Antioxidant farnesylated hydroquinones from *Ganoderma capense*

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ABSTRACT

Phytochemical investigation of the fruiting bodies of *Ganoderma capense* led to isolation of eight aromatic meroterpenoids (**1**–**8**). Ganocapensins A and B (**1**, **2**) possessed a thirteen-membered and a fourteen-membered ether rings, respectively. The structures of new isolates including absolute configuration were elucidated on the basis of extensive spectroscopic technologies and Mosher's method. All isolated compounds showed significant antioxidant effects with IC$_{50}$ values ranging from 6.00 ± 0.11 to 8.20 ± 0.30 μg/ml in the DPPH radical scavenging assay.

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1. Introduction

Mushrooms of the genus *Ganoderma* (Ganodermataceae) are widely used in the areas of medicine and health food, due to their famous bioactivities and low toxicity [1–4]. A large spectrum of chemical constituents (polysaccharides, triterpenoids, steroids, and alkaloids) has been isolated from the mycelia, spores, fruiting bodies and sclerotia [5–7]. Recent years, because of their diverse structures and significant bioactivities (antioxidant, cytotoxic, anti-HIV protease, and anti-AChE activities), aromatic meroterpenoids have attracted attention of many phytochemists, chemists and pharmacologists [8–20].

Ganoderma capense (Lloyd) D. A. Reid is called "Boshuzhi" in China [21]. Research showed that this fungus possessed potential pharmacological effects against hereditary cerebellar ataxia, hepatitis, scleroderma, lupus erythematosus, alopecia areata, atopic dermatitis, myodystrophy and insomnia [22,23]. Phytochemical investigation of this fungus led to isolation of steroids, alkaloids and fatty acids from the liposoluble fraction [24–26], as well as purines and polysaccharides from the water-soluble fraction [27]. However, there is lack of information on the major bioactive constituents from *Glaucidium capense* for explaining its biological effects. Thus, in the present paper, the chemical constituents of *G. capense* are studied and eight aromatic meroterpenoids (Fig. 1), including two new macrocyclic aromatic

meroterpenoids (**1**, **2**), three new aromatic meroterpenoids (**3**–**5**), along with three known analogues (**6**–**8**) were isolated. Meanwhile, their antioxidant activities were evaluated in the DPPH radical scavenging assay.

2. Experimental part

2.1. General

The optical rotations were taken on a JASCO P-1020 polarimeter. UV spectra were record using a Shimadzu UV2401PC spectrophotometres. ^{1}H and ^{13}C NMR spectra were measured on Bruker AV-400 and DRX-500 instruments (Bruker, Zurich, Switzerland) using TMS as internal standard. Chemical shifts (δ) were expressed in *ppm* with reference to the TMS resonance. ESIMS and HRTOF-ESIMS data were recorded on an API QSTAR Pulsar spectrometer. EIMS and HRTOF-EIMS data were acquired on a Waters Auto Spec Premierp 776 spectrometer (America, Waters). Infrared spectra were recorded on a Bruker Tensor-27 instrument by using KBr pellets. CD spectra were taken on a JASCO J-815 spectropolarimeter. An agilent 1100 series instrument equipped with Agilent ZORBAX SB-C18 column (5 μm, 4.6 mm × 250 mm) was used for high-performance liquid chromatography (HPLC) analysis. Chiral chromatography column (CHIRALCEL OD-H, 5 μm, 4.6 mm × 150 mm) was used to analyze enantiomers.

TLC was performed on precoated TLC plates (200–250 μM thickness, F254 Si gel 60, Qingdao Marine Chemical, Inc.) with compounds visualized by spraying the dried plates with 10% aqueous H$_2$SO$_4$ followed by
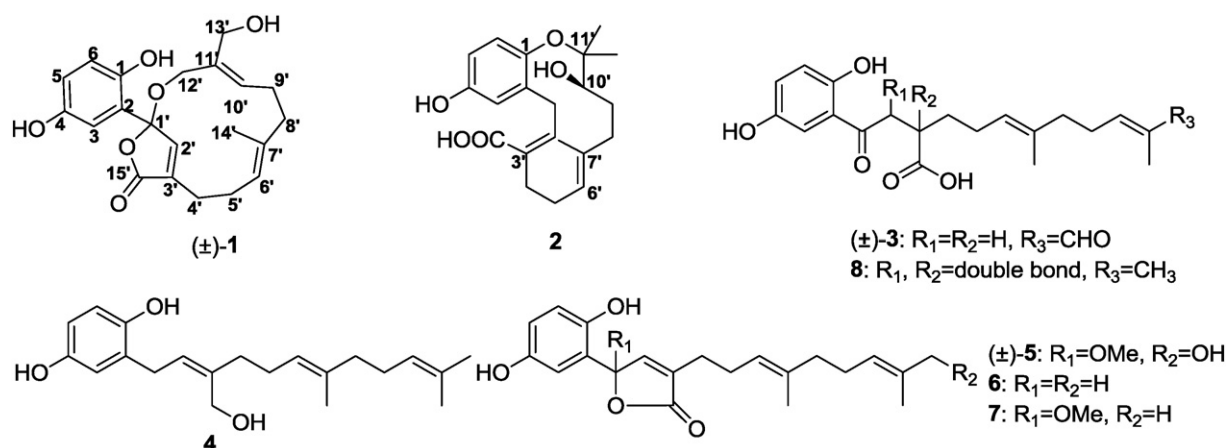
Fig. 1. The structure of compounds **1–8** isolated from *G. capense*.

