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One new cycloartane triterpene glycoside from *Beesia calthaefolia*

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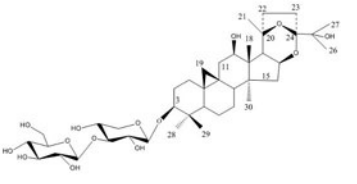

One new cycloartane triterpene glycoside (**1**) was isolated from the whole plant of *Beesia calthaefolia*. Its structure was elucidated on the basis of extensive spectroscopic data analysis. Its inhibitory effect was measured by the classical pathway of the complement system, and compared with those of known related cycloartane glycosides **2** and **3**, previously isolated by us from the same plant. Compounds **1** and **2** exhibited inhibitory activity of complement system with IC_{50} of 395.3 and 214 μM, respectively. The results suggested that OH at C-12, C-18 and C-15 along with the polarity could affect the inhibitory activity.

Keywords: cycloartane triterpene glycoside; *Beesia calthaefolia*; complement system; classical pathway

1. Introduction

Beesia calthaefolia (Maxim.) Ulbr. (Ranunculaceae) is an endemic plant of China and widely grown in southwest and northwest of China. As a herbal medicine of Chinese folk, it possesses anti-inflammatory, analgesic and detoxification functions, relieves fever and can invigorate blood circulation. Its rhizomes or the whole plant have been used to treat colds, rheumatic arthritis, dysentery, sore throats and headaches (Agendae Academiae Sinicae Edita 1979). Previous chemical investigations on this plant revealed the presence of beesiosides I-IV (Sakurai et al. 1986, 1990, 1993; Inoue et al. 1985), beesiosides A-H and beesiosides J-P (Ju, Liu, Lin, Xu, et al. 2002) (all the compounds named as beesiosides are cycloartane triterpene glycosides). A number of cycloartane triterpene glycosides have been reported to show immunomodulatory effects, including anticomplement activity (Ju, Liu, Lin, Zhang, et al. 2002; Lee et al. 2012). Five cycloartane triterpene glycosides were isolated from the whole plant, including two known compounds (beesiosides E and F, already cited in the text), as well as three new compounds, including compounds named herein **2** and **3**, which exhibit anticomplement activity in classical

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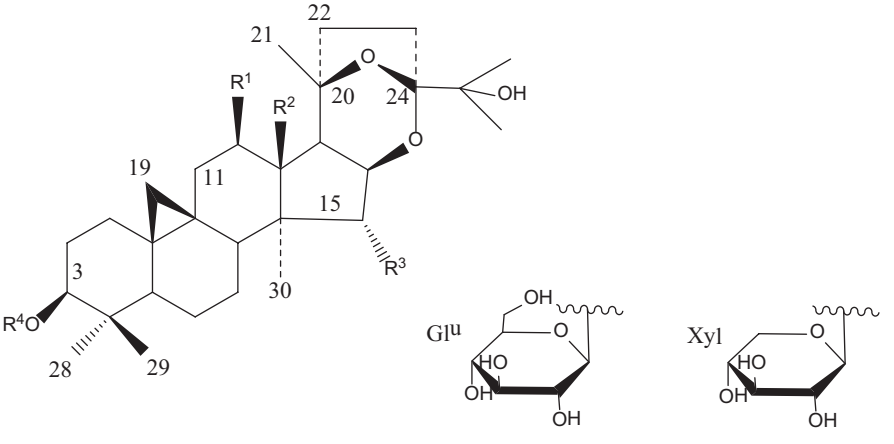
complement pathway (Mu et al. 2014). Subsequent fraction of the n-BuOH fraction yielded a new cycloartane-type saponin (compound **1**). As part of our ongoing investigation on new cycloartane triterpene glycoside from natural source, the chemical studies led to the isolation of one new saponin (**1**). In our previous study, compound **2** and **3** exhibited inhibitory activities of complement system with IC_{50} of 206.0 μM and over 500 μM (Mu et al. 2014). Compounds **1**–**3** have similar structure. In order to compare the activities of **1** with **2** and **3**, ensure data accuracy and dependability, we re-measured the anticomplement activities of compounds **2** and **3**. So we describe herein the isolation and structural elucidation of **1**, as well as the anti-complement activity in classical complement pathway of compounds **1**–**3** (Figure 1).

