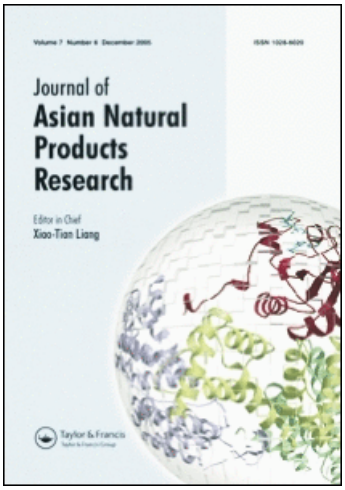


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# Triterpenoid saponins and monoterpenoid glycosides from *Incarvillea delavayi*

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Two new triterpenoid saponins, incarvillosides A (**1**) and B (**2**), and two new monoterpenoid glycosides, incarvillosides C (**3**) and D (**4**), were isolated from the high-polarity fraction of the whole plant of *Incarvillea delavayi*. By means of spectroscopic data and chemical degradation, the structures were established as (3β,21β)-3,19,21,23-tetrahydroxyurs-12-en-28-oic acid 28-*O*-β-D-glucopyranoside (**1**), (2β,3β,19α)-2,3,19,23-tetrahydroxyolean-12-en-28-oic acid 28-*O*-β-D-glucopyranoside (**2**), (2*S*,6*R*)-2,6-dimethyl-1,8-octanediol 1-*O*-β-D-glucopyranoside (**3**), and (2*S*,6*R*)-2,6-dimethyl-1,8-octanediol 8-*O*-β-D-glucopyranoside (**4**).

**Keywords:** *Incarvillea delavayi*; triterpenoid saponins; monoterpenoid glycosides; incarvilloside



## 1. Introduction

In continuation of our chemical studies on the *Incarvillea* species [1–4], we have investigated the high-polarity components of *Incarvillea delavayi* (Bignoniaceae). The whole plant, distributed in Yunnan and Sichuan Provinces of China, has been used in traditional Chinese medicine as an anti-inflammatory and analgesic agent [5]. Previous phytochemical investigations of the genus *Incarvillea* have revealed many acridine-type alkaloids and iridoids (mainly from the low-polarity fractions) with significant antinociceptive bioactivity [6–11]. In the present paper, we report the isolation and structural elucidation of two new triterpenoid saponins, incarvillosides A (**1**) and B (**2**), and two new monoterpenoid glycosides, incarvillosides C (**3**) and D (**4**) (Figure 1), from the high-polarity fraction of the title plant.

## 2. Results and discussion

Compound **1** was obtained as a colorless gum. The positive HR-ESI-MS showed an $[M+Na]^+$ ion at *m/z* 689.3877, in accordance with a molecular formula of $C_{36}H_{58}O_{11}$. Positive results for both Liebermann–Burchard and Molish reactions indicated that **1** should be a triterpenoid saponin. Its EI-MS showed peaks at *m/z* 504, 280, and 224 due to retro Diels–Alder fission, which indicated that the aglycone was an amyrin derivative with two hydroxyl groups in the A/B rings, and two hydroxyl groups as well as one carboxyl group in the D/E rings [12,13].

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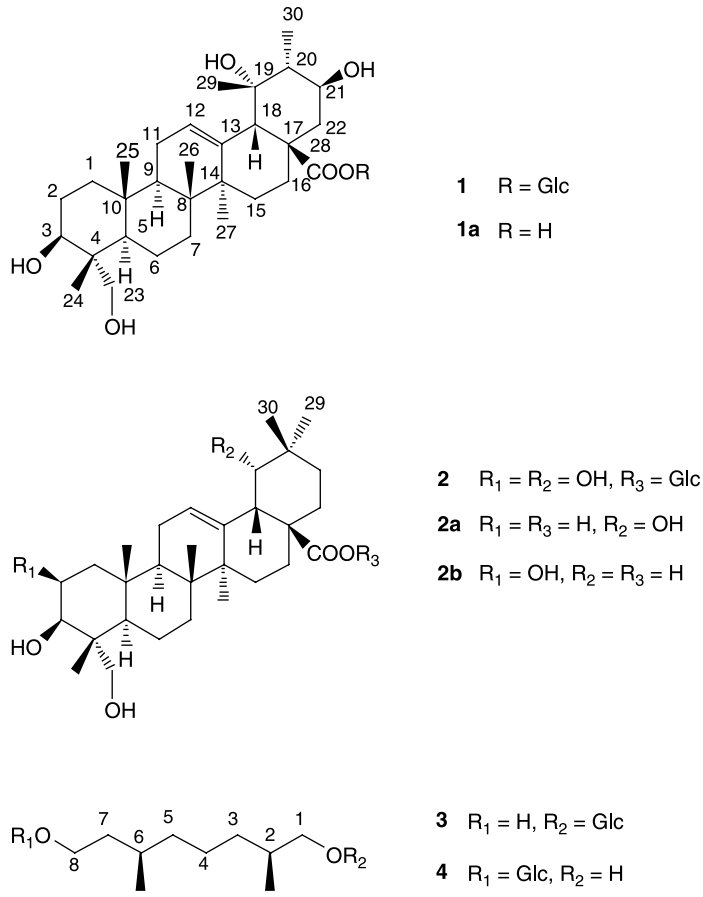


Figure 1. Structures of compounds **1**–**4**.

