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Two new triterpenoid-chromone hybrids from the rhizomes of *Actaea cimicifuga* L. (syn. *Cimicifuga foetida* L.) and their cytotoxic activities

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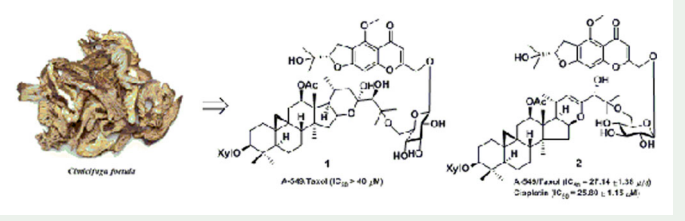
ABSTRACT

Two new triterpenoid-chromone hybrids, cimitriteromones H (**1**) and I (**2**), along with two known analogues (**3**, **4**) were isolated from the phytochemical research on the *n*-butyl alcohol extracts of *Actaea cimicifuga* rhizomes. The new compounds were elucidated by spectroscopic experiments and chemical method. The cytotoxic activities of the isolated compounds were tested on A-549/Taxol cell line. Cimitriteromone I (**2**) showed cytotoxicity with IC_{50} value of $27.14 \pm 1.38\ \mu M$ comparable to positive control group cisplatin (IC_{50} value of $25.80 \pm 1.15\ \mu M$).



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Actaea cimicifuga; Triterpenoid-chromone hybrid; Cytotoxicity

1. Introduction

Plants of the genus *Actaea* comprise about 18 species of perennial herbs distributed among the temperate zone of the Northern Hemisphere (Editorial Committee of Flora of China CAS 1979). Phytochemical and biological studies over the past several decades revealed that triterpenoid glycosides, chromones and cinnamic acid derivatives were the major constituents of this genus (Li and Yu 2006). Those of them were thought to be the major bioactive constituents of functional foods (Agretti et al. 2018) and alternative medicines (Qiu et al. 2012; Sun et al. 2017), including anti-proliferation

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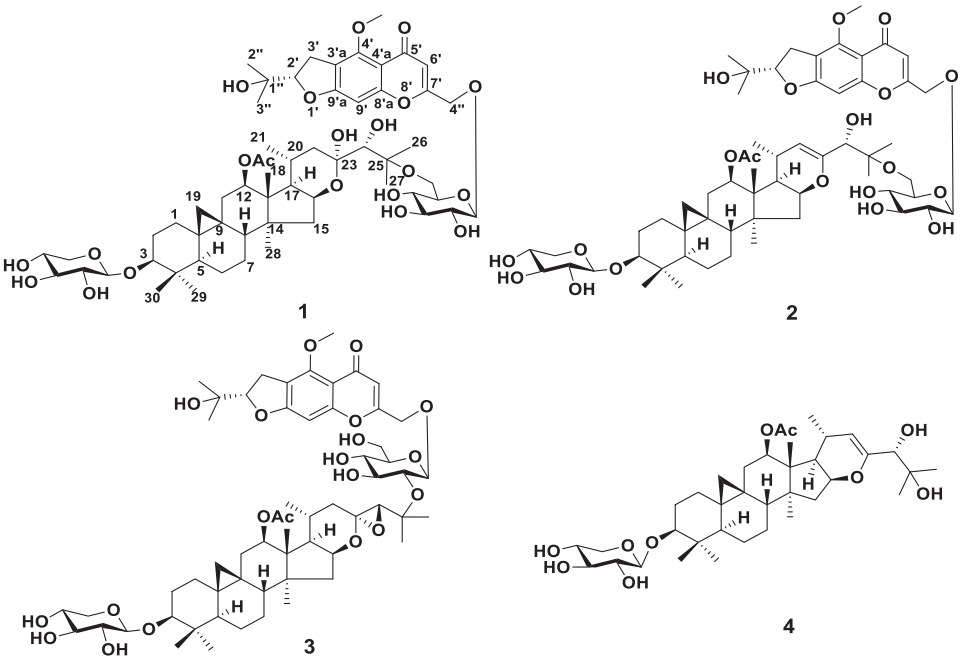

Figure 1. Chemical structures of compounds **1**—**4**.

