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Two New Phenolic Compounds from the Fruiting Bodies of *Ganoderma tropicum*

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Chemical investigation of the fruiting bodies of *Ganoderma tropicum* led to the isolation of two new phenolic compounds, ganodermatropins A (**1**) and B (**2**). Their structures were elucidated by spectroscopic techniques (MS, 1D and 2D NMR). Ganodermatropin A exhibited antimicrobial activity against *Staphylococcus aureus*.

Key Words : *Ganoderma tropicum*, Ganodermatropin A, Ganodermatropin B, Antimicrobial activity

Introduction

Ganoderma is a basidiomycete white rot fungus which has been used for medicinal purposes for centuries particularly in China, Japan and Korea.[1] There are more than 100 species in family Ganodermataceae growing in China and 78 wild species were found in Hainan Province.[2] *Ganoderma tropicum* (Jungh.) Bres. was one of the main wild *Ganoderma* resources growing in Hainan Province, which was used widely as a health supplement and "herbal" medicine. Recent researches on chemical constituents of *Ganoderma* genus showed the presence of natural products including triterpenes, steroids, alkaloids, flavonoids, polysaccharides, and so on.[3] These natural compounds had the pharmacological and biological effects such as antimicrobial,[4] *anti*-HIV,[5] antiaging, antitumor, and immuno-modulating activities.[6-9] During our investigation on potentially bioactive constituents from the fruiting bodies of *G. tropicum*, two new phenolic compounds, ganodermatropins A (**1**) and B (**2**) were isolated from this fungus. In this paper, we mainly report the isolation and structural elucidation of the two new compounds, as well as their antimicrobial activities.

Experimental

General Experimental Procedures. Optical rotation was recorded using a Rudolph Autopol III polarimeter (Rodolph Research Analytical, New Jersey, USA). The UV spectra were measured on a Shimadzu UV-2550 spectrometer. The IR spectra were obtained on a Nicolet 380 FT-IR instrument, as KBr pellets. The NMR spectra were recorded on a Bruker AV-500 spectrometer, using TMS as an internal standard. The HRESIMS were recorded with an API QSTAR Pulsar 1 spectrometer. EIMS and HREIMS were recorded with a Waters Autospec Premier. Column chromatography (CC) was performed with silica gel (Marine Chemical Industry Factory, Qingdao, China) and Sephadex LH-20 (Merck). TLC was preformed with silica gel GF254 (Marine Chemical Industry Factory, Qingdao, China).

Plant Materials. *G. tropicum* were collected from Lingshui County, Hainan Province, China, in May 2011, and authenticated by Professor Xing-Liang Wu of Hainan University. The voucher specimen (No. 2011LZ01) has been deposited in Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Science.

Extraction and Isolation. The dried fruiting bodies of *G. tropicum* (6.5 kg) were powdered and extracted with EtOH (95%) at room temperature for three times. The extract was concentrated and suspended in water followed by successive partition with petroleum ether, EtOAc, and *n*-BuOH, respectively. The EtOAc extract (200 g) was separated by silica gel CC (200-300 mesh) using a gradient solvent petroleum ether-EtOAc (20:1 → 0:1, *v/v*) to afford nine fractions (Fr.1-Fr.9). Fraction 7 (12 g) was separated by silica gel column using a gradient solvent $CHCl_3$-CH_3OH (50:1 → 1:1) to afford fractions A1-A8. Fraction A3 was separated on Sephadex LH-20 column ($CHCl_3$-CH_3OH 1:1) to give five subfractions A3a-A3e. Subfraction A3b was chromatographed with petroleum ether-EtOAc (3:1) to yield compound **1** (30.0 mg). Fraction 8 (18 g) was subjected to CC, with petroleum ether-EtOAc (15:1 → 1:1, v/v) as eluent, to afford nine fractions (B1-B9). Fraction B2 was subjected to Sephadex LH-20, eluting with $CHCl_3$-CH_3OH (1:1), then CC with petroleum ether-EtOAc (20:1) as eluent, to give compound **2** (4.0 mg).

