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Three new hopane-type triterpenoids from the aerial part of *Adiantum capillus-veneris* and their antimicrobial activities

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ABSTRACT

Three new hopane-type triterpenoids (**1**–**3**), fern-7(8)-en-19α, 28-diol (**1**), pteron-14-ene-7α,19α,28-triol (**2**) and 3β,4α,25-trihydroxyfilican (**3**), were isolated from the aerial parts of *Adiantum capillus-veneris*. Their structures were determined by NMR spectroscopic and mass spectrometric data. Compounds **2** and **3** exhibited remarkable antifungal activity against *Helminthosporium maydis* and *Alternaria alternata* with MIC values of 12.5–3.125 μg/mL, and compound **3** also against *Verticillium dahliae* Kleb with an MIC value of 3.125 μg/mL. In addition, compounds **1**–**3** also displayed weak antibacterial activity against *Micrococcus lysodeikticus, Bacterium paratyphosum* B and *Pseudomonas aeruginosa* with an MIC value of 100 μg/mL.

1. Introduction

The genus *Adiantum* (Adiantaceae), commonly known as maidenhair ferns, has been used for medicinal and nutritive purpose, such as respiratory problems treatment, and as an astringent, demulcent, diuretic, emmenagogue, et al. [1] Until now, the chemical constituents of 17 species of this genus have been investigated to afford over 135 compounds, and most of them belonged to the triterpenoids, flavonoids, phenyl propanoids, phenolics, coumarins and phytosterols [1]. *Adiantum capillus-veneris*, one of the most common and widely distributed species, has been traditionally used as single medicine or in multi-herbal formulations for the treatment of various diseases [2]. Previous phytochemical studies of *A. capillus-veneris* have resulted into the isolation of many compounds, and most of which possessed diverse pharmacological activities [3–11].

As a part of our continuing works for searching new bioactive natural products from Chinese medicinal herbs, the chemical constituents of *A. capillus-veneris* were investigated to yield three new triterpenoids (**1**–**3**). Herein, the isolation, structural elucidation, and antimicrobial activities of **1**–**3** are described.

2. Experimental

2.1. General methods

Optical rotations were recorded on a Rudolph Research Analytical polarimeter. IR spectra (KBr pellets) were performed on a Bruker TENSOR-27 spectrophotometer. HRESIMS were obtained from a Bruker SolariX instrument. NMR spectroscopic data were acquired with a Bruker AM-500 using TMS as the internal standard. Column chromatography (CC) was carried out using silica gel (200–300 mesh, Qingdao Marine Chemical Inc., Qingdao, China) and Sephadex LH-20 (GE Healthcare, Uppsala, Sweden).

2.2. Plant material and microbial strains

The aerial parts of *A. capillus-veneris* were collected in April 2014 from Dali, Yunnan, P. R. China, which was identified by Mr. Yu Chen (Kunming Institute of Botany). A voucher specimen (BBP0542) was preserved at Biobiopha Co., Ltd. All microbial strains for biological studies were gifted by Dr. Fei Cao (College of Pharmaceutical Sciences, Hebei University).

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Table 1
^{1}H and ^{13}C NMR data for compounds **1–3** (δ in ppm, J in Hz).