(Nian et al. 2010; Ahn et al. 2013), anti-inflammatory (Thao, Kim, et al. 2017; Thao, Luyen, et al. 2017), anti-osteoporotic (Li et al. 2007; Qiu et al. 2007; Kolios et al. 2010; Ahn et al. 2012), anti-angiogenic (Nian et al. 2015) and relieving the symptoms of the menopause (Mahady et al. 2002, 2010; Wuttke et al. 2003; He et al. 2006; Byron 2007). In our previous work, LC-UV/MS guided separation method was used to analyse the extraction of *Actaea cimicifuga* and seven undescribed hybrid dimers of cycloartane triterpenoid and chromone fused via a glucose moiety, cimitriteromones A – G were isolated from the ethyl acetate extracts (Shi et al. 2018). And, cimitriteromones C and E showed potential anti-proliferative activities against taxol-resistant human lung cancer A-549/taxol with IC_{50} values of 15.73 and 24.21 μM, respectively. So, further phytochemical investigation on the *n*-butyl alcohol extracts of *A. cimicifuga* led to the isolation of three more hybrid dimers of cycloartane triterpenoid and chromone, including two new ones (Figure 1). Herein, we report the structure determination by 1D/2D NMR analyses and cytotoxic activities against A-549/Taxol of the isolated compounds.

2. Results and discussion

Cimitriteromone H (**1**) was obtained to be a white, amorphous powder, and its molecular formula was $C_{59}H_{86}O_{21}$ determined by HRESIMS ion signal at *m/z* 1129.5588 $[M-H]^-$ (calcd for 1129.5589) with 17 degrees of unsaturation. Clearly, the ^{1}H NMR and ^{13}C-DEPT spectra of **1** displayed the characteristic signals of 9,19-cycloartane-type triterpene moiety and chromone moiety (Shi et al. 2018). Direct

comparison of its NMR data (Tables 1S and Tables 2S, Supplementary material) with compound **3** (Shi et al. 2018) suggested compound **1** resemble **3** except for the signals for the side-chain and glucose moiety change a lot. The acid hydrolysis and GC analysis experiments suggested that the D-glucose and D-xylose units occurred in **1**. The ^{1}H-^{1}H COSY associations of δ_H 5.94 (OH-24, 1H, d, $J = 9.7$ Hz) with δ_H 3.75 (H-24, 1H, m), and the HMBC correlations of δ_H 6.27 (OH-23, 1H, s) to δ_C 41.9 (C-22), δ_C 101.9 (C-23) and δ_C 77.7 (C-24) indicated that the epoxy bond between C-23 and C-24 was substituted with two hydroxyl groups. This elucidation was also supported by 18 atomic units (H_2O) more of the molecular weight in compound **1**. The attachment of the two moieties could be observed by the HMBC correlation between proton signal at δ_H 4.40 (glu-6b, 1H, m) and quaternary carbon at δ_C 81.3 (C-25). Thus, the planar structure of **1** was assigned and further verified by a comprehensive analysis of the HSQC, ^{1}H-^{1}H COSY and HMBC spectra (Figure 1S, Supplementary material).

The significant ROESY associations of H-3/H-5, H-12/H-17/CH_3-28, H-16/H-17/CH_3-28 suggested the β-orientation of the substituents at C-3, C-12, C-16. The α-oriented hydroxyl group at C-23 was determined by the ROESY association of OH-23 (δ_H 6.27, 1H, s) with the H-16 (δ_H 4.77, 1H, m) (Figure 1S, Supplementary material). In addition, the stereo structure of C-24 was proposed as '*R*' configuration on the basis of the cleavage pattern of the epoxy ring catalysed by the epoxide hydrolases (Archer 1997) (Figure 3S, Supplementary material). Accordingly, the structure of **1** was characterised as shown (Figure 1).

Cimitriteromone I (**2**) was assigned to the molecular formula $C_{59}H_{84}O_{20}$ with 18 degrees of unsaturation, as established by HRESIMS (*m/z* 1111.5486 $[M-H]^-$, calcd for 1111.5483). Based on the ^{1}H, ^{13}C-DEPT, ^{1}H-^{1}H COSY, HSQC, HMBC NMR data, the integral structure of **2** was closely related to cimifoetidanoside G (**4**) (the configuration of C-24 was not confirmed) (Chen et al. 2014) and (16*S*,20*S*,24*R*)-12β-acetoxy-16,23-epoxy-24,25-dihydroxy-3β-(β-*D*-xylopyranosyloxy)-9,19-cyclolanost-22(23)-ene (**4**) (the configuration of C-24 was confirmed as *R* based on reduced density gradient calculation) (Fang et al. 2019) with an additional chromone moiety. The HMBC correlation of glu-6b (δ_H 4.36, 1H) with C-25 (δ_C 78.2) indicated the connection of the two moieties (Figure 2S, Supplementary material). The relative configuration of compound **2** was determined as 3β, 12β, 16β, 24*R* on the basis of ROESY experiment (Figure 2S, Supplementary material) and comprehensive comparison of the chemical shifts of compounds **2**, **4**, and cimifoetidanoside G with that of (16*S*,20*S*,24*R*)-12β-acetoxy-16,23-epoxy-24,25-dihydroxy-3β-(β-*D*-xylopyranosyloxy)-9,19-cyclolanost-22(23)-ene (Tables 3S).