2. Results and discussion

2.1. *Structure of compound 1*

Compound **1** was obtained as white amorphous powder and the HR-ESI-MS showed an ion peak at *m/z* 805.4353 $[M + Na]^+$, indicating a molecular formula of $C_{41}H_{66}O_{14}$. The ^{1}H-NMR spectrum (Table S1) showed the presence of cyclopropane methylene groups at δ 0.36 (1H, d, $J = 4.2$ Hz) and 0.60 (1H, d, $J = 4.2$ Hz), seven tertiary methyls (δ 0.88, 1.04, 1.69, 1.70, 1.79, 1.83 and 1.95), indicating that **1** might be a cycloartane-type triterpenoid (Mu et al. 2014). The ^{13}C NMR spectrum of **1** displayed 41 carbon signals, three signals attributable to oxygen-bearing quaternary carbons at δ 71.9, 82.8 and 110.3. The singlet at δ 71.9 was assigned to C-25 by comparison with that of beesioside O (Ju, Lin, Yang and Lu, 2002). The oxygen-bearing quaternary carbon at δ 82.8 (C-20), the acetal carbon at δ 110.3 (C-24) and the methine carbon at



	R^1	R^2	R^3	R^4
1	OH	CH_3	H	$^{-}$Xyl—^{3}Glu
2	OH	CH_3	H	$^{-}$Xyl
3	H	CH_2OH	OH	$^{-}$Xyl

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

δ 73.0 (C-16) suggested the presence of a (20*R*, 24*R* or 20*S*, 24*S*)-16β, 24 and 20, 24 diepoxy structure in **1**, by comparison with the ^{13}C NMR spectrum of beesioside IV (Sakurai et al. 1990). The ^{1}H and ^{13}C spectra of **1** are similar to the known compound **2** (Mu et al. 2014). In the ROESY spectrum (Figure S1), the β configuration of the C-12 hydroxyl group was confirmed by the correlation between H-12 and H_3-30. The α configurations of H-16 and H-17 were confirmed by the ROESY correlations between δ_H 1.83 (H-17) and δ_H 0.88 (H_3-30), δ_H 4.60 (H-16) and δ_H 1.83 (H-17). The correlations between H_3-21/H-17, H_3-21/H-22, H-22/H-23, H-16/H-23, H-16/H_3-30 and H-23/H_3-27 in the ROESY spectrum confirmed the 20*S**, 24*S**configuration. Further comparison of the ^{1}H and ^{13}C NMR data assignable to the sugar chain between **1** (Table S1) and **2** suggested one more set of terminal D-glucopyranose moiety signals in **1**. From the coupling constant value of the anomeric signal δ_H 5.33 (1H, d, $J = 7.8$ Hz), the D-glucosyl was deduced to be of β-configuration. The β-D-xylopyranose unit was shown to be attached at C-3 by the observation of a long range cross-peak between H-1′ and C-3 (δ 88.4) in the HMBC spectrum. The attachment point of the xylose residue was established by analysis of HMBC and ROESY spectra. HMBC correlation was observed between δ 99.4 (Glc-C-1) and δ_H 4.14 (Xyl-H-3). ROESY correlation was observed between δ_H 5.33 (Glc-H-1) and δ_H 4.14 (Xyl-H-3). Accordingly, the structure of compound **1** was established as (20*S**, 24*S**)-16β, 24; 20, 24-diepoxy-9, 19-cycloanostane-3β, 12β, 25-triol-3-*O*-[β-D-gluc-opyranosyl-(1→3)]-β-D-xylo-pyranoside.

2.2. *Measuring the anticomplement activity in classical complement pathway*

Cycloartane glycosides **1**–**3** were evaluated for anticomplement activity by measuring their inhibitory activity on the classical complement pathway (Oh et al. 2000). The results (IC_{50} values) are summarised in Table 1. Compounds **1** and **2** exhibited inhibitory activities of complement system with IC_{50} of 395.3 and 214 μM, respectively. On the other hand, compound **3** was inactive. The structures of **1** and **2** are similar, **1** showed less anticomplement activities than **2** suggesting that polarity affects the inhibitory activity. The structures of **2** and **3** are also similar, **2** showed better anticomplement activities than **3** against the classical complement pathway suggesting that OH at C-12, C-18 and C-15 affect the inhibitory activity.

