New Cytotoxic Cycloartane Triterpenes from the Aerial Parts of Actaea heracleifolia (syn. Cimicifuga heracleifolia)

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Key words
Actaea heracleifolia, Ranunculaceae, 15,16-seco-cycloartane triterpene, cycloartane triterpene glycosides, cytotoxicity, NMR

Introduction
The Actaea species, belonging to the family Ranunculaceae, has a long history of uses as a medicinal herb worldwide. In China, the rhizomes of Actaea heracleifolia Kom., Actaea dahurica (Turcz.) Maxim., and Actaea foetida L., officially listed in the Chinese Pharmacopoeia with the name “shengma”, are used as cooling and detoxifying agents for the treatment of headache, sore throat, and toothache [1]. In Europe and the United States, Actaea racemosa (L.) Nutt., commonly called black cohosh, is also used as a dietary supplement for women’s health during climacteric periods [2, 3]. Triterpene glycosides have been considered the main active components of Actaea species, and phytochemical investigations of plant species led to the isolation of a series of 9,19-cycloartane triterpenes characterized with unique structural features [4], including ring A opened cycloartane triterpenes, such as 3,4-seco-4-hydroxy-3-cimigenolate [5]; ring A expanded cimigenol-type triterpenes, such as cimiheraclein A [6]; 15,16-seco-cycloartane triterpenes [5–10]; ring B opened triterpenes [6, 11, 12]; and cycloartane triterpenes with new skeletons, such as cimicifugadine [13], cimicifine B [14], and cimyunnins A–D [15]. In a further investigation of the aerial parts of Actaea species, we found four new cycloartane triterpenes (1–4) and five known ones: cimiheraclein D (5) [6], 25-anhydrocimigenol-3-O-α-L-arabinopyranoside (6) [16], cimacarioside A (7) [17], 2α-hydroxyursolic acid (8) [18], and 1α,3β,19α,23-tetrahydroxys-12-en-28-oic acid-28-O-β-D-xylopyranoside (9) [19] (Fig. 1). Compound 1 was a D ring-cleaved 15,16-seco-cycloartane triterpene. Compounds 2–4 were 9,19-cycloartane triterpenoid saponins with a fused monosaccharide moiety. Herein, we reported the structure determination by 1D/2D NMR analysis of the new compounds and the evaluation of the cytotoxic activity of selected compounds against the HL-60, SMMC-7721, A549, MCF-7, and SW480 cell lines.

* These authors contributed equally to the work reported in this article.
Results and Discussion

Compound 1 was obtained as white powder. The HRTOF-ESIMS ion signal at m/z 525.3180 [M + Na]$^+$ (calcd for 525.3192) indicated the molecular formula C$_{30}$H$_{46}$O$_6$ with eight degrees of unsaturation. Its IR spectra showed the presence of hydroxy (3438 cm$^{-1}$) and carbonyl functional groups (1707 cm$^{-1}$). The $^1$H and $^{13}$C NMR data for 1 (Table 1) were similar to those of cimiheraclein D (5) [6], and the slight difference indicated that compound 1 was a configurational isomer of 5, which was confirmed by its 2D NMR spectra (HSQC, $^1$H–$^1$H COSY, and HMBC) (Fig. 2). The ROESY correlations of H-23 to H-20 and of H-20 to Me-18 suggested a 23R configuration in compound 5, while the correlations of H-23 to H-17 and of H-17 to Me-28 suggested a 23S configuration in compound 1 (Fig. 1). In addition, the configuration of C-24 in compound 5 was proposed to be S by comparison of the chemical shifts and coupling constants of H-23 (5.07, d, $J = 11.2$ Hz) and H-24 (3.76, s) of 5 with 23R, 24S configuration analogue [H-23 (5.01, d, $J = 11.1$ Hz), H-24 (3.70, s)] [8]. The configuration at C-24 in 1 was finally confirmed by molecular modeling, in which $\theta = 73.8^\circ$ in 23R 24S configuration, $\theta = 177.7^\circ$ in 23R 24R configuration, $\theta = 168.9^\circ$ in 23S 24R configuration, and $\theta = 61.1^\circ$ in 23S 24S configuration (Fig. 10S, Supporting Information). The coupling constants of H-23 and H-24 were 10.9 Hz and 0 Hz, so the configuration of C-24 in 1 was proposed as S based on the function of dihedral angle and $\gamma_{HC-C}$. Also, this was consistent with other 15,16-seco-cycloartane derivatives reported previously [5–10]. Therefore, the structure of 1 was established as 15,16-seco-14-formyl-(23S, 24S)-15-oxoheraclein-3-one (Fig. 1) and given the name cimiheraclein E.