heating until dryness. Silica gel (200 − 300) mesh, Qingdao Marine Chemical, Inc.), Lichroprep RP-18 (40–63 μm, Fuji) and Sephadex LH-20 (20–150 μm, Pharmacia) were used for column chromatography. MeOH, CH$_2$Cl$_2$, EtOAc, acetone and *n*-BuOH were purchased from Tianjing Chemical Reagents Co. (Tianjing, China). All other materials were of the highest grade available.

2.2. Fungal material

Fruiting bodies of *G. capense* were purchased in July 2012 from Juhuacun Traditional Chinese Medicine Market in Kunming. This mushroom was identified by Prof. Liu Peigui, a fungal taxonomist, who works at Kunming Institute of Botany, Chinese Academy of Science. A specimen (NO. 12071501) is deposited in the Herbarium of the Department

of Taxonomy, Kunming Institute of Botany, Chinese Academy of Sciences.

2.3. Extraction and isolation

Dry fruiting bodies of *G. capense* (15 kg) were chipped and percolated with acetone at room temperature. The combined acetone extracts were evaporated under reduced pressure to give a residue (173.8 g), which was treated by macroporous resin (D101, MeOH—H$_2$O, 50:50, 70:30, and 90:10) to give three parts (Fr. A → Fr. C). Fr. B (70:30, 103 g) was subjected to silica gel column chromatography (CC), eluting with CHCl$_3$—MeOH (80:1, 50:1, 20:1 and 5:1), to yield four fractions: Fr. B-1 (80:1), Fr. B-2 (50:1), Fr. B-3 (20:1) and Fr. B-4 (5:1). Fr. B-2 (50:1, 13 g) was further separated by silica gel CC, eluting with

Table 1
1D NMR spectroscopic data of compounds **1–5**.

