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New triquinane and gymnomitrane sesquiterpenes from fermentation of the basidiomycete *Antrodiella albocinnamomea*

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ABSTRACT

Five new triquinane-type sesquiterpenes, antrodins A–E (**1**–**5**) and a new gymnomitrane sesquiterpene, antrodin F (**6**), together with three known compounds **7**–**9** were isolated from fermentation of *Antrodiella albocinnamomea*. Their structures were elucidated through extensive spectroscopic methods including 2D NMR and HRMS analyses. The absolute configurations of **1** and **6** were determined using single-crystal X-ray diffraction analyses. The isolated compounds were investigated for their cytotoxicities against five human cancer cell lines.

1. Introduction

Among the many diverse organisms, basidiomycetes were well-known as a major resource of sesquiterpenes. Sesquiterpenes from basidiomycetes produce a rich variation of activities as well. It is worthy to point out that HMAF based on illudane sesquiterpenes from the basidiomycete *Clitocybe illudens* is now in the clinical trial II phase and promises to become an antitumor drug [1]. Inspired by the potential biological activities of different sesquiterpenes from basiodiomycetes, our group focused on sesquiterpene metabolites from higher fungi, and also reported numerous novel sesquiterpenes. Notable examples included trefolane A, a sesquiterpenoid with a new skeleton from *Tremella foliacea* [2], conosilane A, an unprecedented sesquiterpene from *Conocybe siliginea* [3], as well as agrocybone, an illudane–illudane bis-sesquiterpene from the basidiomycete *Agrocybe salicacola*, which exhibited weak antiviral activity against the respiratory syncytial virus [4].

The fungus *Antrodiella albocinnamomea*, belonging to the family Polyporaceae, is widely distributed in temperate to subtropical areas of China [5]. Previous chemical investigations of the *A. albocinnamomea* led to the isolation of novel degraded steroids albocisterols A–C and new humulane-type sesquiterpenes [6,7]. In our continuous search for new and bioactive natural products from higher fungi, five new triquinane-type sesquiterpenes, antrodins A–E (**1**–**5**) and a new gymnomitrane-type sesquiterpene, antrodin F (**6**), together with three known compounds coriolin (**7**) [8], dihydrocoriolin C (**8**) [9], and chloriolin B (**9**) [10] were isolated from fermentation of *A. albocinnamomea* (see Scheme 1). The structures of the new compounds were determined on the basis of extensive spectroscopic analysis and single-crystal X-ray diffraction method. All isolates were tested for their cytotoxic activities. Herein we describe the isolation, structure elucidation, and bioactivity of these new sesquiterpenes.

2. Experimental

2.1. General experimental procedures

Melting points were surveyed with an X-4 microscopic melting point meter. Optical rotations were measured on a Jasco-P-1020 polarimeter. UV spectra were recorded using a Shimadzu UV-2401A spectrophotometer equipped with a DAD

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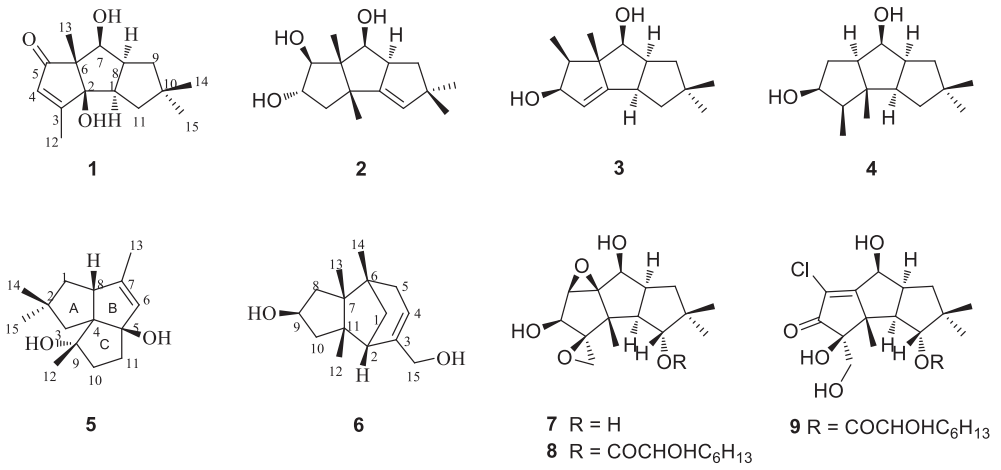