Compound 2 possessed a molecular formula of C$_{31}$H$_{46}$O$_{11}$ based on its HRTOF-ESIMS ion signal at m/z 699.3715 [M + Na]$^+$ (calcd for 699.3720). Its $^1$H NMR spectrum displayed resonances for cyclopropane methylene protons at $\delta_H$ 0.50 (1H, d, $J = 4.0$ Hz) and 1.03 (1H, d, $J = 4.0$ Hz) for CH$_2$-19, six tertiary methyl singlets at $\delta_H$ 0.96, 1.27, 1.35, 1.61, 1.62, and 1.64 (each 3H, s), and one secondary methyl at $\delta_H$ 1.14 (3H, d, $J = 6.3$ Hz) as well as an anomeric proton signal at $\delta_H$ 4.86 (1H, d, $J = 7.6$ Hz), which is characteristic of a 9,19-cyclophanane-type triterpene glycoside. The sugar obtained after acid hydrolysis was identified as L-arabinose by comparing its TLC mobility and specific rotation with those of a standard. Detailed analysis of its NMR data (Table 2) indicated that 2 is a 16a-hydroxyl dahurinol-type triterpene glycoside and is similar to cimidahuside C [20]. The major difference was the acetyl substituent. The observed $^1$H–$^1$H COSY correlation of $\delta_H$ 4.28 (1H, m, H-5) to $\delta_H$ 5.26 (1H, brs, H-4') and the HMBC correlation between $\delta_H$ 5.26 (1H, brs, H-4') and $\delta_H$ 171.2 (OAc) indicated the acetyl group was located in C-4' of the sugar moiety (Fig. 2). The significant ROESY associations (Fig. 3) of H-3/H-5 and H-20/H-17 suggested a 3$\beta$-orientation of the substituents at C-4 and C-15,16-seco-cycloartane-type triterpene glycoside 3$\beta$-D-xylopyranoside (Fig. 1) and named as cimiheraclein F.

