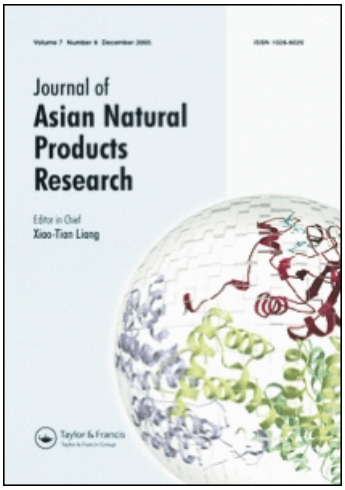


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# Triterpenoid Saponins from *Anemone Begoniifolia*

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# TRITERPENOID SAPONINS FROM *ANEMONE BEGONIIFOLIA*

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A new triterpenoid saponin, begoniifolide D (**1**), along with eight known ones (**2**–**9**) has been isolated from the methanol extracts of *Anemone begoniifolia* Lévl. et Vant. Their structures have been elucidated by spectroscopic and chemical methods.

*Keywords*: *Anemone begoniifolia*; Triterpenoid saponin; Begoniifolide D

## INTRODUCTION

Triterpenoid saponins possess various biological activities, such as hemolysis, uterine contraction, spermaticide, insect sitfast, antineoplastic and antileukemic activities [1]. Saponins are the major and bioactive components in *Anemone*, of which several species are used as folk medicines in China. Therefore, phytochemical studies on the title genus have focused mostly on saponins. We reported previously three new saponins, begoniifolides A, B and C, from *A. begoniifolia* [2]. Further chemical investigation on the methanol extracts of this plant resulted in the isolation of nine other saponins; their structures have been identified on the basis of chemical and spectroscopic evidence as 3-*O*-β-D-glucopyranosyl(1 → 2)-[β-D-glucopyranosyl(1 → 4)]-α-L-arabinopyranosyl oleanolic acid 28-*O*-α-L-rhamnopyranosyl (1 → 4)-β-D-glucopyranosyl(1 → 6)-β-D-glucopyranoside (**1**), oleanolic acid 3-*O*-α-L-arabinopyranoside (fatsiaside $A_1$) (**2**) [3], oleanolic acid 3-*O*-β-D-glucopyranosyl (1 → 3)-α-L-arabinopyranoside (**3**) [4], oleanolic acid 3-*O*-β-D-glucopyranosyl(1 → 4)-α-L-arabinopyranoside (leontoside B) (**4**) [5], oleanolic acid 28-*O*-α-L-rhamnopyranosyl (1 → 4)-β-D-glucopyranosyl(1 → 6)-β-D-glucopyranoside (cussonodide B) (**5**) [6], hederagenin 28-*O*-α-L-rhamnopyranosyl(1 → 4)-β-D-glucopyranosyl(1 → 6)-β-D-glucopyranoside (pulsatilla saponin C) (**6**) [5], 3-*O*-α-L-arabinopyranosyl oleanolic acid 28-*O*-α-L-rhamnopyranosyl (1 → 4)-β-D-glucopyranosyl(1 → 6)-β-D-glucopyranoside (ciwujianoside $C_3$) (**7**) [7], 3-*O*-α-L-arabinopyranosyl hederagenin 28-*O*-α-L-rhamnopyranosyl (1 → 4)-β-D-glucopyranosyl (1 → 6)-β-D-glucopyranoside (cauloside D) (**8**) [5], and 3-*O*-β-D-glucopyranosyl(1 → 4)-α-L-arabinopyranosyl hederagenin 28-*O*-α-L-rhamnopyranosyl(1 → 4)-β-D-glucopyranosyl (1 → 6)-β-D-glucopyranoside (leontoside D) (**9**) [5]

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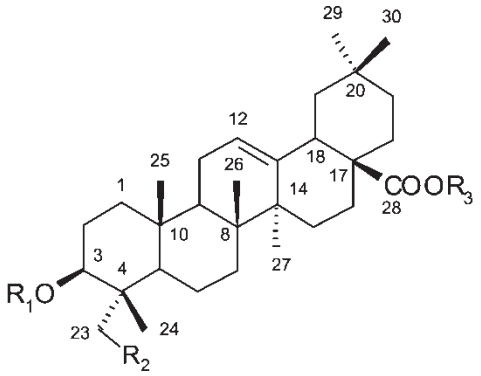


| | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| **1** | glc(2)ara–<br>glc(4) | H | –glc(6)–glc(4)–rha |
| **1a** | H | H | H |
| **1b** | glc(2)ara–<br>glc(4) | H | H |
| **2** | ara– | H | H |
| **3** | glc–(3)ara– | H | H |
| **4** | glc–(4)ara– | H | H |
| **5** | H | H | –glc(6)–glc(4)–rha |
| **6** | H | OH | –glc(6)–glc(4)–rha |
| **7** | ara– | H | –glc(6)–glc(4)–rha |
| **8** | ara– | OH | –glc(6)–glc(4)–rha |
| **9** | glc–(4)ara– | OH | –glc(6)–glc(4)–rha |

FIGURE 1 Structures of **1**–**9**.

