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# Cycloartane Triterpenes from *Beesia calthaefolia* (Maxim.)

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ABSTRACT

Three new cycloartane triterpenoids (**1**–**3**) and two known compounds (**4, 5**) were isolated from the whole plant of *Beesia calthaefolia*. Their structures were elucidated by 1D and 2D NMR, HRESIMS and optical rotation spectral data. All isolates were investigated for their inhibitory effects on the classical pathway of the complement system. Among them, compound **4** showed stronger inhibitory activity ($IC_{50}$ 136.7 μM) than positive control (Rosmarinic acid, $IC_{50}$ 181.8 μM) while compounds **2** and **3** were moderately active with $IC_{50}$ value of 206 μM and 200.9 μM. Chemical compound studied in this article: Rosmarinic acid (PubChem CID: 5281792).



## 1. Introduction

*Beesia calthaefolia (Maxim.)* Ulbr. (Ranunculaceae) is widely distributed in the southwest and northwest of the People's Republic of China. As a well-known Chinese folk herb medicine, it possesses the functions of anti-inflammation, antipyretic, analgetic, detoxification agent and of invigorating blood circulation. Its rhizomes or the whole plant are used to treat colds, rheumatic arthritis, dysentery, sore throats, and headaches [1]. Previously, beesiosides have been isolated from *Beesia calthaefolia* [2–7]. Some beesiosides show immunosuppressive activity and can inhibit angiogenesis [8]. This study is part of an ongoing investigation for identifying compounds from herbal medicines active against the complement system. In our study, extraction and fractionation of the whole plant of *B. calthaefolia* resulted in the isolation of three novel cycloartane glycosides (**1–3**) and two known cycloartane glycosides (**4–5**) (Fig. 1). This paper describes their isolation, structural elucidation, and the evaluation of their inhibitory effects on the complement system.

## 2. Experimental

### 2.1. General

NMR spectra were measured in pyridine $d_5$ on an INOVA 600 spectrometer, using TMS as internal standard. HRESIMS spectra were recorded using Waters SYNATT and an ESI-Q-TOF mass spectrometer. Silica gel H (400–500 mesh, from Haiyang Chemical Group Co., Qingdao, Shandong Province, People's Republic of China) and RI-102 ODS-A-HG (YMC Co., Ltd.) were used. Compounds were finally isolated with the help of a Hanbon Sci.&Tech NP7000 preparative HPLC system equipped with a *Shodex* detector using an ODS column (YMC-ODS, 20 × 250 mm, 5 μm). TLC was carried out on silica gel GF254 (0.15–0.20 mm) (from Jiangyou silica gel Group Co., Yantai, Shandong Province, People's Republic of China) and the spots were visualized by spraying with 10% $H_2SO_4$ and heating.

### 2.2. Plant materials

The whole plant of *B. calthaefolia* was collected at Wu dang, Guiyang City, Guizhou Province, People's Republic of China in 2009 and identified by Dr. *Jianxin Zhang*, Institute of Medicinal Plant Development and Chinese Science Academy. The voucher specimen (collection no 196) is deposited in the Department of

*Abbreviations:* CP, Classical complement pathway; ELSD, Evaporative light scattering detector.

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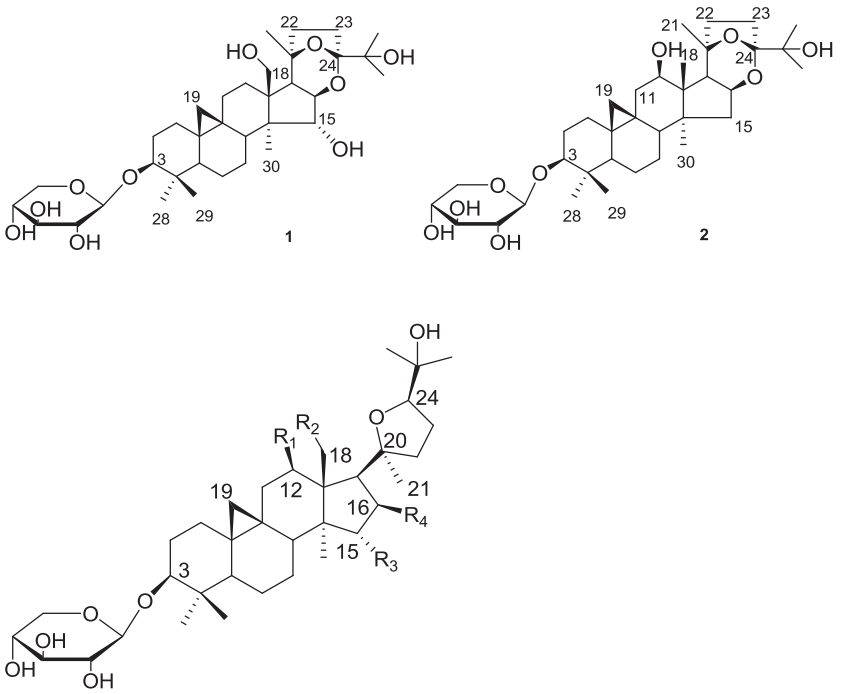


| | $R_1$ | $R_2$ | $R_3$ | $R_4$ |
|---|---|---|---|---|
| **3** | OH | OH | OH | OH |
| **4** | OH | H | H | OAc |
| **5** | H | OH | OH | OH |