Compound 3 was isolated as a white powder and found to have the molecular formula C$_{31}$H$_{46}$O$_{11}$ on the basis of the HRTOF-ESIMS ion peak at m/z 553.3138 [M + Na]$^+$ (calcd for 553.3141). The NMR data (Table 2) of 3 were very similar to those of 3$\beta$-11$\beta$-di-hydroxy-24,25,26,27-tetranor-cycloart-7(8)-en-23,16$\beta$-olide 3$\beta$-D-xylopyranoside [21] except for the absence of the hydroxyl group at C-11. The stereochemistry of 3 was determined from its ROESY spectrum (Fig. 3). The crosspeaks of H-3 ($\delta_H$ 3.50, 1H, dd, $J = 11.7, 4.1$ Hz) with H-5 ($\delta_H$ 1.26, 1H, dd, $J = 12.5, 5.1$ Hz) and H-16 ($\delta_H$ 4.88, 1H, m) with H-17 ($\delta_H$ 1.92, 1H) and CH$_3$-28 ($\delta_H$ 1.06, 3H, s) indicated the $\beta$-orientation of the substituents at C-3 and C-16. Therefore, 3 was elucidated as 3$\beta$-hydroxy-24, 25, 26, 27-tetranor-cycloart-7(8)-en-23,16$\beta$-olide 3$\beta$-D-xylopyranoside (Fig. 1) and given the name cimiheraclein G.
Compound 4 was also obtained as a white powder, and its molecular formula was C\text{39}H\text{58}O\text{11} based on its HRTOF-ESIMS ion signal at \textit{m}/\textit{z} 725.3877 [M + Na]\textsuperscript{+} (calcd for 725.3877), which corresponds to 11 degrees of unsaturation. The NMR spectrum of 4 clearly displayed the signals characteristic of a 9,19-cycloartane-type triterpene. Direct analysis of its NMR data (\textit{▶} Table 2) indicated that compound 4 resembles a 23-O-acetyl-7,8-didehydroshengmanol-3-O-\alpha-L-arabinopyranoside [22] except for the presence of one additional acetyl group. The location of the acetyl group at C-2\textsuperscript{′} (\textit{▶} Fig. 2) was identified by the HMBC correlation between H-2\textsuperscript{′} (δ\textsubscript{H} 5.58, 1H, t, \textit{J} = 8.3 Hz) and the carbonyl signal at δ\textsubscript{C} 170.4. The similarity between the chemical shifts and coupling constants of C-23 and C-24 in compound 4 with those of 23-O-di-acetyl-7,8-didehydroshengmanol-3-O-\alpha-L-arabinopyranoside indicated the configurations of C-23 and C-24 were \textit{R} and \textit{S}, respectively. Finally, the structure of 4 was confirmed as 23-O-acetyl-7(8)-en-shengmanol-3-O-[2′-O-acetyl]-\alpha-L-arabinopyranoside, as shown (\textit{▶} Fig. 1).

The new compounds (1–4) were evaluated for their cytotoxicities against HL-60, SMMC-7721, A549, MCF-7, and SW480 cell lines (\textit{▶} Table 3). Compounds 1 and 2 did not show cytotoxic activity with \textit{IC}_{50} value > 40 \mu M. Compound 3 showed weak activity against A549 and MCF-7 cell lines with \textit{IC}_{50} value 27.75 and 22.45 \mu M, respectively. Compound 4 also showed antitumor activity against the HL-60, A549, and MCF-7 cell lines with \textit{IC}_{50} value 26.54, 36.98, and 21.34 \mu M, respectively.

The NMR, IR, UV, and HRTOF-ESIMS spectra of compounds 1–5, as well as the dose-response curves of cytotoxic activity, are available as Supporting Information.