Scheme 1. Structures of compounds **1**–**9**.

and a 1 cm pathlength cell. Samples in methanol solution were scanned from 190 to 400 nm in 1 nm steps. IR spectra were obtained by a Bruker Tensor 27 FT-IR spectrometer with KBr pellets. 1D and 2D NMR spectra were recorded on a Bruker Avance III 600 MHz spectrometer (Karlsruhe, Germany). HR-EI-MS was recorded on a Waters Auto Premier P776 spectrometer. HPLC analysis was performed on an Agilent 1200 HPLC system using an ODS column (C18, 250 × 4.6 mm, YMC Pak, 5 μm; detector: UV) with a flow rate of 1.0 mL/min. Preparative HPLC was performed on an Agilent 1100 series with a Zorbax SB-C18 column (5 μm, 9.4 × 150 mm, Agilent, America) with a flow rate of 10.0 mL/min. Column chromatography (CC) was performed using silica gel (200–300 mesh; Qingdao Marine Chemical Inc., Qingdao, China), RP-18 gel (40–63 μm, Merck, Darmstadt, Germany) and Sephadex LH-20 (Amersham Biosciences, Sweden). Fractions were monitored by TLC (GF254, Qingdao Marine Chemical Co. Ltd., Qingdao), and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in EtOH. All solvents were distilled prior to use.

2.2. Fungal material

Fruiting bodies of *A. albocinnamomea* were collected at Changbai Mountain, Northeast of China in 1997 and were identified by Prof. Yu-Cheng Dai (Beijing Forestry University). The voucher specimen (no. CGBWSHF00182) was deposited at the herbarium of Kunming Institute of Botany. Culture medium was composed of glucose (5%), pork peptone (0.15%), yeast (0.5%), KH_2PO_4 (0.05%) and $MgSO_4$ (0.05%). Initial pH was adjusted to 6.0; the fermentation was first carried out on an erlenmeyer flask for 6 days till the mycelium biomass reached to the maximum. Then it was transferred to a fermentation tank (100 L) at 24 °C and 250 rpm for 20 days; ventilation was set to 1.0 vvm (vvm: air volume/culture volume/min).

2.3. Extraction and isolation

The fermented whole broth (80 L) was filtered through cheesecloth to separate into supernatant and mycelia. The former was concentrated under reduced pressure to about a quarter of the original volume and then extracted three times with ethyl acetate (20 L × 3) at room temperature, and the organic solvent was evaporated to dryness under reduced pressure to afford a brown crude extract. The mycelia were extracted three times with acetone (10 L × 3). The acetone solution was concentrated under reduced pressure to afford an aqueous solution. The aqueous solution was extracted three times with ethyl acetate (3 × 3 L), and the ethyl acetate solution was evaporated to dryness under vacuum to obtain the crude extract of the mycelia. Both of the extracts were combined for further purification (55 *g*).

The combined crude extract was then fractionated by silica gel CC eluted with a gradient of petroleum ether–acetone (100:0–0:100) to obtain eight fractions. Fraction 2 was eluted with petroleum ether–acetone (5:1), and was then purified by silica gel CC ($CHCl_3$–MeOH, 50:1) and Sephadex LH-20 CC (acetone) to give **6** (3.7 mg). Fraction 3 was eluted with petroleum ether–acetone (3:1), and was then purified into five subfractions (3A–3E) by MPLC using MeOH/H_2O as eluent. Fraction 3C was then separated by Sephadex LH-20 CC eluting with acetone to give **1** (6.8 mg). Fraction 4, was eluted with petroleum ether–acetone (2:1), and was then purified into four subfractions (4A–4D) by MPLC using MeOH/H_2O as eluent. Fraction 4B purified by preparative HPLC (CH_3CN/H_2O, 20:80 → 50:50, 30 min) to afford **4** (3.7 mg), **5** (2.1 mg) and **7** (7.1 mg). Fraction 5 was eluted with petroleum ether–acetone (1:1), and was then purified into seven subfractions (5A–5G) by MPLC using MeOH/H_2O as eluent. Fraction 5B was then purified by repeat silica gel CC to give **8** (15.2 mg), **9** (54.6 mg) and **10** (110.0 mg). Fraction 5C was purified by preparative HPLC (CH_3CN/H_2O, 0:100 → 24:76, 30 min) to give **2** (1.8 mg).