**Fig. 1.** The structures of compounds **1**–**5**.

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### 2.3. Extraction and isolation

The air-dried and pulverized whole plant of *B. calthaefolia* (6.8 kg) was extracted two times with 95% EtOH for 2 h under reflux (80 L × 2) and then extracted with 50% EtOH for 2 h under reflux (80 L × 2). After removal of solvent, the residue (1.0 kg) obtained by 50% EtOH was suspended in water (1000 mL) and partitioned successively with petroleum ether (1000 mL × 6), $CHCl_3$ (1000 mL × 6), EtOAc (1000 mL × 6) and n-BuOH (1000 mL × 6). The $CHCl_3$-soluble fraction (170 g) was subjected to low-pressure column chromatography on silica gel (4 kg, 200–300 10 × 110 cm). Gradient elution with petroleum ether-EtOAc = 1:4 (10 L × 3 times), ether-EtOAc = 1:6 (10 L × 3 times), petroleum ether-EtOAc = 1:9 (10 L × 3 times), EtOAc (10 L × 3 times), EtOAc-MeOH = 20:1 (10 L × 3 times), EtOAc-MeOH = 15:1 (10 L × 3 times), EtOAc-MeOH = 10:1 (10 L × 3 times) and MeOH (30 L) gave nine fractions, A (8 g), B (9 g), C (10 g), D (12 g), E (10 g), F (15 g), G (12 g), H (15 g) and I (9 g). Fraction F was fractionated by LPLC over Silica gel H (450 g, 4 × 100 cm) to give six smaller fractions, eluting with $CHCl_3$–MeOH (40:1–8:1). The fifth was further separated by ODS column chromatography [MeOH:$H_2O$(65:35 → 68:32 → 70:30 → 75:25 → 80:20)] to afford **4** (105 mg, $t_R$ = 21.4 min, 75% MeOH) and by preparative HPLC at a flow rate of 15.0 mL/min to afford **1** (45 mg, $t_R$ = 6.8 min, 75% MeOH).The fraction D further separated by Silica gel (400 g, 4 × 80 cm) eluting with $CHCl_3$–MeOH (40:1–8:1) and preparative HPLC at a flow rate of 15.0 mL/min to afford **2** (300 mg, $t_R$ = 19.8 min, 75%MeOH). Fraction H was isolated by LPLC over Silica gel H (450 g, 4 × 100 cm), eluting with $CHCl_3$–MeOH (40:1–9:1) to give four small fractions. The fourth fraction was further separated by preparative HPLC at a flow rate of 15.0 mL/min to afford **3** (36.7 mg, $t_R$ = 4.5 min, 75% MeOH) and **5** (149 mg, $t_R$ = 11.5 min, 75% MeOH). The purity of all compounds was assessed by HPLC as more than 95%.

Compound **1**: White amorphous powder; $[\alpha]^{20}_D$ + 7.7 ($c$ = 0.20, MeOH); HR-ESI-MS $m/z$ 637.3962 ($[M + H]^+$, $C_{35}H_{57}O_{10}^+$; calc. 637.3946). $^1$H-NMR ($C_5D_5N$, 600 MHz) and $^{13}$C-NMR($C_5D_5N$,150 MHz) are given in Table 1.

Compound **2**: White amorphous powder; $[\alpha]^{20}_D$ + 2.5 ($c$ = 0.15, MeOH); HR-ESI-MS $m/z$ 621.3995 ($[M + H]^+$, $C_{35}H_{57}O_9^+$; calc. 621.3997). $^1$H-NMR ($C_5D_5N$, 600 MHz) and $^{13}$C-NMR ($C_5D_5N$, 150 MHz) are given in Table 1.

Compound **3**: White amorphous powder; $[\alpha]^{20}_D$ + 20.0 ($c$ = 0.16, MeOH); HR-ESI-MS $m/z$ 655.4039 ($[M + H]^+$, $C_{35}H_{59}O_{11}^+$; calc. 655.4052). $^1$H-NMR ($C_5D_5N$, 600 MHz) and $^{13}$C-NMR ($C_5D_5N$, 150 MHz) are given in Table 1.