No.	1[a]		2[b]		3[b]	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	1.58, m 0.88, brd (3.8)	39.1, CH_2	1.57, m 0.90, brd (3.6)	39.3, CH_2	1.57, m 1.76, brd (3.3)	17.3, CH_2
2	1.54, m 1.42, m	19.8, CH_2	1.60, m 1.43, m	18.5, CH_2	1.66, m 1.95, m	31.1, CH_2
3	1.14, m 1.39, m	42.9, CH_2	1.21, m 1.39, m	41.9, CH_2	3.55, m	76.5, CH
4		33.7, C		32.6, C		76.2, C
5	1.36, m	52.0, CH	1.49, m	47.4, CH		41.7, C
6	2.16, m 1.88, m	25.3, CH_2	1.85, m 1.69, dd (13.0, 1.1)	24.1, CH_2	1.48, m	34.5, CH_2
7	5.46, brd (3.4)	117.6, CH	3.87, m	72.7, CH	1.44, m	17.0, CH_2
8		145.9, C		45.5, C	1.40, m	49.5, CH
9	2.53, m	49.0, CH	2.13, m	42.1, CH		42.0, C
10		36.2, C		38.0, C	1.69, m	53.3, CH
11	1.68, m	16.9, CH_2	1.82, m 1.48, m	15.4, CH_2	1.85, m 1.05, m	30.0, CH_2
12	1.57, m	32.2, CH_2	2.07, m 1.31, m	32.2, CH_2	1.51, m	30.1, CH_2
13		37.6, C		36.6, C		39.0, C
14		43.6. C		154.3, C		39.7, C
15	1.57, m 1.72, m	32.1, CH_2	5.36, t (3.7)	118.6, CH	1.97, m	29.2, CH_2
16	2.48, m 1.79, m	44.5, CH_2	2.13, m 2.02, m	40.0, CH_2	1.62, m	35.8, CH_2
17		48.0, C		44.9, C		42.7, C
18	1.54, overlap	58.2, CH	1.36, brs.	61.7, CH	1.55, m	51.6, CH
19	4.55, m	70.5, CH	4.39, m	70.7, CH	1.33, m	19.9, CH_2
20	1.78, m 1.50, m	35.5, CH_2	2.43, m 1.39, m	43.7, CH_2	1.83, m 1.18, m	28.4, CH_2
21	1.01, m	59.7, CH	1.08, m	58.2, CH	0.97, m	60.0, CH
22	2.18, m	30.5, CH	1.89, m	29.7, CH	1.42, m	30.8, CH
23	0.84, s	33.4, CH_3	0.87, s	33.0, CH_3	1.23, s	21.5, CH_3
24	0.90, s	21.8, CH_3	0.81, s	21.5, CH_3	1.13, s	18.0, CH_3
25	0.83, s	13.6, CH_3	0.88, s	15.4, CH_3	3.89, d (11.8) 3.82, d (11.8)	64.2, CH_2
26	1.16, s	24.9, CH_3	1.04, s	28.1, CH_3	0.94, s	15.1, CH_3
27	1.74, s	23.8, CH_3	1.49, s	22.4, CH_3	0.96, s	15.8, CH_3
28	3.90, d (11.6) 4.33, d (11.6)	63.2, CH_2	3.84, d (11.4) 3.73, d (11.4)	63.3, CH_2	0.77, s	16.3, CH_3
29	1.06, d (6.5)	24.2, CH_3	0.97, d (6.5)	22.8, CH_3	0.82, d (6.5)	22.9, CH_3
30	0.96, d (6.5)	23.7, CH_3	0.89, d (6.5)	22.8, CH_3	0.88, d (6.5)	21.9, CH_3

[a] Measured in pyridine-d_5.
[b] Measured in $CDCl_3$.

2.3. Extraction and isolation

The air-dried and powdered plants of *A. capillus-veneris* (9.5 kg) were extracted three times with 95% EtOH (15 L/each time), and the combined extracts were evaporated in vacuum to afford 1.17 kg of crude extract. The crude extract was directly subjected to chromatography column (CC) on silica gel using a petroleum ether/acetone gradient elution to yield ten fractions (A_1-A_{10}) according to TLC detection on silica gel plates. Subfraction A_3 (3.5 g) was fractionated by Sephadex LH-20 (MeOH), and further purified by silica gel CC (petroleum ether/acetone, 10:1, v/v) to yield compound **2** (10 mg). Compound **1** (8.5 mg) was isolated from subfraction A_5 (15.8 g) by repeated silica gel CC and Sephadex LH-20 ($CHCl_3$/MeOH, 1:1, v/v). Subfraction A_8 (10.3 g) was separated by silica gel CC eluted with a solvent system of $CHCl_3$/acetone (50:1, v/v), and further purified by repeated Sephadex LH-20 (MeOH) to afford compound **3** (6.3 mg).

Fern-7(8)-en-19α, 28-diol (**1**). White amorphous powder; $[\alpha]_D^{20.8}$ −72.3° (*c* 0.1, MeOH); IR (KBr) ν_{max} 3210 (OH), 2922, 1471, 1455, 1383, 1366, 1066, 1018 cm^{-1}; for ^{1}H (pyridine-d_5, 500 MHz) and ^{13}C NMR (pyridine-d_5, 125 MHz) spectroscopic data, see Table 1; positive HRESIMS *m/z* 465.3705 $[M + Na]^+$ (calcd for $C_{30}H_{50}O_2Na^+$, 465.3703).