(Fig. 1). All these compounds were first separated from the title plant and among them **1** is a new compound, named begoniifolide D.

## RESULTS AND DISCUSSION

Begoniifolide D (**1**) was obtained as a white amorphous powder; its molecular formula was determined as $C_{65}H_{106}O_{31}$ by HR-FABMS. Acid hydrolysis of **1** affords glucose, rhamnose and arabinose, together with oleanolic acid (**1a**), all of which were identified by comparison with authentic samples on TLC. $^{13}$C NMR signals due to the aglycone of **1** are identical to those of 3-*O*-α-L-arabinopyranosyl oleanolic acid 28-*O*-α-L-rhamnopyranosyl(1 → 4)-β-D-glucopyranosyl(1 → 6)-β-D-glucopyranoside (ciwujianoside $C_3$) (**7**) [7], indicating that **1** is a bisdesmoside of oleanolic acid with the same glycosidation sites at C-3 (δ 88.9) and C-28 (δ 176.5) as ciwujianoside C3 [7,8]. The $^1$H NMR spectrum of **1** displays six anomeric proton signals at δ 6.25 (d, $J$ = 7.8 Hz), 5.87 (brs), 5.19 (d, $J$ = 7.7 Hz), 5.15 (d, $J$ = 7.6 Hz), 5.01

(d, $J = 7.6$ Hz) and 4.94 (d, $J = 5.5$ Hz), correlating with the anomeric carbon signals of those sugar moieties at δ 105.7, 105.6, 104.8, 104.3, 102.7 and 95.6, respectively. Therefore, **1** was confirmed to be a bisdesmoside of oleanolic acid composed of six sugars.

Alkaline hydrolysis of **1** gives **1b**, and further acid hydrolysis of **1b** on TLC affords glucose and arabinose. Two β-D-glucopyranoses and one α-L-arabinopyranose are suggested by the $^{13}$C NMR data at δ 105.6, 105.5 and 104.4 and the coupling constants of the anomeric protons at δ 5.14 (d, $J = 7.6$ Hz), 5.09 (d, $J$=7.7 Hz) and 4.92 (d, $J$=5.2 Hz). In the FAB-MS spectrum of **1**, followed the loss of the sugar chain at C-28 (*m/z* 911 [M − glc × 2 − rha]$^-$), two glucoses (*m/z* 749 [911 − glc]$^-$ and 587 [749 − glc]$^-$) and one arabinose (*m/z* 455 [587 − ara]$^-$) were lost successively, suggesting that there was at least one terminal glucose, while arabinose was attached to the aglycone directly. Comparing the $^{13}$C NMR data of arabinose moiety in **1b** with those of compound **2**, the former shifted downfield at C-2 ($\Delta\delta + 8.2$) and C-4 ($\Delta\delta + 8.1$), indicating that both C-2 and C-4 of arabinose are glycosidated. The $^{13}$C NMR data due to the sugar moiety agree well with those of pulsatiloside A, the sugar chain of which is glc (1 → 2) [glc (1 → 4)]-ara- [5]. After alkaline hydrolysis of **1**, the remaining aqueous layer was further acid hydrolyzed to give glucose and rhamnose, which were detected by PC. Comparison of the $^{13}$C NMR spectra of **1** and **1b** reveals that the signals due to the sugar chain linked to C-28 of the aglycone are identical to those of oleanolic acid 28-*O*-α-L-rhamnopyranosyl(1 → 4)-β-D-glucopyranosyl(1 → 6)-β-D-glucopyranoside (cussonodide B) (**5**) [6]. The FAB-MS fragmentation patterns comply with this conclusion: *m/z* 1235 [M − rha]$^-$, 1073 [M − rha − glc]$^-$, 911 [M − rha − glc × 2]$^-$ Therefore, the structure of begoniifolide D (**1**) is established as 3-*O*-β-D-glucopyranosyl(1 → 2)-[β-D-glucopyranosyl(1 → 4)]-α-L-arabinopyranosyl oleanolic acid 28-*O*-α-L-rhamnopyranosyl(1 → 4)-β-D-glucopyranosyl(1 → 6)-β-D-glucopyranoside.