Antrodin A (**1**): lamellar crystals (petroleum ether/Me_2CO, 10:1); mp 151–153 °C; [α]25 D −49.0 (*c* 0.34, MeOH); UV (MeOH) λ_{max} (log ε) 221.8 (3.89) nm; IR (KBr) ν_{max} 3431, 2954, 2931, 2865, 1658, 1619, 1461, 1366 cm^{-1}; ^1H NMR data see Table 2; ^{13}C NMR data see Table 1; HR-EI-MS (+) *m/z* 250.1570 (calcd for $C_{15}H_{22}O_3$, 250.1569).

Antrodin B (**2**): colorless oil; [α]25 D −73.7 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 203 (3.70), 240 (3.01) nm; IR (KBr) ν_{max} 3441, 2955, 2926, 2863, 1631, 1462, 1384 cm^{-1}; ^1H NMR data see Table 2; ^{13}C NMR data see Table 1; HR-EI-MS (+) *m/z* 275.1616 (calcd for $C_{15}H_{24}O_3Na$, 275.1623).

Table 1
^{13}C NMR data for compounds **1**–**6** (150 MHz, acetone-d_6).

No.	1	2	3	4	5	6
1	53.2 d	159.6 s	42.0 d	48.4 d	44.2 t	41.7 t
2	89.7 s	42.1 s	157.9 s	51.4 s	40.9 s	48.1 d
3	180.9 s	45.2 t	125.9 d	55.3 d	41.7 t	145.7 s
4	127.0 d	74.9 d	86.2 d	77.5 d	71.1 s	120.4 d
5	210.4 s	83.5 d	60.3 d	38.2 t	93.3 s	41.0 t
6	66.6 s	61.8 s	58.9 s	60.6 d	132.3 d	45.2 s
7	77.4 d	75.1 d	81.6 d	79.6 d	143.7 s	54.4 s
8	47.4 d	51.3 d	51.4 d	50.3 d	60.0 d	46.4 t
9	39.4 t	39.2 t	43.1 t	39.0 t	82.2 s	72.8 d
10	41.3 s	50.3 s	42.3 s	43.4 s	37.7 t	48.2 t
11	43.1 t	126.7 d	48.5 t	44.5 t	41.0 t	57.8 s
12	14.1 q	24.2 q	12.1 q	25.0 q	23.1 q	28.6 q
13	15.6 q	13.2 q	16.2 q	11.9 q	15.0 q	25.0 q
14	29.4 q	28.9 q	27.1 q	29.0 q	32.4 q	24.8 q
15	30.8 q	29.9 q	29.1 q	29.6 q	29.3 q	66.8 t

Antrodin C (**3**): white powder; [α]25 D +23.5, (*c* 0.37, MeOH); UV (MeOH) λ_{max} (log ε) 206 (3.77) nm; IR (KBr) ν_{max} 3440, 2954, 2933, 2866, 1630, 1462, 1382 cm^{-1}; ^{1}H NMR data see Table 2; ^{13}C NMR data see Table 1; HR-EI-MS (+) *m/z* 259.1674 (calcd for $C_{15}H_{24}O_2Na$, 236.1674).

Antrodin D (**4**): white powder; [α]25 D −3.94 (*c* 0.21, MeOH); UV (MeOH) λ_{max} (log ε) 203 (3.02) nm; IR (KBr) ν_{max} 3440, 2953, 2927, 2866, 1630, 1462, 1380 cm^{-1}; ^{1}H NMR data see Table 3; ^{13}C NMR data see Table 1; HR-ESI-MS (−) *m/z* 237.1854 (calcd for $C_{15}H_{25}O_2$, 237.1855).

Antrodin E (**5**): white powder; [α]25 D +14.3 (*c* 0.37, MeOH); UV (MeOH) λ_{max} (log ε) 203 (3.62) nm; IR (KBr) ν_{max} 3439, 2948, 2929, 2862, 1631, 1459, 1377 cm^{-1}; ^{1}H NMR data see Table 3; ^{13}C NMR data see Table 1; HR-EI-MS (+) *m/z* 259.1673 (calcd for $C_{15}H_{24}O_2Na$, 259.1674).

Antrodin F (**6**): prism crystals (EtOAc/MeOH, 10:1); mp 126–128 °C; [α]25 D +49.4 (*c* 0.36, MeOH); UV (MeOH) λ_{max} (log ε) 203 (3.60), 251 (2.86) nm; IR (KBr) ν_{max} 3440, 2948, 2930, 2867, 1631, 1461, 1384 cm^{-1}; ^{1}H NMR data see Table 3; ^{13}C NMR data see Table 1; HR-EI-MS (+) *m/z* 259.1664 (calcd for $C_{15}H_{24}O_2Na$, 259.1674).