Position	**1**[a]		**2**[b]		**3**[a]		**4**[a]		**5**[c]	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1		146.7, C		151.6, C		148.1, C		147.4, C		149.7, C
2		124.1, C		128.0, C		118.6, C		127.4, C		123.7, C
3	6.99, br s	112.3, CH	6.77, d (2.5)	124.6, CH	6.75, m	119.1, CH	6.58, m	117.1, CH	7.83, d (2.4)	115.6, CH
4		149.7, C		149.1, C		155.9, C		149.3, C		152.1, C
5	6.69, br s	117.8, CH	6.60, dd (2.5, 8.5)	123.3, CH	6.98, m	125.3, CH	6.55, m	116.7, CH	7.18, dd (2.4, 8.4)	118.6, CH
6	6.69, overlap	117.7, CH	6.62, d (8.5)	115.7, CH	7.16, m	114.5, CH	6.49, m	114.0, CH	7.16, d (8.4)	118.7, CH
1′		107.4, C	3.66, m; 3.59, m	29.7, CH$_2$		203.5, C	3.27, d (7.6)	29.7, CH$_2$		108.3, C
2′	7.27, br s	146.6, CH	5.88, t (7.8)	138.0, CH	3.35, m	39.8, CH$_2$	5.33, t (7.8)	127.5, CH	7.93, s	147.7, CH
3′		134.4, C		135.0, C	2.97, m	39.4, CH		138.3, C		135.8, C
4′	2.02, m; 2.18, m	23.2, CH$_2$	2.59, m; 2.38, m	34.8, CH$_2$	1.65, m; 1.55, m	31.6, CH$_2$	2.06, m	36.3, CH$_2$	2.33, m	26.1, CH$_2$
5′	2.15, m; 2.39, m	25.0, CH$_2$	2.40, m; 2.19, m	26.6, CH$_2$	2.09, m	25.4, CH$_2$	1.92, m	26.7, CH$_2$	2.30, m	26.5, CH$_2$
6′	4.94, d (9.7)	125.8, CH	5.19, m	127.7, CH	5.11, m	124.2, CH	5.04, m	123.6, CH	5.67, t (8.3)	123.6, CH
7′		135.5, C		136.3, C		134.8, C		135.5, C		137.0, C
8′	2.12, m; 2.24, m	38.8, CH$_2$	2.28, m; 2.10, m	36.8, CH$_2$	2.15, m	37.8, CH$_2$	1.92, m	39.6, CH$_2$	2.01, m	40.2, CH$_2$
9′	2.49, m; 2.05, m	23.4, CH$_2$	1.85, m; 1.43, m	29.2, CH$_2$	2.44, m	27.2, CH$_2$	2.04, m	26.6, CH$_2$	2.17, m	26.9, CH$_2$
10′	5.19, d (10.6)	136.9, CH	3.75, dd (10.3 and 1.4)	74.0, CH	6.78, m	155.9, CH	5.04, m	124.2, CH	5.19, m	124.3, CH
11′		133.3, C		83.5, C		139.3, C		131.3, C		135.6, C
12′	4.28, d (13.4); 3.89, d (13.4)	70.2, CH$_2$	1.19, s	24.3, CH$_3$	9.33, s	196.5, CH	1.65, s	25.6, CH$_3$	4.30, s	68.4, CH$_2$
13′	4.46, d (11.8); 4.13, d (11.8)	59.6, CH$_2$	1.11, s	22.9, CH$_3$	1.72, s	9.2, CH$_3$	1.56, s	17.6, CH$_3$	1.34, s	16.5, CH$_3$
14′	1.62, s	14.8, CH$_3$	1.56, s	16.0, CH$_3$	1.59, s	15.9, CH$_3$	1.52, s	15.9, CH$_3$	1.54, s	14.4, CH$_3$
15′		172.4, C		171.9, C		180.3, C	4.19, s	60.4, CH$_2$		172.4, C
OMe									3.62, s	52.3, CH$_3$

^{1}H and ^{13}C NMR spectra (δ) were measured at 400 (100) MHz for **1–4** and at 600 (150) MHz for **5**. The assignments were based on COSY, HSQC, and HMBC experiments.

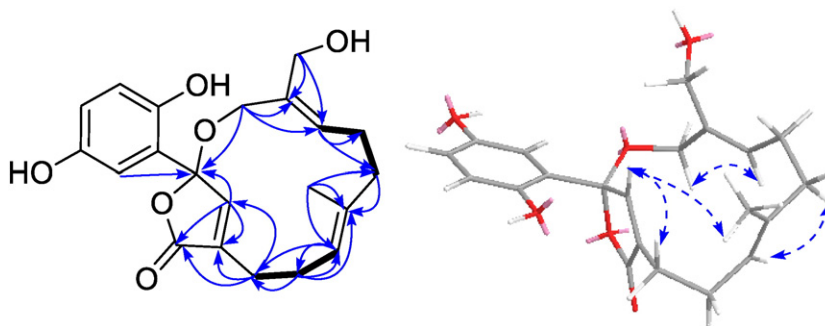 [a] Measured in CDCl$_3$.
  [b] Measured in MeOD.
  [c] Measured in C$_5$D$_5$N.

Fig. 2. Key HMBC (H → C), ^{1}H—^{1}H COSY (a) and ROESY (b) correlations of $(\pm)-$**1**.

CHCl$_3$—acetone (100:1, 80:1, 60:1, 40:1 and 20:1) to get four sub-fractions (Fr. B-2-1 → Fr. B-2-4). Fornicin B (**7**, 56 mg) was obtained from Fr. B-2-1 by CC (gel, LH-20, MeOH). Fr. B-2-2 was also treated by CC (gel, LH-20, MeOH) to afford ganocapensin A (**1**, 103 mg) and ganomycin I (**6**, 50 mg). Fr. B-2-3 was separated by CC (silica gel, CHCl$_3$—acetone, 70:1) to give three fractions (Fr. B-2-3a → Fr. B-2-3c). Fr. B-3-3a was further purified by preparative TLC to yield ganomycin F (**4**, 15 mg) and fornicin E (**5**, 7 mg).