Pteron-14-ene-7α,19α,28-triol (**2**). White amorphous powder; $[\alpha]_D^{20.8}$ −80.6° (*c* 0.13, MeOH); IR (KBr) ν_{max} 3355 (OH), 2929, 1470, 1441, 1389, 1046 cm^{-1}; for ^{1}H ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) spectroscopic data, see Table 1; positive HRESIMS *m/z* 481.3650 $[M + Na]^+$ (calcd for $C_{30}H_{50}O_3Na^+$, 481.3652).

3β,4α,25-Trihydroxyfilican (**3**). White amorphous powder; $[\alpha]_D^{20.4}$ + 2.0° (*c* 0.1, MeOH); IR (KBr) ν_{max} 3395 (OH), 2946, 1468, 1452, 1377, 1055, 1034 cm^{-1}; for ^{1}H ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) spectroscopic data, see Table 1; positive HRESIMS *m/z* 483.3806 $[M + Na]^+$ (calcd for $C_{30}H_{52}O_3Na^+$, 483.3809).

2.4. Antifungal assay

Compounds **1–3** were evaluated for their antifungal activity against eight agricultural pathogenic fungi, including *Sclerotinia sclerotiorun*, *Helminthosporium maydis*, *Verticillium dahliae* Kleb, *Phytophthora parasitica*, *Gibberella saubinetii*, *Alternaria alternata*, *Botrytis cinerea* Pers., and *Colletotrichum acutatum* Simmonds, by using the microbroth dilution method according to the procedures previously described in the literature [12,13].

2.5. Antibacterial assay

Compounds **1–3** were also evaluated for their antibacterial activity. Eight bacterial strains, including *Micrococcus lysodeikticus, Micrococcus luteus, Bacillus megaterium*, *Bacterium paratyphosum* B, methicillin-resistant *Staphyloccocus aureus, Pseudomonas aeruginosa*, *Escherichia coli*, and *Vibrio Parahemolyticus*, were used in this study. The minimum inhibitory concentrations (MIC) of samples and positive control were determined in sterile 96-well plates by the modified broth dilution test as previously described procedures [12,13].

3. Results and discussion

Compound **1** was obtained as a white amorphous powder. Its molecular formula, $C_{30}H_{50}O_2$, was determined by (+) HRESIMS at *m/z* 465.3705 $[M + Na]^+$ (calcd for $C_{30}H_{50}O_2Na^+$, 465.3703), suggesting 6 indices of hydrogen deficiency. The IR spectrum showed the presence of hydroxy group (3210 cm^{-1}) and aliphatic C—H stretching group (2922 cm^{-1}). The ^{1}H NMR data of **1** (Table 1) revealed characteristic resonances for two secondary methyls [δ_H 1.06 (d, H_3–29) and 0.96 (d, H_3–30)], five tertiary methyls [δ_H 0.84 (s, H_3–23), 0.90 (s, H_3–24), 0.83 (s, H_3–25), 1.16 (s, H_3–26) and 1.74 (s, H_3–27)], a pair of oxygenated methylenes (δ_H 3.89, 4.33), an oxygenated methine (δ_H 4.55), and one olefinic proton (δ_H 5.46). Its ^{13}C NMR spectroscopic data (Table 1) exhibited 30 carbons, which were assigned to a trisubstituted double bond, seven methyls, ten methylenes (including one oxygenated), six methines (one of which was oxygenated), and five sp^3 quaternary carbons. Comparison of the NMR data of **1** with those of one closely related compound, fern-9(11)-en-28-ol previously reported from this plant [8], revealed that both compounds possessed the same hopane-type triterpene skeleton, as confirmed by 2D NMR experiments (Figs. 2 and 3). The key differences were found that the chemical shifts at C-7, C-8, C-9, C-11, and C-19 in **1** were quite different from those of fern-9(11)-en-28-ol. The observed HMBC correlations (Fig. 2) of H-7 with C-5 and C-9, H-19 with C-13, C-17 and C-21, in conjunction with ^{1}H, ^{1}H COSY correlations (Fig. 2) of H-5/H_2-6/H-7 and H-18/H-19/H_2-20/H-21/H-22/H_3-29(/H_3-30), revealed that the $\Delta^{9,11}$ double bond in fern-9(11)-en-28-ol