3. Experimental

3.1. *General experimental procedures*

NMR spectrum was measured in pyridine d_5 on an INOVA 600 spectrometer (Varian Associates, Inc., NMR Instruments, Palo Alto, CA, USA), using TMS as internal standard. IR spectrum was obtained on a Nicolet 6700 FT-IR (by a KBr disk method) spectrometer (Thermo-Fisher, Wultham, MA, USA). HR-ESI-MS spectrum was recorded using Waters SYNATT and an ESI-Q-TOF mass spectrometer. Silica gel (200-300 mesh) (from Haiyang Chemical Group Co., Qingdao, Shandong Province, People's Republic of China) and ODS-A-HG (YMC Co., Ltd) were used.

Table 1. Inhibitory effects of the isolated compounds **1**–**3** on the complement system.

Compounds	IC_{50} (μM)
1	395.3 ± 8.5
2	214.0 ± 6.9[b]
3	≥500.0[b]
Rosmarinic acid[a]	178.0 ± 15.6

[a] This compound was used as a positive control.
[b] The reported IC_{50} values for **2** and **3** were 206.0 and ≥500 μM, respectively (Mu et al. 2014).

Compounds were finally isolated with the help of a medium pressure liquid chromatography (MPLC) (BUCHI-ODS 3.6 cm × 45 cm, 10 μm). Thin-layer chromatography 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 visualised by spraying with 10% H_2SO_4 and heating.

3.2. *Plant material*

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. Voucher specimen (NO. 2009010) has been deposited in the department of clinical pharmacology, General Hospital of People's Liberation Army, Beijing.

3.3. *Extraction and isolation*

The air-dried and pulverised whole plant of *B. calthaefolia* (6.9 kg) was extracted two times with 95% EtOH for 2 h under reflux and then extracted two times with 50% EtOH for 2 h under reflux. 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 mL), $CHCl_3$ (1000 mL × 6 mL), EtOAc (1000 mL × 6 mL) and n-BuOH (1000 mL × 6 mL). The *n*-BuOH-soluble fraction (106 g) was subjected to low-pressure column chromatography (CC) on Si gel (200–300 mesh). Gradient elution with petroleum $CHCl_3$–MeOH (15:1, 10:1) and $CHCl_3$–MeOH–H_2O (8:2:0.2, 7:3:0.5) gave six fractions, A (13.0 g), B (10.0 g), C (10.4 g), D (9.9 g), E (10.4 g) and F (9.0 g). Fraction A was isolated by LPLC over Si gel (200–300 mesh, 400 g, 4 cm × 100 cm) to give seven fractions, eluting with $CHCl_3$–MeOH (12:1–4:1) and $CHCl_3$–MeOH–H_2O (8:2:0.2). The third was separated by MPLC (MeOH: H_2O = 10:90–70:30) at a flow rate of 15.0 mL/min (every 14 min to collect a fraction) to afford **1** (168 mg). Compounds **2** and **3** were isolated from the $CHCl_3$-soluble fraction by silica gel and ODS CC as previously reported (Li et al. 2013).

Compound **1**: White amorphous powder; −7.1 (c = 0.29, MeOH); IR (KBr) ν_{max}: 3412, 2940, 1638, 1384, 1164 and 1052 cm^{-1}; HR-ESI-MS m/z 805.4353 ($[M + Na]^+$, $C_{41}H_{66}$; calc. 805.4350). ^{1}H NMR (C_5D_5N, 600 MHz, δ): 3.48 (1H, dd, J = 11.4, 4.2 Hz, H-3), 3.94 (1H, m, H-12), 4.60 (1H, m, H-16), 1.83 (1H, d, J = 6.0 Hz, H-17), 1.79 (3H, s, H-18), 0.36 (1H, d, J = 4.2 Hz, Ha-19), 060 (1H, d, J = 4.2 Hz, Hb-19), 1.95 (3H, s, H-21), 1.70 (3H, s, H-26), 1.69 (3H, s, H-27), 1.33 (3H, s, H-28), 1.04 (3H, s, H-29), 0.88 (3H, s, H-30), 4.85 (1H, d, J = 7.8 Hz, H-1′), 4.05 (1H, m, H-2′), 4.14 (1H, t, J = 9.0 Hz, H-3′), 4.22 (1H, m, H-4′), 3.72 (1H, t, J = 10.8 Hz, Ha-5′), 4.34 (1H, dd, J = 11.4, 5.4 Hz, Hb-5′), 5.33 (1H, d, J = 7.8 Hz, H-1″), 4.07 (1H, m, H-2″), 4.21 (1H, m, H-3″), 4.23 (1H, m, H-4′), 3.97 (1H, m, H-5′), 4.38 (1H, m, Ha-6′), 4.49 (1H, m, Hb-6′). ^{13}C NMR (C_5D_5N, 150 MHz, δ): 32.2 (C-1), 30.0 (C-2), 88.4 (C-3), 41.3 (C-4), 47.5 (C-5), 20.9 (C-6), 26.1 (C-7), 46.3 (C-8), 20.2(C-9), 26.0 (C-10), 29.4 (C-11), 72.3 (C-12), 49.1 (C-13), 51.3 (C-14), 45.5 (C-15), 73.0 (C-16), 54.6 (C-17), 14.7 (C-18), 29.8 (C-19), 82.8 (C-20), 40.6 (C-22), 40.9 (C-23), 110.3 (C-24), 71.9 (C-25), 26.9, 23.1, 23.4, 25.8, 15.4, 20.9 (C-21, 26, 27, 28, 29, 30). 107.6 (C-1′), 75.6 (C-2′), 79.7 (C-3′), 71.3 (C-4′), 67.1 (C-5′), 99.4 (C-1″), 78.6 (C-2″), 78.9 (C-3″), 75.5 (C-4″), 78.1 (C-5″), 63.0 (C-6″).