Fr. B-3 (20:1, 30 g) was divided into four fractions (Fr. B-3-1 → Fr. B-3-4) by a reversed-phase C18 column (MeOH — H$_2$O, 45:55 → 75:25). Fr. B-3-2 (50:50) was separated by CC (silica gel, CHCl$_3$—MeOH, 70:1 → 10:1) to give five sub-fractions (Fr. B-3-2a → Fr. B-3-2e). Fr. B-3-2c, Fr. B-3-2d and Fr. B-3-2e were subjected to LH-20 (MeOH) CC to provide ganocapensin B (**2**, 30 mg), ganomycin C (**8**, 230 mg) and ganomycin E (**3**, 62 mg), respectively.

Compounds **1**, **3** and **5**, which are racemic, were subjected to chiral HPLC to yield $(+)$-**1** (1.2 mg, t$_R$ = 18.6 min) and $(-)-$**1** (1.0 mg, t$_R$ = 23.9 min) (n-hexane/isopropanol, 90:10), $(+)-$**3** (0.98 mg, t$_R$ = 15.7 min) and $(-)-$**3** (1.01 mg, t$_R$ = 17.3 min) (n-hexane/isopropanol, 85:15), and $(+)-$**5** (1.56 mg, t$_R$ = 16.7 min) and $(-)$ $-$**5** (1.34 mg, t$_R$ = 17.5 min) (n-hexane/isopropanol, 90:10).

2.4. Spectral data

Ganocapensin A (**1**): yellow powder (MeOH); $[\alpha]^{25}_D - 3.96$ (c 0.18, CHCl$_3$/MeOH); UV (MeOH): λ_{max} (log ε): 311 (3.53), 236 (3.78), 221 (3.32) nm; IR (KBr) v_{max}: 3426, 2988, 1702, 1434 cm^{-1}; For ^{1}H and ^{13}C-DEPT NMR spectroscopic data, see Table 1; HREIMS m/z 372.1588 [M]$^+$ (calcd for C$_{21}$H$_{24}$O$_6$, 372.1573).

Ganocapensin B (**2**): yellow powder (MeOH); $[\alpha]^{25}_D + 47.50$ (c 0.3, MeOH); UV (MeOH) λ_{max} (log ε): 283 (3.40), 203 (4.35) nm; IR (KBr) v_{max}: 3400, 2992, 1719, 1399 cm^{-1}; For ^{1}H and ^{13}C-DEPT NMR spectroscopic data, see Table 1; HRESIMS m/z 359.1864 [M − H]$^-$ (calcd for C$_{21}$H$_{27}$O$_5$, 359.1859).

Preparation of the (R)- and (S)-MTPA ester derivatives of **2** by Mosher ester procedure [28]. Two portions (each 1.0 mg) of **2** were treated with (R)-(−)- and (S)-(+)-α-methoxy-α-(trifluoromethyl) phenylacetyl chloride (1 μl) in deuterated pyridine (20 μl) directly in

separate the NMR tube, and then the NMR tube was shaken carefully to mix the sample and MTPA chloride at room temperature, which afforded the (R)- and (S)-MTPA ester derivatives (**2r** and **2s**) after 24 h. ^{1}H NMR data of the (R)-MTPA ester derivative (**2r**) of **2** (600 MHz, pyridine-d_5, data were obtained from the reaction NMR tube directly): δ 6.385 (1H, m, H-2′), δ 4.624 (1H, m, H-6′), δ 3.499 (1H, m, H-9′), δ 1.228 (3H, s, H$_3$-12′), δ 1.447 (3H, s, H$_3$-13′). 1H NMR data of the (S)-MTPA ester derivative (**2s**) of **2**: δ 6.403 (1H, m, H-2′), δ 4.629 (2H, m, H-6′), δ 3.495 (1H, m, H-10′), δ 1.226 (3H, s, H$_3$–12′), δ 1.453 (3H, s, H$_3$–13′).

Ganomycin E (**3**): yellow powder (MeOH); $[\alpha]^{25}_D - 9.69$ (c 0.08, MeOH); UV (MeOH) λ_{max} (log ε): 226 (4.25), 202 (4.21) nm; IR (KBr) v_{max}: 3415, 2972, 1649, 1461 cm^{-1}; For ^{1}H and ^{13}C-DEPT NMR spectroscopic data, see Table 1; HREIMS m/z 374.1720 [M]$^+$ (calcd for C$_{21}$H$_{26}$O$_6$, 374.1729).