Table 2
^{1}H NMR data for compounds **1**–**3** (600 MHz, acetone-d_6).

No.	1	2	3
1	2.69 dt (12.7, 7.9)		3.05 m
3		1.69 dd (12.7, 7.3)	5.26 br s
		1.43 dd (12.7, 11.1)	
4	5.71 q (1.3)	3.82 m	4.46 t (7.5)
5		3.49 dd (9.1, 5.1)	1.65 m
6			
7	3.82 t (5.4)	3.60 t (5.4)	3.88 dd (8.9, 4.8)
8	2.10 m	3.46 m	3.02 m
9	1.91 dd (13.2, 3.5)	1.84 dd (12.0, 8.3)	1.37 m
	1.36 dd (13.2, 9.3)	1.53 dd (12.0, 7.8)	1.90 dd (13.1, 9.7)
11	1.94 t (12.3)	4.99 d (2.6)	1.70 m
	1.30 dd (11.9, 7.4)		1.37 m
12	2.12 d (1.3)	1.04 s	1.05 d (7.0)
13	1.07 s	0.95 s	1.03 s
14	1.08 s	1.06 s	0.92 s
15	0.96 s	1.07 s	1.06 s
2-OH	3.86 s		
4-OH		3.74 d (4.6)	3.75 d (7.0)
5-OH		3.89 d (5.1)	
7-OH	4.16 d (5.2)	3.12 d (5.4)	3.66 d (4.8)

Table 3
^{1}H NMR data for compounds **4**–**6** (600 MHz, acetone-d_6).

No.	4	5	6
1	2.38 m	1.42 dd (12.5, 9.3)	1.31 d (10.6)
		1.60 overlapped	1.82 dd (10.6, 4.5)
2			1.79 d (4.5)
3	1.45 dq (9.9, 6.8)	2.00 dd (14.1, 1.6)	
		1.58 overlapped	
4	3.66 m		5.43 br s
5	1.53 m		2.22 dd (18.6, 2.0)
	1.65 ddd		1.91 ddd
	(13.4, 12.0, 8.5)		(18.6, 5.1, 2.8)
6	2.25 dd (12.0, 3.9)	5.12 br s	
7	3.66 m		
8	2.54 m	2.58 d (9.3)	2.01 dd (13.5, 6.5)
			1.06 m
9	1.26 ddd		4.13 m
	(12.5, 9.1, 1.4),		
	1.80 dd (12.5, 6.9)		
10		1.38 dd (7.0, 3.1)	1.17 m
		1.56 m	2.07 m
11	1.09 m	1.81 dd (12.5, 7.2)	
	1.53 m	1.97 dd (12.5, 7.0)	
12	1.15 s	1.21 s	1.06 s
13	0.94 d (6.8)	1.63 br s	0.95 s
14	0.96 s	1.03 s	0.89 s
15	1.04 s	0.99 s	3.93 m
4-OH	3.60 d (5.7)		
5-OH		2.99 s	
7-OH	3.58 d (3.1)		
9-OH		2.87 s	3.39 d (5.0)
15-OH			3.78 t (5.7)

2.4. X-ray diffractometry

The lamellar crystals of antrodin A (**1**) and the prism crystals of antrodin F (**6**) were obtained from petroleum ether/Me_2CO (10:1) and EtOAc/MeOH (10:1), respectively. Data were collected at 100 K on a Bruker APEX DUO diffractometer equipped with an APEX II CCD using Cu Kα radiation. Cell refinement and data reduction were performed with Bruker SAINT software. The structure was solved by direct methods using SHELXL-97. Refinements were performed with SHELXL-97 using full-matrix least-squares, with anisotropic displacement parameters for all the non-hydrogen atoms. The H atoms were placed in calculated positions and refined using a riding model. Molecular graphics were computed with PLATON. Crystallographic data have been deposited in Cambridge Crystallographic Data Centre (CCDC 1017903 for **1**, CCDC 1017904 for **6**). Copies of the data can be obtained free of charge on application to the Director, CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