The chromone glycoside moiety of compounds **1** and **2** was identical to that of *prim-O*-glucosylcimifugin (Sasaki et al. 1982), as evidenced by comparison of the 1 D NMR data, and further established on the basis of 2D NMR correlations (^{1}H-^{1}H COSY, HMBC, and HSQC) (Figures 1S and 2S, Supplementary material). The isolated compounds were evaluated against taxol-resistant human lung cancer A-549/taxol for their cytotoxicities, using the MTS methods with cisplatin and taxol as positive controls. Compound **2** showed anti-proliferative activity with IC_{50} value of 27.14 ± 1.38 μM comparable to the positive control cisplatin (IC_{50} 25.80 ± 1.15 μM).

3. Experimental

3.1. General experimental procedures

All of the 1D and 2D NMR spectra were obtained on Bruker Ascend^{TM} 600 and 800 MHz spectrometers in pyridine-d_5 with TMS as an internal standard. HRESIMS data were measured on a Waters API QSTAR Pulsar spectrometer. Optical rotation was obtained in methanol with a JASCO P-1020 digital polarimeter. UV spectra were measured using a Shimadzu UV-2401PC spectrometer. A Bruker Tensor-27 Fourier transformed infrared spectrometer was used to scan IR spectra with KBr pellets. Column chromatography (CC) was performed on Silica gel (200 – 300 mesh, Qingdao Marine Chemical, Ltd.), Lichroprep RP-18 (40 – 75 μm, Merck), and Sephadex LH-20 (20 – 150 μm, Pharmacia). Semi-preparative high performance liquid chromatography was performed on an Agilent 1260 liquid chromatography system equipped with an SB-PHENYL column (5 μm, 9.4 mm × 250 mm, 3 mL/min) and a DAD detector.

3.2. Plant material

Rhizomes of *Actaea cimicifuga L.* (syn. *Cimicifuga foetida* L.) were collected from Lijiang County, Yunnan Province, China, in August 2014. The material was identified by Prof. Shengji Pei, Kunming Institute of Botany, Chinese Academy of Science. A Voucher specimen (KUN No. 20140828) has been deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Kunming, China.

3.3. Extraction and isolation

The air-dried rhizomes of *A.cimicifuga L.* (15 kg) were milled and extracted with 70% aqueous methanol (3 × 50 L, 2 hours each) at 60 °C. The solvent was evaporated in vacuo to afford a crude extracts (1 kg), which was suspended in H_2O, and then extracted successively with EtOAc and *n*-BuOH. The *n*-BuOH extracts (50 g) were fractionated by RP-18 (MeOH/H_2O 50:50, 60:40, 70:30, 80:20, 90:10, 100:0) to yield five fractions, Fr.A – E. Fr.C (MeOH/H_2O 70:30, 5 g) was further fragmented by a silica gel column ($CHCl_3$/MeOH/H_2O 9:1:0.1, 8:2:0.2, 7:3:0.5 and 6:4:1) and yield fractions C1 – C4. Fraction C2 (2 g) was separated by RP-18 with a gradient elution of MeOH/H_2O (60:40 to 80:20) to give five sub-fractions, Fr.C2.1 – C2.5. Fraction C2.2 (500 mg) was purified by Sephadex LH-20 (MeOH), followed by semi-preparative HPLC (3 mL/min, detector UV λmax 210, 254, 285 and 360 nm, MeCN/H_2O 37:63) to afford **3** (2 mg with purity over 95%, 15.7 min). Fraction C3 (1.3 g) was fragmented by Sephadex LH-20 to give two parts (Fr.C3.1 and C3.2), Fr.C3.2 was further subjected on a silica gel column ($CHCl_3$/MeOH/H_2O 8.5:1.5:0.15) to obtain fractions C3.2.1 to C3.2.3. Fraction C3.2.1 was further purified by HPLC (3 mL/min, detector UV λmax 210, 254, 285 and 360 nm, MeCN/H_2O 60:40)to obtain compound **4** (17.7 mg with purity over 95%, 15.3 min). Compounds **1** (7.7 mg with purity over 95%, 27.3 min) and **2** (3.3 mg with purity over 95%, 30.5 min) were yield from Fraction C3.2.2 using semi-preparative HPLC (3 mL/min, detector UV λmax 210, 254, 285 and 360 nm, MeCN/H_2O 40:60).