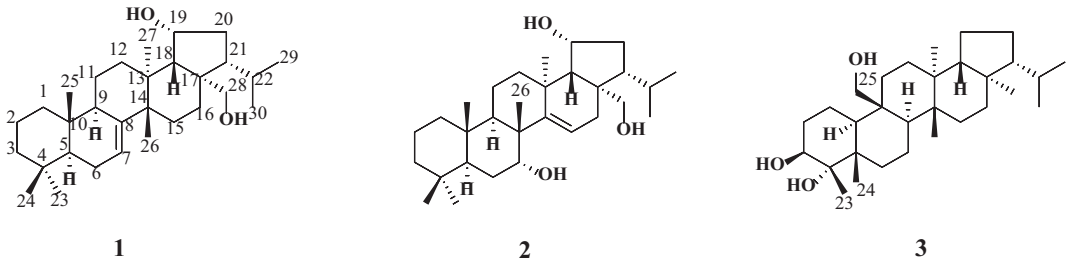

Fig. 1. Structures of compounds **1–3.**

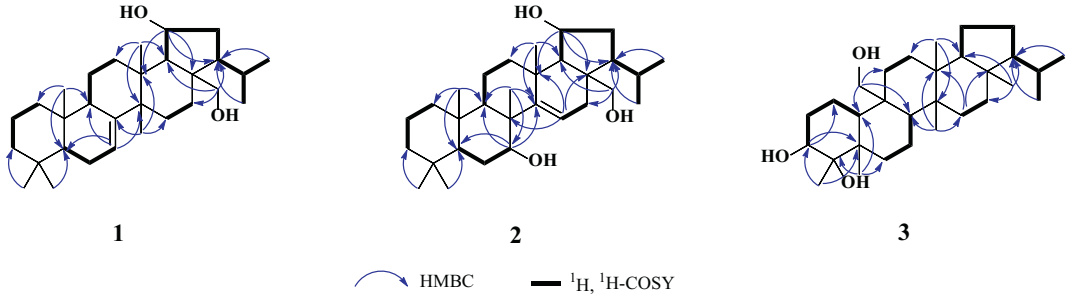

Fig. 2. ^{1}H, ^{1}H COSY and key HMBC correlations of compounds **1–3.**

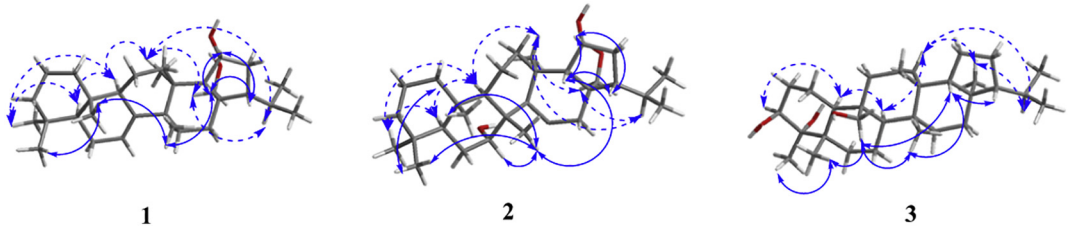

Fig. 3. NOESY correlations of compounds **1–3.**

was shifted at C-7 and C-8 in **1**, as well as the hydroxyl group was substituted at C-19 in **1**. Therefore, the gross structure of **1** was characterized as shown in Fig. 1. The relative stereochemistry of C-5, C-10, C-13, C-14, C-17, C-21, and C-28 were determined to be 5*S**,10*S**,13*S**,14*S**,17*S**, 21*R** by comparison of NMR data in conjunction with NOESY experiment (Fig. 3), which was the same as those found in fern-9(11)-en-28-ol as well as in agreement with hopane-type triterpene skeleton. Furthermore, the observation of NOESY correlations of H-9 with H-5 and H_3–27, and H-19 with H-8 and H-21, suggested the relative configurations of C-9 and C-19 should be 9*R**,19*R**. Thus, compound **1** was established as fern-7(8)-en-19α, 28-diol (Fig. 1).