Crystallographic data of antrodin A (**1**): colorless crystal, $C_{15}H_{22}O_3$, M = 250.33, orthorhombic, a = 6.6694(3) Å, b = 12.6257(5) Å, c = 16.3326(7) Å, α = 90.00°, β = 90.00°, γ = 90.00°, V = 1375.30(10) Å^3, T = 100(2) K, space group $P212121$, Z = 4, μ(CuKα) = 0.662 mm^{-1}, 9045 reflections measured, 2299 independent reflections (R_{int} = 0.0597). The final R_1 values were 0.0557 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.1566 ($I > 2\sigma(I)$). The final R_1 values were 0.0582 (all data). The final $wR(F^2)$ values were 0.1593 (all data). The

goodness of fit on F^2 was 1.068. Flack parameter = 0.1(3). The Hooft parameter is 0.03(13) for 915 Bijvoet pairs. Cu Kα radiation, wavelength = 1.54178 A.

Crystallographic data of antrodin F (**6**): colorless crystal, $C_{15}H_{24}O_2$, M = 236.34, monoclinic, a = 13.8420(3) Å, b = 7.42280(10) Å, c = 14.5141(3) Å, α = 90.00°, β = 116.43°, γ = 90.00°, V = 1335.39(4) Å^3, T = 100(2) K, space group $P21$, Z = 4, μ(CuKα) = 0.591 mm^{-1}, 13510 reflections measured, 4232 independent reflections (R_{int} = 0.0284). The final R_1 values were 0.0344 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.0904 ($I > 2\sigma(I)$). The final R_1 values were 0.0345 (all data). The final $wR(F^2)$ values were 0.0906 (all data). The goodness of fit on F^2 was 1.029. Flack parameter = 0.06(16). The Hooft parameter is 0.06(5) for 1623 Bijvoet pairs. Cu Kα radiation, wavelength = 1.54178 A.

2.5. Cytotoxic activity

Human myeloid leukemia HL-60, hepatocellular carcinoma SMMC-7721, lung cancer A-549 cells, breast cancer MCF-7 and colon cancer SW480 cell lines were used in the cytoxic assay. All the cells were cultured in RPMI-1640 or DMEM medium (Hyclone, USA), supplemented with 10% fetal bovine serum (Hyclone, USA) in 5% CO_2 at 37 °C. The cytotoxicity assay was performed according to the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) method in 96-well microplates. Briefly, 100 μL adherent cells were seeded into each well of 96-well cell culture plates and allowed to adhere for 12 h before drug addition, while suspended cells were seeded just before drug addition with initial density of 1×10^5 cells/mL. Each tumor cell line was exposed to the tested compounds dissolved in DMSO at concentrations of 0.0625, 0.32, 1.6, 8, and 40 μmol in triplicates for 48 h, with cisplatin (Sigma, USA) as a positive control. After compound treatment, cell viability was detected and a cell growth curve was graphed. IC_{50} values were calculated by Reed and Muench's method.

3. Results and discussion

Compound **1** was obtained as colorless crystals (petroleum ether/Me_2CO, 10:1). Its molecular formula was determined to be $C_{15}H_{22}O_3$ by HR-EI-MS at m/z 250.1570 (calcd for $C_{15}H_{22}O_3$, 250.1569), indicating five degrees of unsaturation. Its IR data indicated the presence of hydroxyl (3431 cm^{-1}) and α, β-unsaturated ketone (1658, 1619 cm^{-1}) groups. The 1D NMR spectra revealed 15 carbon resonances ascribable to four methyls, two methylenes, four methines (one oxygenated and one olefinic) and five quarternary carbons (one carbonyl and one olefinic carbon). The aforementioned functionalities (a carbonyl and a double bond) accounted for two degrees of unsaturation, and the remaining three degrees of unsaturation required **1** to be tricyclic.

The 1D NMR spectra data of **1** were similar to those of chondrosterin E [11], which was isolated from a coral-associated fungus *Chondrostereum* sp., except a methine at δ_H (2.34, d, J = 2.5 Hz, H-2) and δ_C (63.8, C-2) in chondrosterin E was replaced by an oxyquaternary carbon δ_C (89.7, C-2) in **1**. In the HMBC spectrum (Fig. 1), significant correlations from the hydroxyl proton at δ_H (3.81, 2-OH) to C-1 (δc 53.2), C-2 (δc 89.7), and C-3 (δc 180.9) were corroborated the change. Furthermore, a single crystal X-ray diffraction experiment not only confirmed the structure of **1** but also determined the absolute configuration of **1** as shown in Fig. 3. Therefore, compound **1** was determined to be antrodin A.