Ganomycin F (**4**): yellow oil (PE-acetone); $[\alpha]^{21}_D - 10.3$ (c 0.3, CH$_2$Cl$_2$); UV (CH$_2$Cl2) λ_{max} (log ε): 294 (3.59), 229 (3.71); IR (KBr) v_{max}: 3381, 2923, 1503, 1452 cm^{-1}; For ^{1}H and ^{13}C-DEPT NMR spectroscopic data, see Table 1; HRESIMS m/z 353.2088 [M + Na]$^+$ (calcd for C$_{21}$H$_{30}$O$_3$Na, 353.2092).

Fornicin E (**5**): yellow oil (MeOH); $[\alpha]^{21.2}_D - 5.67$ (c 0.12, MeOH); UV (MeOH) λ_{max} (log ε): 307 (3.32), 202 (4.13); IR (KBr) v_{max}: 3431, 2922, 1766, 1748, 1633, 1547, 1452 cm^{-1}; For ^{1}H and ^{13}C-DEPT NMR spectroscopic data, see Table 1; HREIMS m/z 388.1877 [M]$^+$ (calcd for C$_{22}$H$_{28}$O$_6$, 388.1886).

2.5. DPPH radical scavenging assay

The DPPH (α,α-Diphenyl-β-picrylhydrazyl) radical scavenging activity was measured using a modified previously established methodology [29]. 100 μM DPPH radical was dissolved in 80% aqueous methanol. Compounds (150 μl) at different concentrations in methanol were added to 4.35 ml of the methanolic DPPH radical solution. The mixture was shaken vigorously and allowed to stand at 23 °C in the dark for 30 min. The decrease in absorbance of the resulting solution was monitored at 515 nm at 30 min. A control consisted of 150 μl of 80% aqueous methanol and 4.35 ml of DPPH radical solution. The scavenging activity was estimated based on the percentage of DPPH radical scavenged using
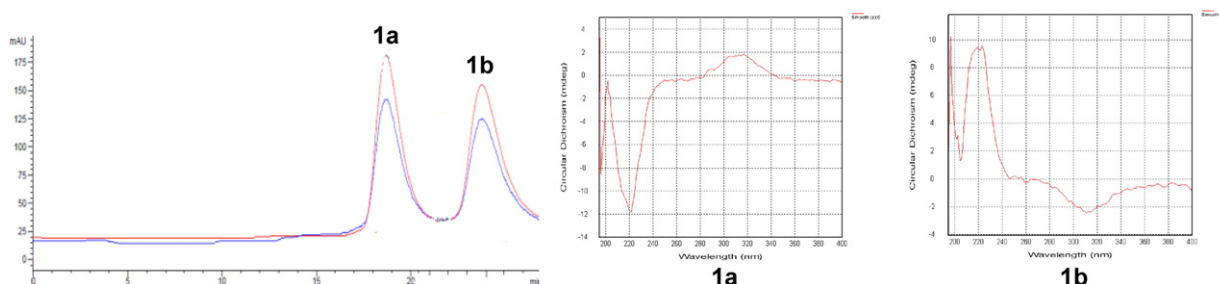

Fig. 3. The chiral analytical HPLC chromatography and CD spectra of **1a** and **1b**.
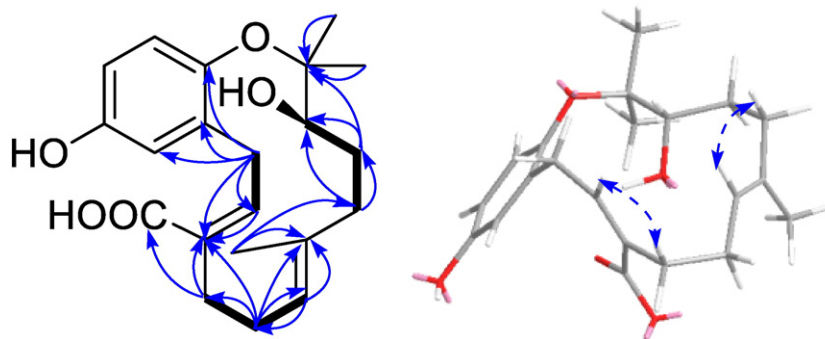
Fig. 4. The selected HMBC (H → C), ^{1}H—^{1}H COSY (a) and ROESY (b) correlations of compound **2**.

the following equation: DPPH radical scavenging effect (%) = [(control absorbanc − sample absorbance) / control absorbance] × 100. The IC$_{50}$ value (mg/ml) is the concentration of inhibition at which the DPPH radicals were scavenged by 50%. Trolox was used as positive control. Each sample was assayed in triplicate.