3.3.1. Cimitriteromone H (1)

White, amorphous powder; $[\alpha] + 32.5$ (*c* 0.38, MeOH); UV (MeOH) λmax (log ε) 215.0 (0.65), 243.5 (0.49), 294.0 (0.31); IR (KBr) ν_{max} 3429, 2966, 2935, 1717, 1656, 1629, 1469, 1383, 1075, 1047, 565 cm^{-1}; ^{1}H and ^{13}C NMR data (pyridine-d_5) (see Supplementary material Tables 1S and Tables 2S); HRESIMS *m/z* $[M-H]^-$ calcd for $C_{59}H_{85}O_{21}$ 1129.5589, found at 1129.5588.

3.3.2. Cimiriteromone I (2)

White, amorphous powder; $[\alpha] - 81.1$ (*c* 0.10, MeOH); UV (MeOH) λmax (log ε) 211.0 (0.54), 241.5 (0.30), 293.0 (0.18); IR (KBr) ν_{max} 3440, 2966, 2934, 1725, 1656, 1630, 1468, 1384, 1077, 1046, 566 cm^{-1}; ^{1}H and ^{13}C NMR data (pyridine-d_5) (see Tables 1S and Tables 2S, Supplementary material); HRESIMS *m/z* $[M-H]^-$ calcd for $C_{59}H_{83}O_{20}$ 1111.5483, found at 1111.5486.

3.4. Cytotoxicity bioassay

Drug-resistant human lung cancer A-549/Taxol was used in the cytotoxic assay. The cell viability was assessed by MTS method in 96-well micro plates (Monks et al. 1991). 100 μL of adherent cells was seeded in 96-well plate and allowed to be adhere for 12 h before test compounds addition. The tumor cell line was exposed to the tested compounds at various concentrations of 0.064, 0.32, 1.6, 8, and 40 μM in triplicate for 48 h, with cisplatin (Sigma) as positive control. After the incubation, cell viability was detected and a cell growth curve was graphed. IC_{50} values were calculated by Reed and Muench's method (Reed and Muench 1938).

3.5. Hydrolysis and identification of the sugar moieties in compound 1

The hydrolysis experiment was conducted as we described previously (Shi et al. 2018). Briefly, compound **1** (2.5 mg) was hydrolysed with 1.0 N HCl (2 mL) at 90 °C for 2 h. After neutralising with 1.0 N NaOH, the reaction mixture was extracted by $CHCl_3$, and the aqueous layer was evaporated under reduced pressure to give the residue of monosaccharide. The residue was dissolved in pyridine (150 μl) containing (L)-cysteine methyl ester hydro chloride (1.5 mg), and the mixture was heated at 80 °C for 1.5 h. Then, the trimethylsilylation reagents HMDS (100 µL) and TMCS (50 µL) were added successively, and the mixture was heated at 60 °C for 30 min. The reaction product was extracted by *n*-hexane (1 mL), and the upper layer was subjected to gas chromatography (GC) analysis under the following conditions: carrier gas, N_2; constant flow 1.2 mL/min; The GC oven was set at 120 °C to 270 °C, 8 °C/min. Transfer line temperature: 200 °C; injector temperature, 250 °C; injector volume, 1 µL; and split ratio, 1/30. The sugar moieties of compound **1** corresponding to those of D-xylose (t_R 6.45, 7.31 min) and D-glucose (t_R 11.28, 12.46 min).

4. Conclusions

Further phytochemical investigation on the rhizomes of *Actaea cimicifuga* led to the acquisition of three triterpenoid-chromone hybrids, including two new ones. The new compound **2** showed anti-proliferative activity against taxol-resistant human lung cancer A-549/Taxol with IC_{50} value of 27.14 μM comparable to positive control group cisplatin (IC_{50} value of 25.80 μM).

Disclosure statement

No potential conflict of interest was reported by the authors.

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