **1** and **2**. This mixture was purified by reversed-phase semipreparative HPLC using 67% acetonitrile in H_2O as eluent (retention time: 21.5 and 22.3 min) to yield **1** (25.0 mg) and **2** (27.0 mg), respectively. Fr. 13.3 (5.0 g) was subjected to a silica gel column, eluted with petroleum ether–EtOAc (5:1 – 1:1, v/v) to give five subfractions (Fr. 13.3.1–Fr. 13.3.5). Fr. 13.3.1 (107.0 mg) was subjected to a RP-C_{18} column, eluted with MeOH/H_2O (50:50 – 100:0, v/v) to give compound **3** (6.0 mg) and compound **4** (16.0 mg).

3.4. Spectral data

Ganocalidoins A (**1**): yellow amorphous powder; [α]21D –22.3 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3432, 2962, 2932, 2878, 1708, 1641, 1430, 1375, 1304, 1233, 1184, 1113, 1039,

1003 cm^{-1}; UV (MeOH) λ_{max} (log ε) 375 (4.81), 235 (5.68), 195 (5.81); ^{1}H NMR data, see Supporting Information Table S1; ^{13}C NMR data, see Supporting Information Table S1; positive ESIMS: *m/z* 869 $[M + Na]^+$; HRESIMS: *m/z* 869.5189 $[M + Na]^+$ (calcd for $C_{51}H_{74}O_{10}Na$, 869.5174).

Ganocalidoins B (**2**): yellow amorphous powder; [α]21D −15.2 (*c* 0.15, MeOH); IR (KBr) ν_{max} 3439, 2965, 2932, 2879, 1705, 1642, 1432, 1375, 1305, 1234, 1185, 1113, 1041, 1003 cm^{-1}; UV (MeOH) λ_{max} (log ε) 375 (5.45), 235 (6.33), 196 (6.40); ^{1}H NMR data see Supporting Information Table S1; ^{13}C NMR data, see Supporting Information Table S1; positive ESIMS: *m/z* 869.5 $[M + Na]^+$; HRESIMS: *m/z* 869.5185 $[M + Na]^+$ (calcd for $C_{51}H_{74}O_{10}Na$, 869.5174).

3.5. In vitro anti-oxidant capacity

Dilute samples to 1000 μg/mL in Ethanol. Add 30 μL Trolox (1 mM) and sample into 96 well plate. Assay each test article in triplicate. Initiate reaction by adding 270 μL/well DPPH solution (100 μM). Incubate at 30 °C for 1 h. Read plate at 515 nm. Anti-oxidation (%) = (1 - Mean OD515 nm of Sample/Mean OD515nm of untreated Control) × 100% (Brand-Williams et al. 1995; Prior et al. 2005).

3.6. Mushroom tyrosinase in vitro inhibition

Test samples (Final Conc. = 20 μM) were mixed with L-DOpa (Final Conc. = 1.25 mM), the reaction was initiated by adding tyrosinase (Final Conc. = 25 U/mL), Incubated at room temperature on shaker for 5 min. Read plate at 490 nm. Tyrosinase Inhibition (%) = (1-Mean OD490nm of Sample/Mean OD490 nm of untreated Control) × 100% (Kertesz and Wietek 2001; Lim et al. 2009; Nakashima S, et al. 2010).

4. Conclusions

Two new lanostane-type triterpenoids characterized with farnesyl hydroquinone moieties, ganocalidoins A and B, were isolated from the fruiting body of *Ganoderma calidophilum*, together with two known compounds. Ganocalidoins A and B showed anti-oxidant capacity, and may be used to in cosmetics as antioxidants.

Disclosure statement

No potential conflict of interest was reported by the authors.

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