3. Results and discussion

Ganocapensin A (**1**) displayed an $[M + Na]^+$ ion at m/z 395 in the positive ESIMS, consistent with a molecular formula of $C_{21}H_{24}O_6$. The 1D NMR spectra showed signals for one methyl, six methylenes (two oxymethylenes), six aromatic/olefinic methines, eight quaternary carbons (six aromatic/olefinic carbons, one lactone carbon and one oxygenated quaternary carbon). The characteristic proton and carbon resonances at δ 6.99, br s, δ 112.3, CH (C-3); δ 6.69, br s, δ 117.8, CH (C-5) and δ 6.69, overlap, δ 117.7, CH (C-6) showed the presence of 1,2,4-trisubstituted dihydroxylbenzene moiety. Comparing the 1D NMR data (Table 1) of **1** to that of fornicin B [9] displayed that they have the similar structure, in terms of containing a 1,2,4-trisubstituted dihydroxylbenzene and a 15-carbon subfraction. However, the obvious difference between **1** and fornicin B was two oxymethylenes (δ 59.6 and δ 70.2) in **1** instead of two methyls at C-12′ and C-13′ in fornicin B, which was confirmed by the HMBC correlations (Fig. 2) of H-10′ (δ 5.19, d, $J = 10.6$ Hz) with C-11′ (δ 133.3), C-12′ (δ 70.2) and C-13′ (δ 59.6); of H$_2$-12′ (δ 4.28, d, $J = 13.4$ Hz; δ 3.89, d, $J = 13.4$ Hz) and H$_2$-13′ (δ 4.46, d, $J = 11.8$ Hz; δ 4.13, d, $J = 11.8$ Hz) with C-10′ and C-11′, together with the ^{1}H—^{1}H COSY correlations of H-8′/H-9′/H-10′. Furthermore, the key HMBC correlation of H$_2$-12′ with C-1′ (δ 107.4, C), and its molecular weight illustrated that C-1′ was linked to C-12′ *via* an ether bond. Thus, the planar structure of **1** was determined.

The ROESY correlations of H-2′/H$_2$-4′, of H-6′/H$_2$-8′ and of H-10′/H$_2$-12′ assigned the configurations of double bonds as 2′Z, 6′E and 10′E, respectively. The specific rotation of **1** ($[\alpha]^{25}_D$ − 3.96) indicated that it could be a raceme. Subsequently, analytical chiral HPLC chromatography gave two peaks (**1a** and **1b**, Fig. 3), which had opposite specific rotation ($[\alpha]$ + 1.03 for **1a**; $[\alpha]$ − 4.67 for **1b**) and CD spectra (Fig. 3). In view of only one chiral center in **1**, the absolute configuration of **1** was consequently established to be 1′R and 1′S.

Compound **2** was obtained as a yellow powder with a molecular ion peak at m/z 359.1864 $[M − H]^-$ in the HRESIMS, coincided with the molecular formula of $C_{21}H_{28}O_5$ with eight degrees of unsaturation. The 1D NMR spectra of **2** showed many similarities to ganomycin B [8]. However, the ^{13}C-DEPT spectra of **2** obviously had one oxymethine (δ 74.0) and one oxygenated quaternary carbon (δ 83.5), suggesting that $\Delta^{10'}$ in ganomycin B were replaced by an oxymethine and an oxyquaternary carbon in **2**, respectively. This was further confirmed by HMBC correlations (Fig. 4) of H-10′ (δ 3.75, dd, $J = 10.3$ and 1.4 Hz) with C-11′ (δ 83.5), C-12′ (δ 24.3), C-13′ (δ 22.9), C-9′ (δ 29.2) and C-8′ (δ 36.8); of H$_3$-12′ (δ 1.19, s) and H$_3$-13′ (δ 1.11, s) with C-10′ (δ 74.0) and C-11′, together with ^{1}H—^{1}H COSY correlations of H$_2$-8′/H$_2$-9′/H-10′.

Apart from seven degrees of unsaturation occupied by one phenyl group, two double bonds and one carboxyl group, the remaining one degree of unsaturation was representative of an additional ring. The observed downfield shift of C-1 (149.3 → 151.6), C-3 (117.7 → 124.6) and C-5 (114.7 → 123.3), as well as a highfield shift of C-4 (151.1 → 149.1) and C-6 (116.9 → 115.7) in **2** indicated that an ether ring could be between C-1 and C-10′ or C-11′. Nevertheless, no HMBC correlation of H-10′ with C-1 in **2** illustrated that the ether bond should be located at C-1 and C-11′. The 2′Z and 6′E was established by the ROESY correlations (Fig. 4) of H-2′/H$_2$-4′, and of H-6′/H$_2$-8′. The absolute configuration of C-10′ was determined to be R based on Mosher''s method (Fig. 5). Thus, the structure of **2** was finally determined and named as ganocapensin B (**2**).