Ganodermatropin A (1): Yellow oil. $[\alpha]_D^{27}$ –27.0 (c = 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 223 (3.00), 255 (1.48), 364 (1.46) nm; IR (KBr) ν_{max} 3630, 1722, 1593, 1484, 1441 cm^{-1}; HREIMS *m/z* 306.1104 $[M]^+$ (calcd. for $C_{16}H_{18}O_6$, 306.1103); ^1H and ^{13}C NMR see Table 1.

Ganodermatropin B (2): Yellow oil. $[\alpha]_D^{27}$ –31.5 (c = 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 225 (4.49), 258 (3.12), 367 (1.39) nm; IR (KBr) ν_{max} 3617, 1742, 1692, 1540 cm^{-1}; HRESIMS *m/z* 343.1150 $[M + Na]^+$ (calcd. for $C_{17}H_{20}O_6Na$, 343.1157); ^1H and ^{13}C NMR see Table 1.

Antibacterial Activity. Two new compounds were tested for *in vitro* antibacterial activities against *Staphylococcus aureus* (SA, obtained from National Institutes for Food and

Drug Control) strain by the filter paper disc agar diffusion method.[10] The media nutrient agar was used to culture the bacteria. The sterile agar media was poured into Petri-plates to a uniform depth of 5 mm and was allowed to solidify. The bacterial suspensions were streaked over the surface of media using a sterile cotton swab. Fifty μL (20 μg/μL) of the test compound was impregnated on sterile filter paper discs of 6 mm size. These discs were then aseptically applied to the surface of the agar plates at wellspaced intervals. Control discs impregnated with 50 μL of acetone and 50 μL of kanamycin sulfate (0.64 μg/μL) were also used alongside the test discs in the experiment. The plates were incubated at 36 °C for 24 h. The MICs was performed by dilution of test compound. Experiments were done in triplicate, and the results were presented as mean values of the three measurements.

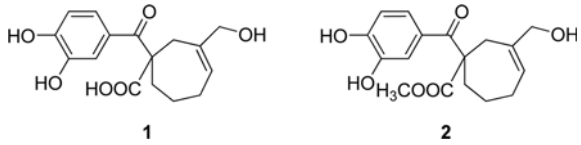
Results and Discussion

Compound **1** was obtained as yellow oil. $[\alpha]_D^{27}$ −27.0 (c = 0.2, MeOH). Its molecular formula was assigned to be $C_{16}H_{18}O_6$ from its HREIMS (*m/z* 306.1104 $[M]^+$, calcd. for $C_{16}H_{18}O_6$, 306.1103) and NMR data (Table 1), with eight degrees of unsaturation. The IR spectrum displayed the presence of hydroxyl (3630 cm^{-1}), carbonyl (1722 cm^{-1}) and double bond (1593 cm^{-1}) function groups. The ^1H NMR spectrum of compound **1** (Table 1) displayed the presence of three aromatic protons [δ_H 7.21 (1H, d, *J* = 3.0 Hz, H-2'), 6.79 (1H, d, *J* = 9.0 Hz, H-5'), 7.00 (1H, dd, *J* = 3.0, 9.0 Hz, H-6')] for one typical 1,3,4-trisubstituted phenyl group, an olefinic proton [δ_H 5.82 (1H, s, H-4)], two proton signals of oxygen-bearing methylene [δ_H 3.95 (2H, s, H-10)]. The ^{13}C NMR and DEPT spectra (Table 1) displayed a total of 16 carbon signals including five methylenes (one oxygenated),

Table 1. ^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) data of **1** and **2** (δ in ppm, *J* in Hz)

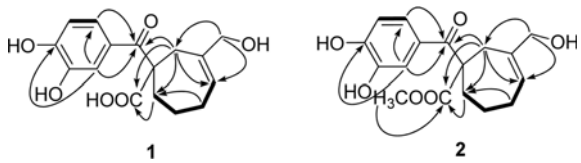
No.	**1**[a]		**2**[b]	
	δ_C	δ_H	δ_C	δ_H
1	45.6	-	44.6	-
2	49.0	3.43 s	47.9	3.36 s
3	141.6	-	140.7	-
4	124.8	5.82 s	123.5	5.79 s
5	26.3	2.03 t (*J* = 5.8)	25.5	2.89 br s
6	20.3	1.86 m, 1.76 m	19.3	1.83 m, 1.73 m
7	33.1	2.20 m, 1.69m	32.1	2.20 m, 1.66 m
8	205.0	-	203.1	-
9	179.9	-	176.6	-
10	67.1	3.95 s	67.0	4.04 s
1'	119.7	-	119.1	-
2'	115.4	7.21 d (*J* = 3.0)	114.7	7.13 d (*J* = 1.2)
3'	150.6	-	147.8	-
4'	156.6	-	156.6	-
5'	120.6	6.79 d (*J* = 9.0)	119.5	6.84 d (*J* = 8.9)
6'	125.9	7.00 dd (*J* = 3.0, 9.0)	125.3	7.01 dd (*J* = 1.2, 8.9)
OMe	-	-	52.5	3.69 s