Compound **2** was also isolated as a white amorphous powder. It was assigned the molecular formula $C_{30}H_{50}O_3$ on the basis of its (+) HRESIMS at *m/z* 481.3650 $[M + Na]^+$ (calcd for $C_{30}H_{50}O_3Na^+$, 481.3652), requiring 6 degrees of unsaturation. The ^{13}C NMR spectroscopic data of **2** (Table 1) displayed the presence of 30 carbon signals, which were classified into a trisubstituted double bond, seven methyls, nine methylenes (one of which was oxygenated), seven sp^3 methines (two of which were oxygenated), and five sp^3 quaternary carbons. Detailed by comparison of its ^{1}H and ^{13}C NMR data with those of **1**, we found that the structure of **2** is very similar to that of **1**, except for the differences in the locations of a trisubstituted double bond and a tertiary methyl, and the presence of one additional hydroxy group in **2**. The trisubstituted double bond at C-7 and C-8 in **1** was shifted to C-14 and C-15 in **2**, as evident from HMBC correlations of H-15 with C-8, C-13 and C-17, together with COSY correlation of H-15/H_2-16. Furthermore, key HMBC correlations of H-26 with C-7, C-9 and C-14 implied that a tertiary methyl at C-14 in **1** was migrated at C-8 in **2**. In addition, the position of the additional hydroxy group was located at C-7, as deduced from COSY correlations of H-5/H_2–6/H-7 and HMBC correlations of H-7 with C-5, C-9 and C-14. Thus, the gross structure of **2** was elucidated. The relative stereochemistry of **2** were determined to be 5*S**,7*R**,8*R**,9*R**,10*S**,13*S**,17*S**18*S**,19*R**,21*R** by comparison of NMR data with one related known compound, pteron-14-en-7-ol previously reported from this plant [8], combing with NOE correlations of H-5 with H-9, CH_3–27 with H-9 and H_2-28, H-7 with CH_3-25 and CH_3-26, H-18 with H-19, H-21 and CH_3–26. Finally, compound **2** was elucidated as pteron-14-ene-7α,19α,28-triol.

Compound **3** was isolated as a white amorphous powder, with molecular formula $C_{30}H_{52}O_3$ as determined by (+) HRESIMS at *m/z* 483.3806 $[M + Na]^+$ (calcd for $C_{30}H_{52}O_3Na^+$, 483.3809), corresponding to 5 degrees of unsaturation. The ^{13}C NMR data showed 30 carbon signals, which were categorized into seven methyls, eleven methylenes (one of which was oxygenated), six methines (one of which was oxygenated), and six quaternary carbons including one oxygenated. Detailed analysis of its ^{1}H and ^{13}C NMR data revealed that compound **3** closely resembled to one known compound, 3,4-dihydroxyfilicane [6]. The only difference was found that the tertiary methyl at C-9 in 3,4-dihydroxyfilicane was replaced by a hydroxymethylene group in **3**, as supported by HMBC correlations of H-25 with C-8, C-10 and C-11. The relative stereochemistry of **2** were determined to be 3*S**,4*S**,5*S**,8*R**,9*S**,10*S**, 13*S**,14*R**,17*R**,18*R**,21*R** by comparison of NMR data and NOESY experiment. Thus, compound **3** was determined to be 3β,4α,25-trihydroxyfilicane.

Compounds **1–3** were evaluated for their antimicrobial activity against eight agricultural pathogenic fungi and eight human pathogenic bacteria (see Tables S1-S2). The antifungal assays indicated that compound **1** displayed moderate activity against *H. maydis*, *V. dahliae* Kleb

and *B. cinerea* Pers. with an MIC value of 50 μg/mL (positive control ketoconazole: 0.78125, 1.5625, and 0.78125 μg/ml, respectively), while compounds **2** and **3** exhibited significant activity against *H. maydis* and *A. alternata* with MIC values of 12.5–3.125 μg/mL (ketoconazole: 0.78125 μg/mL). In addition, compound **3** also showed significant activity against *V. dahliae* Kleb with an MIC value of 3.125 μg/mL (ketoconazole: 1.5625 μg/mL). The antibacterial assays indicated that compounds **1–3** showed weak activity against *M. lysodeikticus, B. paratyphosum* B and *P. aeruginosa* with an MIC value of 100 μg/mL (positive control ciprofloxacin: 0.78125 μg/mL).

In summary, three new triterpenoids (**1–3**) were isolated from the aerial parts of *A. capillus-veneris*. Compounds **1–3** belonged to the hopane-type skeleton. Compounds **2** and **3** exhibited remarkable antifungal activity.

Conflict of interest

Authors declare no conflict of interest.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fitote.2019.01.006.

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