3.4. *Acid hydrolysis of compound 1 and determination of absolute configuration of monosaccharides*

Compound **1** (5 mg) was heated in 2 M trifluoroacetic acid (5 mL) at 95°C for 5 h. The reaction mixture was extracted with $CHCl_3$ (5 mL × 3 mL). The remaining aqueous layer was

concentrated to dryness with EtOH to give a residue. After that, the residue was dissolved in pyridine (2 mL), to which of 3 mg of **L**-cysteine methyl ester hydrochloride was added. The mixture was kept at 60°C for 1 h. After the reaction, the residue was added with trimethylchlorosilane (0.5 mL) and hexamethyldisilane (1 mL) at 60°C for 0.5 h. Finally, the supernatant (0.5 mL) was analysed by GC-MS (GC-MS-QP 2012, Shimadzu Corporation, Japan) under the following conditions: capillary column, Rtx-5MS (30 m × 0.25 mm × 0.25 μm), temperature gradient: 60°C–300°C, rate, 20°C/min; carrier, helium gas (1.0 mL/min); and injection volume: 1.0 μL. The presence of D-xylose and D-glucose in the acid hydrolysate of **1** was confirmed by comparison of their respective retention times with those of standard samples. The retention times (t_R, min) of monosaccharide derivatives were as follows: D-xylose (9.18, 9.38 min) and D-glucose (10.24, 10.64 min).

3.5 *Anti-complement bioassays*

The anti-complement assay used in this study measures inhibition of sheep red blood cell hemolysis following induction of the complement binding reaction (Kabat & Mayer 1961; Klerx et al. 1983). This assay was carried out according to the method of Kabat and Mayer and Klerx et al. with modifications. The rate of hemolysis was measured spectrophotometrically and compared to the untreated control. SRBC (two percent sensitized sheep erythrocytes) were used for the complement binding reaction of the classical pathway. A solution of complement serum (100 μL) came from a healthy guinea pig (female). Each sample was dissolved in DMSO, which was also used as the negative control. The sample (25 μL) was mixed with BBS (barbitol buffer solution, 175 μL), serum (100 μL) and SRBC (200 μL) together. The mixture was cultured at 37°C for 30 min in a water bath. And then, the mixture was centrifuged (4°C, 5000 rpm, 20 min). Last the optical density of the supernatant (200 μL) was measured at 405 nm (Xu et al. 2007). The anticomplement activity was determined in triplicate measurements and was expressed as the concentration inhibiting 50% of the complement dependent hemolysis in the control (IC_{50} value) (Oh et al. 2000). Purity of the isolated compounds used in the assay was above 95% as determined by analytical HPLC/ELSD.

4. Conclusion

Compound **1** was a new cycloartane triterpene glycoside, which was isolated from the whole plant of *B. calthaefolia*. Its structure was elucidated on the basis of 1D-NMR, 2D-NMR, HR-ESI-MS, IR, GC-MS and optical rotation spectral data. Compounds **1**–**3** were evaluated for their inhibitory effects on the complement system by the classical pathway.

Supplementary material

Experimental details relating to this article are available online http://dx.doi.org/10.1080/14786419.2015.1058791, alongside Table S1 and Figures S1–S9.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Note

1. This author contributed equally to this work.

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