Compound **2** was isolated as colorless oil with a quasi-molecular ion peak at m/z [M + Na]$^+$ (calcd 275.1623) in its HR-EI-MS, in agreement with the molecular formula $C_{15}H_{24}O_3$. The IR absorptions at 3441 and 1631 cm^{-1} indicated the presence of hydroxyl and double bond groups. The ^{13}C and DEPT spectra (Table 1) of **2** exhibited 15 signals attributed to four methyls, two methylenes, five methines and four quarternary carbons. The ^1H NMR data of **2** displayed diagnostic signals for four tertiary methyls [δ_H 0.95 (s), 1.04 (s), 1.06 (s) and 1.07 (s)], three oxygenated methines [δ_H 3.82 (1H, m, H-4); 3.60 (1H, t, J = 5.4 Hz, H-7); 3.49(1H, dd, J = 9.1, 5.1 Hz, H-5)], and an olefinic proton [δ_H 4.99 (1H, d, J = 2.6 Hz, H-11)]. The COSY spectrum showed two spin systems corresponding to H-3/H-4(4-OH)/H-5(5-OH) and H-7(7-OH)/H-8/H-9. The structure of **2** was finally assigned using HMBC spectrum, which showed critical long-range correlations from H-14 and H-15 to C-9, C-10 and C-11, from H-11 to C-1, C-9, C-10, and C-2, from H-12 to C-1, C-2, C-3 and C-6, from H-13 to C-5, C-6, C-7 and C-2, as shown in Fig. 1. The ROESY experiment showed correlations between H-4

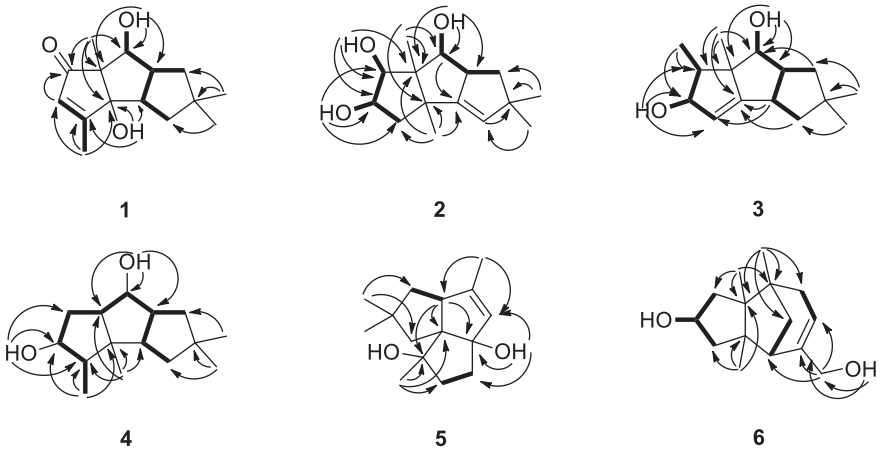

Fig. 1. The key ^1H–^1H COSY and HMBC correlations of compounds **1**–**6**.

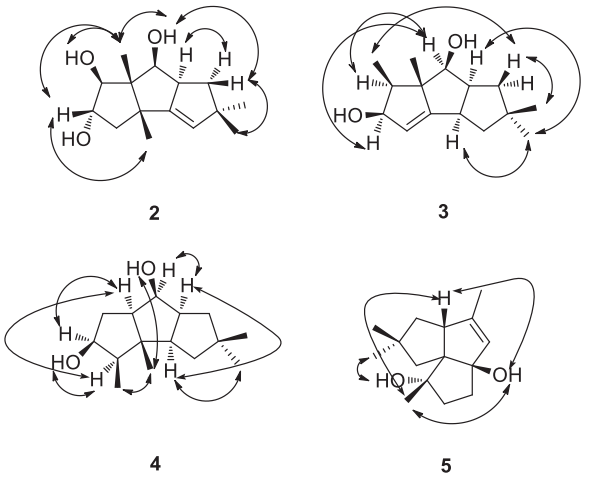

Fig. 2. The key ROESY correlations of compounds **2**–**5**.

and H-12, H-13; H-13 and 5-OH, 7-OH; H-9β and 7-OH, 15-Me, which indicated that H-4, H-12, H-13, 5-OH, 7-OH and H-15 were β-orientated. In addition, the ROESY correlations between H-8 and H-14 allowed to assignment of H-8 in α-orientation. Accordingly, the relative structure of **2** was established, and named antrodin B.