Saponins **2**–**9** were identified by comparison of their physical and spectral properties with those reported in the literature [3–7].



## EXPERIMENTAL

### General Experimental Procedures

NMR spectra were recorded on a Bruker AC-300P spectrometer using TMS as internal standard. Optical rotation was measured on a Perkin–Elmer 341 Polarimeter. FAB-MS and HR-FAB-MS spectra were recorded on a VG AutoSpec-3000 instrument. Column chromatography was performed on macroporous resin D-101 (Tianjin Pesticide Factory, China), silica gel (Qingdao Haiyang Chemical Co., China), Lichroprep RP-18 and RP-8 (40–63 μm) (Merck). TLC was conducted on Silica gel 60 $GF_{254}$ (Qingdao Haiyang Chemical Co., China), RP-18 $F_{254}$ and RP-8 $F_{254}$ plates (Merck).

### Plant Material

Whole plants of *Anemone begoniifolia* Lévl. et Vant. were collected from Jinfo Mountain of Chongqing in April 1998, and were identified by Professor Shunchang Xiao. A voucher specimen (no. 1999150) has been deposited in the herbarium of Chengdu Institute of Biology, Chinese Academy of Sciences.

### Extraction and Isolation

Dried, powdered plants of *A. begoniifolia* (4.0 kg) were extracted with methanol 3 times at room temperature, and the solvent was then evaporated *in vacuo*. The resultant residue was

suspended in $H_2O$ and extracted with light petroleum (b.p. 60–90°C), EtOAc and *n*-BuOH successively. The *n*-BuOH extract (A) was subjected to silica-gel column chromatography, eluting with $CHCl_3$–MeOH–$H_2O$ (40:10:1, 30:10:1, 20:10:1), to yield eight fractions. Each fraction was subjected to silica gel, eluting with $CHCl_3$–MeOH–$H_2O$ gradiently, and further to RP-8 and RP-18 chromatography with MeOH–$H_2O$ gradiently (60–80%) to yield compounds **1**–**9**. The aqueous layer was chromatographed over macroporous resin D-101 eluted with ethanol (95%) to yield B, which was processed with the same ways as extract A to yield compounds **7**–**9**. Altogether, saponins **1** (2.5 g), **2** (20 mg), **3** (70 mg), **4** (190 mg), **5** (170 mg), **6** (140 mg), **7** (520 mg), **8** (4.2 g), and **9** (8.2 g) were obtained.

### *Saponin 1*

White amorphous powder; $[\alpha]_D^{20}$ −8.8 (*c* 0.17, MeOH); FAB-MS *m/z*: 1381 $[M-H]^-$, 1235 $[M-rha]^-$, 1219 $[M-glc]^-$, 1073 $[M-rha-glc]^-$, 1057 $[M-glc\times2]^-$, 911 $[M-rha-glc\times2]^-$, 749 $[911-glc]^-$, 587 $[749-glc]^-$ and 455 $[587-ara]^-$; HR-FABMS *m/z*: 1405.6614 $[M+Na]^+$ (calcd for $C_{65}H_{106}O_{31}+Na$, 1405.6616); $^1H$ NMR (300 MHz) δ: 6.25 (1H, d, $J=7.8$ Hz, glc-1-H), 5.87 (1H, brs, rha-1-H), 5.42 (1H, brs, 12-H), 5.19 (1H, d, $J=7.7$ Hz, glc-1-H), 5.15 (1H, d, $J=7.6$ Hz, glc-1-H), 5.01 (1H, d, $J=7.6$ Hz, glc-1-H), 4.94 (1H, d, $J=5.5$ Hz, ara-1-H), 1.71 (3H, d, $J=6.0$ Hz, rha-$CH_3$), 1.23, 1.18, 1.11, 1.03, 0.90×2 and 0.88 (each 3H, $CH_3\times7$). $^{13}C$ NMR (75 MHz) data see Table I.