The HREIMS of **3** exhibited a molecular ion peak at m/z 374.1720 $[M]^+$, suggesting a molecular formula of $C_{21}H_{26}O_6$, with nine degrees of unsaturation. The IR spectrum showed the presence of hydroxyl (3415 cm^{-1}), carbonyl (1725 cm^{-1}), and aldehyde (1698 cm^{-1}) groups, which were consistent with the 1D NMR spectroscopic data (Table 1). The ^{13}C-DEPT spectra of **3** exhibited 21 carbon resonances, of which signals at δ 196.5 (CHO), δ 139.3 (C) and δ 155.9 (CH) were characteristic for an α,β-unsaturated aldehyde group. Meanwhile, two methyls were observed in the ^{13}C-DEPT spectra of **3**, with one less than those of fornicin C [9], suggesting that one methyl in fornicin C was replaced by the aldehyde group in **3**. The HMBC correlations of H$_3$–13′ (δ 1.72, s) with C-12′ (δ 196.5), C-10′ (δ 155.9) and C-11′ (δ 139.3), of H-12′ (δ 9.33, s) with C-13′, C-10′ and C-11′, together with ^{1}H—^{1}H COSY correlations of H$_2$-8′/H$_2$-9′/H-10′ confirmed that C-12′ was the aldehyde group (Fig. 6). 10′Z was determined by the ROESY correlation of H-10′/H-12′.

Compound **3** had only one chiral center (C-3′), which was same as that of fornicin C. However, compound **3** was also a pair of enantiomers, which was analyzed by chiral analytical HPLC. Yang *et al* synthesized (+)-fornicin C and determined its absolute configuration to be R [18]. Accordingly, the absolute configuration at the chiral center was assigned as R for (+)-ganomycin E and S for (−)-ganomycin E, respectively.

The molecular formula of **4** was determined as $C_{21}H_{30}O_3$ by its HRESIMS and ^{13}C-DEPT NMR data (Table 1). Its 1D NMR spectra indicated that compound **4** was an aromatic meroterpenoid similar to
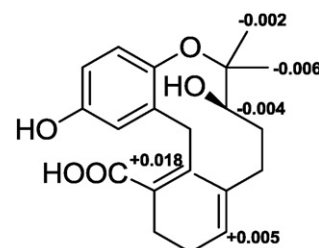

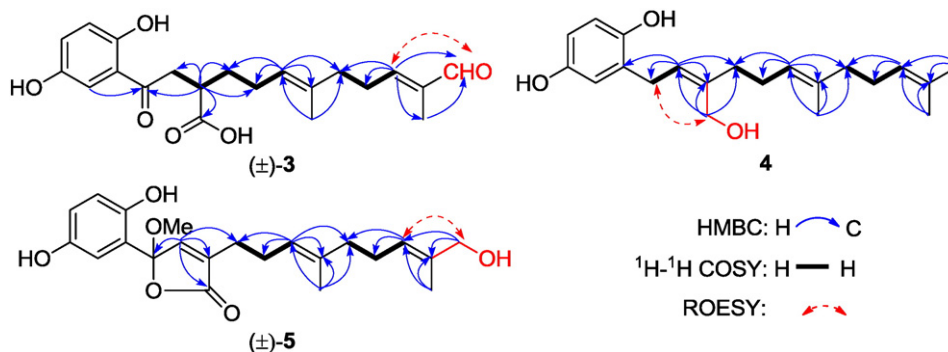**Fig. 5.** Values of δ_S–δ_R of the MTPA esters of **2**.

Fig. 6. Key HMBC, ^{1}H–^{1}H COSY and ROESY correlations of compounds $(\pm)$−**3**, **4** and $(\pm)$−**5**.

ganomycin B [8]. However, the characteristic carboxyl signal and absorption band were not observed in the 1D NMR and IR spectra of **4**, whereas an oxygenated methylene (δ 60.4) was present. This indicated an oxymethylene in **4** instead of a carboxyl group at C-15′ in ganomycin B, which was confirmed by the HMBC correlations (Fig. 6) of H$_2$-15′ (δ 4.19, s) with C-2′ (δ 127.5), C-3′ (δ 138.3), and C-4′ (δ 36.3); of H-2′ (δ 5.33, t, $J = 7.8$ Hz), H-4′ (δ 2.06, m) with C-15′ (δ 60.4); of H$_2$-1′ with C-2′, C-3′, C-1 (δ 147.4), C-2 (δ 127.4) and C-3 (δ 117.1). Thus, the structure of **4** was determined and named ganomycin F.