Compound **3** was obtained as a white powder, and exhibited the molecular formula $C_{15}H_{24}O_2$ by HR-ESI-MS, indicating four degrees of unsaturation. The IR spectrum of compound **3** showed the absorptions for hydroxyl (3440 cm^{-1}) and double bond (1630 cm^{-1}) groups. The 1D NMR displayed 15 carbon resonances due to four methyls, two methylenes, six methines (one sp^2) and three quarternary carbons (one sp^2). Apart from one degree of unsaturation occupied by the double bond, the remaining three degrees of unsaturation indicated that compound **3** should possess a tricyclic system. The ^1H–^1H COSY spectrum displayed the following cross-peaks H-3/H-4(4-OH)/H-5/H-12, H-7(7-OH)/H-8/H-9, and H-8/H-1/H-11, indicating two partial fragments as shown in bold in Fig. 1. The HMBC correlations from H-14 and H-15 to C-9, C-10 and C-11, from H-1 to C-2, C-3, and C-4, from H-12 to C-3, C-4, C-5, from H-13 to C-5, C-6, C-7 and C-2 established the planar structure of **3**. In the ROESY spectrum (Fig. 2), the correlations between H-4 and H-13, H-7; H-7 and H-5, H-1; H-1 and H-8, H-15 revealed that H-12 and H-13 had a β-orientation, while H-1, H-7, H-8 and H-15 were in the opposite orientation (α). Therefore, compound **3** was established as depicted, and named as antrodin C.

Compound **4** was obtained as a white powder. HR-EI-MS analysis of **4** showed a $[M - H]^-$ ion peak at m/z 237.1854 consistent with a molecular formula of $C_{15}H_{26}O_2$ (calcd for $C_{15}H_{25}O_2$, 237.1855), indicating three degrees of unsaturation. The IR spectrum of compound **4** showed the absorptions for hydroxyl (3440 cm^{-1}). The ^{13}C NMR and DEPT spectra displayed 15 carbon signals, including four methyls, three methylenes, six methines (two oxygenated) and two quarternary carbons. The ^1H NMR spectrum showed three singlet methyls (δ_H 0.96, 1.04, 1.15), one doublet methyl (δ_H 0.94, d, $J = 6.8$ Hz), and two oxygenated protons (δ_H 3.66, m). Based on the 1D NMR spectra, we deduced that compound **4** also has a tricyclic system. Detailed analyses of the 2D NMR spectra (COSY, HSQC and HMBC) established the planar structure of **4** as shown in Fig. 1. The stereochemistry of **4** was determined by the ROESY experiment. The ROESY correlations between H-13 and H-12, 4-OH; H-12 and 7-OH; H-6 and H-3, H-4; H-8 and H-1, H-7 implied that H-13, H-12, 4-OH and 7-OH were in β-orientation, whereas H-1, H-7 and H-8 were in α-orientation (Fig. 2). Thus, compound **4** was established to be antrodin D.

Compound **5** was isolated as a colorless oil. Its molecular formula was assigned to be $C_{15}H_{24}O_2$ by HR-ESI-MS at m/z 259.1673 (calcd for $C_{15}H_{24}O_2Na$, 259.1674). Its IR showed the absorptions for hydroxyl (3439 cm^{-1}) and double bond (1631 cm^{-1}). The ^{13}C NMR and DEPT spectra displayed 15 carbon signals, including four methyls, four methylenes, two methines (one sp^2) and five quarternary carbons (one sp^2 and two oxygenated). The ^1H-^1H COSY spectrum showed two spin systems corresponding to H-1/H-8 and H-10/H-11. 13-Me attached to quaternary carbon C-7, based on the chemical shift δ_H (1.63, s) and a weak allylic coupling to H-5 in ^1H-^1H COSY. In the HMBC spectrum, germinal dimethyl proton H-14 and H-15 showed correlations to C-1, C-2 and C-3, which indicated that germinal dimethyl Me-14 and Me-15 were attached to a quarternary carbon C-2, and C-1 and C-3 were linked via C-2. The HMBC correlations from H-8 to C-8, C-4 and C-1, from H-3 to C-4 and C-8 established a five membered ring A. The HMBC correlations from H-13 to C-7 and C-8, from H-6 to C-4, C-5 and C-8 established another five membered ring B. The HMBC correlations from angular methyl proton H-12 to C-4, C-9 and C-10 implied that 12-Me was attached at a quarternary carbon