\begin{table}
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\begin{tabular}{llllllllllllll}
\hline
\textbf{Position} & \textbf{1} & \textbf{5} & \textbf{Position} & \textbf{1} & \textbf{5} \\
\hline
\multirow{2}{*}{\textbf{δH\textsuperscript{a}}} & 1.81\textsuperscript{a} & 1.82\textsuperscript{a} & 3 & 215.2 s & 32.2 t \\
& 1.51\textsuperscript{a} & 1.53\textsuperscript{a} & 2 & 37.4 t & 32.2 t \\
\multirow{2}{*}{\textbf{δH\textsuperscript{b}}} & 2.73 m & 2.72 m & 4 & 50.3 s & 37.4 t \\
& 2.37 brd & 2.38 brd & 5 & 45.4 d & 37.4 t \\
\multirow{2}{*}{\textbf{δH\textsuperscript{c}}} & 1.82\textsuperscript{a} & 1.81\textsuperscript{a} & 6 & 45.4 d & 1.92 t \\
& 1.36\textsuperscript{a} & 1.38\textsuperscript{a} & 5 & 45.4 d & 1.92 t \\
\multirow{2}{*}{\textbf{δH\textsuperscript{d}}} & 1.42\textsuperscript{a} & 1.16 m & 7 & 22.0 t & 22.0 t \\
& 1.39\textsuperscript{a} & 1.16 m & 8 & 39.2 d & 22.0 t \\
\multirow{2}{*}{\textbf{δH\textsuperscript{e}}} & 2.47 m & 2.44 dd & 9 & 22.1 s & 22.0 s \\
& (5.2 7.4) & 22.1 s & 10 & 25.7 s & 22.0 s \\
\multirow{2}{*}{\textbf{δC\textsuperscript{f}}} & 1.64\textsuperscript{a} & 1.54\textsuperscript{a} & 11 & 27.1 t & 27.1 t \\
& 1.64\textsuperscript{a} & 1.54\textsuperscript{a} & 12 & 32.7 t & 27.1 t \\
\multirow{2}{*}{\textbf{δC\textsuperscript{g}}} & 1.56 s & 1.56 s & 13 & 47.9 s & 47.9 s \\
& 1.56 s & 1.56 s & 14 & 55.2 s & 47.9 s \\
\multirow{2}{*}{\textbf{δC\textsuperscript{h}}} & 9.97 s & 9.94 s & 15 & 208.0 s & 175.3 s \\
& 9.94 s & 175.3 s & 16 & 175.3 s & 175.3 s \\
\multirow{2}{*}{\textbf{δC\textsuperscript{i}}} & 2.76 d (5.5) & 2.74 d (5.6) & 17 & 55.8 d & 55.8 d \\
& 2.74 d (5.6) & 175.5 s & 18 & 18.4 q & 18.7 q \\
\multirow{2}{*}{\textbf{δC\textsuperscript{j}}} & 0.80 d (4.7) & 0.80 d (4.7) & 19 & 21.9 t & 21.9 t \\
& 0.80 d (4.7) & 0.80 d (4.7) & 19 & 21.9 t & 21.9 t \\
\multirow{2}{*}{\textbf{δC\textsuperscript{k}}} & 0.99 d (6.2) & 0.99 d (6.2) & 20 & 28.8 d & 28.8 d \\
& 1.02 d (6.4) & 28.8 d & 21 & 25.7 q & 25.1 q \\
\multirow{2}{*}{\textbf{δC\textsuperscript{l}}} & 2.57 m 1.82\textsuperscript{a} & 2.07\textsuperscript{a} & 22 & 35.0 t & 37.0 t \\
& 1.90 m & 35.0 t & 23 & 28.0 s & 78.9 d \\
\multirow{2}{*}{\textbf{δC\textsuperscript{m}}} & 5.02 d (10.9) & 5.07 d (11.2) & 24 & 79.1 d & 80.2 d \\
& 5.02 d (10.9) & 25 & 72.2 s & 80.2 d \\
\multirow{2}{*}{\textbf{δC\textsuperscript{n}}} & 4.00 s & 3.76 s & 26 & 27.7 q & 72.7 s \\
& 3.76 s & 26 & 27.7 q & 26.2 q \\
\multirow{2}{*}{\textbf{δC\textsuperscript{o}}} & 1.59 s & 1.67 s & 27 & 28.0 q & 26.2 q \\
& 1.62 s & 28 & 28.0 q & 29.5 q \\
\multirow{2}{*}{\textbf{δC\textsuperscript{p}}} & 1.53 s & 1.51 s & 28 & 14.8 q & 14.8 q \\
& 1.53 s & 28 & 14.8 q & 14.6 q \\
\multirow{2}{*}{\textbf{δC\textsuperscript{q}}} & 1.01 s & 1.01 s & 29 & 20.6 q & 14.6 q \\
& 1.01 s & 29 & 20.6 q & 20.6 q \\
\multirow{2}{*}{\textbf{δC\textsuperscript{r}}} & 1.08 s & 1.08 s & 30 & 22.6 q & 20.6 q \\
& 1.08 s & 30 & 22.6 q & 22.5 q \\
\hline
\end{tabular}
\caption{The NMR data of compounds 1 and 5 (δ in ppm).}
\end{table}

\textsuperscript{a} Signals overlapped. \textsuperscript{b} Recorded at 600 MHz in Pyridine-d\textsubscript{5}. \textsuperscript{c} Recorded at 150 MHz in Pyridine-d\textsubscript{5}. 

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### Table 2 The NMR data of compounds 2–4 (δ in ppm, J in Hz).