Fornicin E (**5**) was assigned a molecular formula of C$_{22}$H$_{28}$O$_6$ by HREIMS and 1D NMR spectroscopic data (Table 1). The hydroxyl (3431 cm^{-1}), carbonyl (1748 cm^{-1}) and conjugated carbonyl groups (1766 cm^{-1}) were identified from the IR spectrum. Its 1D NMR spectroscopic data resembled those of fornicin B [9], except for the presence of an oxymethylene and the absence of a methyl in **5**. The observed HMBC correlations (Fig. 6) of the oxymethylene proton signal at δ 4.30 with C-13′ (δ 16.5), C-10′ (δ 124.3) and C-11′ (δ 135.6); of H-10′ (δ 5.19, m) with the oxymethylene (δ 68.4), C-13′ and C-11′ illustrated that a hydroxyl group was connected to C-12′. Meanwhile, H$_2$-12′ showed a ROESY correlation with H-10′, suggesting that $\Delta^{10'}$ was Z configuration. Racemic **5** was subjected to chiral analytical HPLC to yield a pair of enantiomers. Thus, structure **5** was established and named $(\pm)$-fornicin E.

The known isolates were identified by comparing their physical and spectroscopic data from the literature. They are ganomycin I (**6**) [9], fornicin B (**7**) [10] and ganomycin C (**8**) [13].

Both ganocapensin A (**1**) and ganocapensin B (**2**) possess a macrocyclic structure. Detailed analysis of their structural features showed that they have the same skeleton as fornicin B (**7**) and ganomycin B (**6**), respectively. Meanwhile, similarities are also shown in compounds **3–7** and ganomycin C (**8**). Thus, a possible biosynthetic pathway for compounds **1–7** is proposed and shown in Scheme S1 (see Supporting information).

Antioxidant activities of compounds **1–8** were evaluated in the DPPH radical scavenging assay. All the isolates showed comparable DPPH radical scavenging effects with IC$_{50}$ values of 6.00 $\pm$ 0.11–

8.20 $\pm$ 0.30 µg/ml, when compared to the positive control (trolox) with IC$_{50}$ values of 3.63 $\pm$ 0.17 µg/ml (Table 2).

Actually, farnesylated hydroquinones were also isolated from some plants (Lithospermum and Phacelia), marine species (Amaroucium), and other macrofungi (*Lactarius lignyotus*) [30–33]. Moreover, these constituents showed various biological activities, such as anti-tumor, anti-oxidant, antibacterial, and anti-inflammatory effects [30–33]. Since 2000, many structurally diverse aromatic meroterpenoids were found from different species of *Ganoderma* and attracted many interests of chemists and pharmacologist [20,34]. In the present study, eight aromatic meroterpenoids (**1–8**) were first isolated and identified from the fruiting bodies of *G. capense*. Among them, compounds **1** and **2** had a macrocyclic substructure. Moreover, all of them showed strong antioxidant activities. The antioxidant property of *Ganoderma* has been the focus of attention by people due to its low toxicity [35–37]. Meanwhile, research found that the total phenol fraction had stronger antioxidant activities than other fractions [38,39]. Our previous and present research further exhibited that aromatic meroterpenoids had excellent *in vitro* antioxidant effects [13]. This will lay foundation for the further *in vivo* bioactive research and provide a theoretical basis to the application of *Ganoderma* on anti-aging.

Conflict of interest

We declare that there is no conflict of interest.

Acknowledgements

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.fitote.2016.04.006.

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Table 2
DPPH radical scavenging activities of compounds **1–8**. (IC$_{50}$: µg/ml).

Compounds	IC$_{50}$ (µg/ml)
1	7.79 $\pm$ 0.15
2	8.20 $\pm$ 0.13
3	7.64 $\pm$ 0.34
4	7.44 $\pm$ 0.13
5	6.00 $\pm$ 0.09
6	6.83 $\pm$ 0.12
7	7.12 $\pm$ 0.15
8	7.32 $\pm$ 0.23
Trolox[a]	3.63 $\pm$ 0.17

[a] Positive control

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