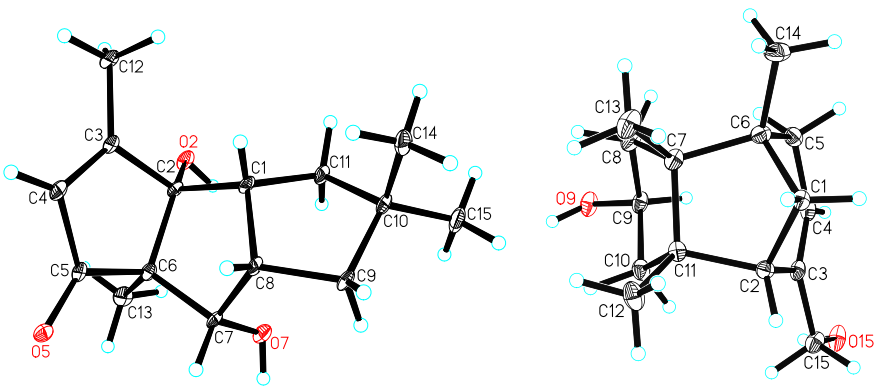

Fig. 3. X-ray crystallographic structures of compounds **1** (left) and **6** (right).

C-9, and C-4 was directly linked to C-9; from H-11 to C-4, C-5 and C-9 constructed the cyclopentyl ring C. The large chemical shift of C-5 (δc 93.3) and C-6 (δc 82.2) indicated that both of them were oxygenated. The HMBC correlations from 5-OH to C-5, C-6 and C-11, from 9-OH to C-9, C-10 and C-4 further confirmed the assignments. Therefore, the planar structure of **5** was established. In ROESY spectrum (Fig. 2); the correlations between H-8 and H-12, 5-OH, H-15 and 9-OH determined the relative structure of **5** as shown in Fig. 1, and has been named antrodin E.

Compound **6** was obtained as colorless crystals (EtOAc: MeOH = 10:1, *v/v*). Its molecular formula was assigned to be $C_{15}H_{24}O_2$ by HRESIMS, indicating four degrees of unsaturation. The ^{13}C NMR and DEPT spectra displayed 15 carbon signals, including three methyls, five methylenes (one oxygenated), three methines (one sp^2) and four quarternary carbons (one sp^2), indicative of a tricyclic skeleton. The 1D NMR spectra of **6** were resembled to the known compound 3-gymnomitren-15-ol [12], except that the ^{13}C signal at 27.2 ppm (C-9) in 3-gymnomitren-15-ol was shifted to 72.8 ppm in **6**, suggesting an oxygen-bearing methine instead of a methylene existed in **6**. Finally, the X-ray diffraction determined the absolute structure of **6** as shown in Fig. 3. It was named to be antrodin F.

Interestingly, antrodin E (**5**) was a pentalenene-type sesquiterpene, which was produced by a variety of streptomycete species with broad biological properties such as antibiotic, antiviral and inhibitory of the enzyme glyceraldehyde-3-phosphate dehydrogenase [13–16]. To the best of our knowledge, antrodin E (**5**) was the first example of pentalenene-type sesquiterpene isolated from the cultures of fungus. In addition, gymnomitrane sesquiterpenes were mainly found in liverwort [12,17], and antrodin F (**6**) was the first report of this type sesquiterpene isolated from higher fungus.

Triquinane-type sesquiterpenes were structurally unique secondary metabolites, including two types: linear and angular. The first example of triquinane-type sesquiterpene was hirsutane, which was isolated from a higher fungus *Stereum hirsutum* in 1947 [18]. Up to date, a series of triquinane-type sesquiterpenes were isolated from fungi [4,5,19], plants [20], as well as Streptomyces [21,22]. Those sesquiterpenes were formed via the humulane-protoilludane biosynthetic pathway. However, gymnomitranes were another type of tricyclic sesquiterpenes with 5/5/6 skeleton, which were known as characteristic constituents of liverworts. The gymnomitranes were synthesized from farnesyl diphosphate (FDP) and nerolidyl diphosphate (NDP), and the cuparanes were assumed as precursors [23]. In the literature survey, this report was the first co-occurrence of triquinane and gymnomitrane sesquiterpene.

Compounds **1**–**9** were tested for their cytotoxic activities against five tumor cell lines HL-60, A-549, SMMC-7721, MCF-7 and SW-480 using the MTT method as reported previously [24]. However, no compounds showed significant activity ($IC_{50} > 40$ μmol).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.fitote.2015.02.005.

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