### *Acid Hydrolysis of 1*

Compound **1** (50 mg) was dissolved in 7% $H_2SO_4$ alcohol–$H_2O$ (1:1) (5 mL), and then boiled under reflux at 100°C for 4 h. The alcohol was evaporated thoroughly *in vacuo*, and the remaining aqueous layer was extracted with chloroform. Aglycone **1a** was obtained from

TABLE I $^{13}C$ NMR spectral data in pyridine-$d_5$ at 75 MHz for compounds **1** and **1b**.

| *No.* | ***1*** | ***1b*** | *No.* | ***1*** | ***1b*** | *No.* | ***1*** | ***1b*** |
|---|---|---|---|---|---|---|---|---|
| 1 | 38.7 | 38.6 | 25 | 15.6 | 15.4 | 5‴ | 78.1 | 78.0 |
| 2 | 26.3 | 26.3 | 26 | 17.5 | 17.4 | 6‴ | 62.6 | 62.5 |
| 3 | 88.9 | 88.9 | 27 | 26.0 | 26.1 | Glc (1 → C-28) | | |
| 4 | 39.9 | 39.7 | 28 | 176.5 | 180.1 | 1″ | 95.6 | |
| 5 | 55.8 | 55.7 | 29 | 33.1 | 33.1 | 2″ | 73.8 | |
| 6 | 19.1 | 18.4 | 30 | 23.7 | 23.7 | 3″ | 78.7 | |
| 7 | 32.5 | 33.1 | Ara (1 → C-3) | | | 4″ | 70.8 | |
| 8 | 39.4 | 39.4 | 1′ | 104.3 | 104.4 | 5″ | 78.0 | |
| 9 | 48.0 | 48.0 | 2′ | 80.6 | 80.6 | 6″ | 69.1 | |
| 10 | 36.9 | 36.9 | 3′ | 72.4 | 72.4 | Glc (1 → 6-glc) | | |
| 11 | 23.3 | 23.6 | 4′ | 77.1 | 77.1 | 1⁗ | 104.8 | |
| 12 | 122.8 | 122.5 | 5′ | 63.1 | 63.6 | 2⁗ | 75.3 | |
| 13 | 144.1 | 144.8 | Glc (1 → 2-ara) | | | 3⁗ | 76.5 | |
| 14 | 42.1 | 42.1 | 1″ | 105.7 | 105.6 | 4⁗ | 78.3 | |
| 15 | 28.2 | 28.2 | 2″ | 75.6 | 75.5 | 5⁗ | 77.1 | |
| 16 | 23.8 | 23.8 | 3″ | 78.0 | 78.5 | 6⁗ | 61.3 | |
| 17 | 47.0 | 46.6 | 4″ | 71.3 | 71.3 | Rha (1 → 4-glc) | | |
| 18 | 41.7 | 41.9 | 5″ | 78.6 | 77.9 | 1‴ | 102.7 | |
| 19 | 46.2 | 46.4 | 6″ | 62.6 | 62.5 | 2‴ | 72.7 | |
| 20 | 30.7 | 30.9 | Glc (1 → 4-ara) | | | 3‴ | 72.5 | |
| 21 | 34.0 | 34.2 | 1‴ | 105.6 | 105.5 | 4‴ | 73.9 | |
| 22 | 32.5 | 33.1 | 2‴ | 76.1 | 76.0 | 5‴ | 70.3 | |
| 23 | 28.2 | 28.1 | 3‴ | 78.2 | 78.2 | 6‴ | 18.5 | |
| 24 | 16.7 | 16.7 | 4‴ | 71.5 | 71.5 | | | |

the chloroform layer and identified as oleanolic acid by comparison with an authentic sample on TLC. The aqueous layer was neutralized to pH 7 with saturated $Ba(OH)_2$ solution. After filtration and concentration of the filtrate, glucose, rhamnose and arabinose were detected by PC.

### *Alkaline Hydrolysis of 1*

Compound **1** (60 mg) was dissolved in ammonia water (5.0 mol $L^{-1}$, 8 mL), and then boiled under reflux at 100°C for 5 h. The reaction mixture was evaporated to dryness *in vacuo* and dissolved in $H_2O$ which was then extracted with saturated *n*-BuOH to give **1b** (32 mg); **1b** was obtained as a white powder, its $^{13}C$ NMR data are shown in Table I. The aqueous layer was concentrated and then further hydrolyzed with acid (the same as for the acid hydrolysis of **1**). Glucose and rhamnose were detected from the resulting solution by PC.

### *Saponin 1b*

White amorphous powder; $^1H$ NMR (300 MHz) $\delta$(ppm): 5.48 (1H, brs, 12-H), 5.14 (1H, d, $J = 7.6$ Hz, glc-1-H), 5.09 (1H, d, $J = 7.7$ Hz, glc-1-H), 4.92 (1H, d, $J = 5.2$ Hz, ara-1-H), 1.27, 1.17, 1.02, 1.00, 0.98, 0.97 and 0.84 (each 3H, $CH_3 \times 7$). $^{13}C$ NMR (75 MHz) data is shown in Table I.

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