[a]Measured in CD_3OD. [b]Measured in $CDCl_3$.



1 2

Figure 1. Structures of compounds **1** and **2**.



1 2

Figure 2. Selected ^1H, ^1H-COSY (—) and key HMBC (→) correlations of **1** and **2**.

four methines and seven quaternary carbons (two carbonyls at δ_C 205.0 and 179.9), among which six carbon signals at δ_C 119.7, 115.4, 150.6, 156.6, 120.6 and 125.9 indicated the presence of one phenyl group. The other remained ten carbons suggested the presence of a monoterpene skeleton. The connectivity of the monoterpene skeleton was deduced by the 2D NMR data. In the ^1H-^1H COSY spectrum, a proton spin system (H-4/H-5/H-6/H-7) was observed, indicating the connection of C-3 to C-7 as drawn in Figure 1. The key HMBC correlations from H-2 (δ_H 3.43) to C-4, C-7 (δ_C 33.1), and C-1 (δ_C 45.6) led to the establishment of seven-member ring similar to those of cyclohexenones,[11] which accorded with one remained degree of unsaturation apart from seven degrees of unsaturation occupied by one double bond, two carbonyls and one pheny. In the HMBC spectrum (Figure 2), the correlations from H-10 to C-2 (δ_C 49.0) and C-4, from H-2 to C-8 (δ_C 205.0) and C-9 (δ_C 179.9), and from H-7 (δ_H 2.20, 1.69) to C-9 further confirmed the assignment of the monoterpene skeleton as drawn in Figure 1, indicative of the attachments of C-8 and C-9 to C-1 and C-10 to C-3. The linkage of the phenyl group to the carbonyl (C-8) was elucidated by the key HMBC correlations of H-2' (δ_H 7.21) and H-6' (δ_H 7.00) with C-8. Based on the above evidence, the structure of compound **1** was established as shown in Figure 1, named ganodermatropin A.

Compound **2** was isolated as yellow oil. $[\alpha]_D^{27}$ −31.5 (c = 0.2, MeOH). Its molecular formula was assigned to be $C_{17}H_{20}O_6$ by HRESIMS (*m/z* 343.1150 $[M + Na]^+$, calcd. for $C_{17}H_{20}O_6Na$, 343.1157) and NMR data (Table 1), indicating one more carbon and two more protons than **1**. The IR spectrum of **2** showed absorption bands at 1742 and 1692 cm^{-1} ascribable to ester carbonyl and carbonyl groups, 1540 cm^{-1} for double bond, and 3617 cm^{-1} for hydroxyl group. The ^1H and ^{13}C NMR spectroscopic data of **2** was extremely similar to those of **1** except for an additional methoxyl signals at δ_C 52.5 and δ_H 3.69 (s), which hinted compound **2** was the methylated derivative of **1**. The methoxyl group was attached to carboxylic carbon at C-9 (δ_C 176.6) as established by the key HMBC correlation from methoxyl signal at δ_H 3.69 (s) to carboxylic carbon (C-9) at δ_C 176.4, which was also supported by carboxylic carbon at δ_C 179.9 in **1** shifted upfield to δ_C 176.6 (C-9) in **2**. Therefore, the structure of

compound **2** was determined as shown in Figure 1, named ganodermatropin B.

The antimicrobial test demonstrated that compound **1** possessed inhibitory effect on SA with the diameter of the inhibition zone 8.34 mm with the positive control (kanamycin sulfate) 17.23 mm, while compound **2** show no inhibitory activity on SA.

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