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^a Signals overlapped. ^b Recorded at 600 MHz in Pyridine-d_5. ^c Recorded at 150 MHz in Pyridine-d_5.
Materials and Methods

General experimental procedures

Optical rotations were recorded on a Horiba SEPA-300 polarimeter. UV spectra were acquired on a Shimadzu UV-2401A instrument. IR spectra were collected on Bruker Tensor 27 FTIR spectrometers with KBr pellets. NMR spectra were recorded on Bruker Avance III-600 spectrometers with tetramethylsilane as an internal standard at room temperature. HRTOF-ESIMS were recorded on an Agilent G6230 TOF spectrometer. TLC was performed on precoated TLC plates (200 µm, 250 g, 5 × 50 cm) eluted with MeOH; UV (MeOH) \( \lambda_{\text{max}} (\log \varepsilon) \) 204 (3.78) nm; IR (KBr) \( \nu_{\text{max}} \) 3434, 2926, 2869, 1721, 1632, 1549, 1359, 1247, 1065 cm\(^{-1}\); \( ^1H \) and \( ^13C \) NMR data (CD\(_3\)OD) see Table 1; HRTOF-ESIMS m/z 525.3180 [M + Na]\(^+\) (calcd for C\(_{20}\)H\(_{28}\)O\(_{11}\)Na, 525.3192).

Physicochemical properties of 1–4


cimihercalen E (1): white powder; \( [\alpha]_{D}^{25} = -69.25 \) (c 0.08, MeOH); UV (MeOH) \( \lambda_{\text{max}} (\log \varepsilon) \) 204 (3.79) nm; IR (KBr) \( \nu_{\text{max}} \) 3440, 2942, 2871, 1700, 1632, 1457, 1380, 1032, 977 cm\(^{-1}\); \( ^1H \) and \( ^13C \) NMR data (CD\(_3\)OD) see Table 1; HRTOF-ESIMS m/z 725.3877 [M + Na]\(^+\) (calcd for C\(_{37}\)H\(_{56}\)O\(_{11}\)Na, 725.3870).

Hydrolysis and identification of the sugar moieties in compounds 2 and 4

Compounds 2 and 4 (2.5 mg of each) were dissolved in methanol (3 mL) and refluxed with 1.0 N HCl (2 mL) at 90°C for 2 h. After neutralizing with 1.0 N NaOH, the reaction mixtures were extracted with CHCl\(_3\), and the aqueous layers were concentrated under reduced pressure to give the monosaccharides, which had \( \bar{R}f \) values (EtOAc/CHCl\(_3\)/MeOH/H\(_2\)O, 3:2:2:1) and specific rotations \( [\alpha]_{D}^{25} = 82.78 \) (c 0.05, MeOH) that were consistent with those of L-arabinopyranose (Sigma-Aldrich).
Biological assays

Cytotoxic activity was investigated using five human cancer cell lines, human leukemia HL-60, hepatocellular carcinoma SMMC-7721, lung cancer A549, breast cancer MCF-7, and colon cancer SW480 (Cell Bank of Chinese Academy of Sciences). Cells were cultured at 37°C in a humidified atmosphere of 5% CO2 in RPMI-1640 medium (HyClone) supplemented with 10% (v/v) FBS (HyClone) and dispersed in identical 96-well plates. Compounds were dissolved in DMSO and serially diluted in saline to give final DMSO concentrations below 1%. Each tumor cell line was exposed to the test compounds at concentrations of 0.064, 0.32, 1.6, 8, and 40 µM for 48 h with cisplatin (DPP; Sigma, > 98%) as the positive control; cell viability was determined by MTT cytotoxicity assay by measuring the absorbance at 570 nm with a microplate reader (Bio-Rad 680) [23]. Three independent trials were conducted for each compound (n = 3). The IC50 values and 95% confidence interval (CI) were estimated using GraphPad Prism 6.

Supporting Information

The NMR, IR, UV, and HRTOF-ESIMS spectra of compounds 1–5, as well as the dose-response curves of cytotoxic activity, are available as Supporting Information.

Acknowledgements

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Conflict of Interest

The authors declare no conflicts of interest.

References