Furthermore, compound **1** afforded D-glucose on acid hydrolysis.

The $^{1}$H NMR spectrum (Table 1) indicated the presence of five tertiary methyl groups at $\delta$ 0.71, 0.75, 0.88, 1.03, and 1.20, one secondary methyl group at $\delta$ 0.93 ($J = 6.5$ Hz), two oxymethine groups at $\delta$ 3.31 (dd, $J = 12.0$, 4.5 Hz, H-3) and 3.68 (ddd, $J = 4.5$, 10.5, 10.5 Hz, H-21), one oxymethylene group at $\delta$ 3.27 and 3.51 (AB, $J = 11.0$ Hz, $H_2$-23), and one olefinic proton at $\delta$ 5.31 (br t, H-12) in the aglycone moiety. The $^{13}$C NMR spectrum for the aglycone moiety of **1** exhibited the signals due to one carboxyl group at $\delta$ 178.6 (s), one oxygenated quaternary C-atom at $\delta$ 73.9 (s), two oxymethine groups at $\delta$ 69.7 (d) and 73.7 (d), one oxymethylene group at $\delta$ 66.8 (t), and one C=C group at $\delta$ 129.6 (d) and 139.8 (s). The $^{1}$H and $^{13}$C NMR spectra of **1** showed a close resemblance to those of ilexolic acid A (**1a**) [14], and the main difference was observed for the signals of the sugar moiety. The NMR spectra of the sugar unit indicated it to be a D-glucose, which was confirmed by acid hydrolysis and GC analysis of the thiazolidine derivative. The anomeric proton at $\delta$ 5.32 (d, $J = 7.8$ Hz) demonstrated the β-configuration of the glucose. The β-D-glucose was attached to C-28 of the aglycone, as indicated by a HMBC correlation of the anomeric proton to the signal at $\delta$ 178.6 (C-28). Hence, the structure of **1** was established as (3β,21β)-3,19,21,23-tetrahydroxyurs-12-en-28-oic

Table 1. $^{1}H$ (600 MHz) and $^{13}C$ NMR (150 MHz) spectral data of compounds **1** and **2** in $CD_3OD$ ($\delta$ in ppm, $J$ in Hz).

| | **1** | | **2** | |
|---|---|---|---|---|
| Position | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ |
| 1 | 38.3 (t) | α 1.04–1.08 (m)<br>β 1.81–1.85 (m) | 47.8 (t) | α 1.29–1.33 (m)<br>β 1.91–1.95 (m) |
| 2 | 27.2 (t) | α 1.62–1.65 (m)<br>β 2.62–2.67 (m) | 71.3 (d) | 3.35–3.39 (m) |
| 3 | 73.7 (d) | 3.31 (dd, 12.0, 4.5) | 74.0 (d) | 3.32 (d, 3.6) |
| 4 | 44.1 (s) | – | 44.1 (s) | – |
| 5 | 48.8 (d) | 1.78 (d, 10.2) | 49.1 (d) | 1.82–1.86 (m) |
| 6 | 19.3 (t) | 1.30–1.33 (m) | 19.3 (t) | 1.32–1.35 (m) |
| 7 | 33.3 (t) | α 1.28–1.30 (m)<br>β 1.76–1.78 (m) | 33.6 (t) | α 1.29–1.31 (m)<br>β 1.75–1.78 (m) |
| 8 | 40.9 (s) | – | 41.3 (s) | – |
| 9 | 48.4 (d) | 1.77–1.79 (m) | 48.5 (d) | 1.90–1.92 (m) |
| 10 | 35.9 (s) | – | 39.0 (s) | – |
| 11 | 24.8 (t) | 1.98–2.00 (m) | 24.9 (t) | 1.93–1.95 (m) |
| 12 | 129.6 (d) | 5.31 (br t) | 124.9 (d) | 5.32 (br t) |
| 13 | 139.8 (s) | – | 144.5 (s) | – |
| 14 | 42.8 (s) | – | 42.8 (s) | – |
| 15 | 29.6 (t) | α 1.08–1.10 (m)<br>β 1.78–1.81 (m) | 28.6 (t) | α 1.07–1.10 (m)<br>β 1.86–1.89 (m) |
| 16 | 26.6 (t) | 2.24–2.28 (m) | 24.9 (t) | 2.28–2.30 (m) |
| 17 | 49.2 (s) | – | 47.2 (s) | – |
| 18 | 55.0 (d) | 2.52 (s) | 45.1 (d) | 3.06 (s) |
| 19 | 73.9 (s) | – | 82.6 (d) | 3.27 (br s) |
| 20 | 42.9 (d) | 1.97–2.01 (m) | 35.9 (s) | – |
| 21 | 69.7 (d) | 3.68 (ddd, 4.5, 10.5, 10.5) | 28.6 (t) | 1.05–1.12 (m) |
| 22 | 48.0 (t) | α 1.34–1.36 (m)<br>β 1.93–1.96 (m) | 33.4 (t) | α 1.26–1.28 (m)<br>β 1.53–1.55 (m) |
| 23 | 66.8 (t) | a 3.27 (d, 11.0)<br>b 3.51 (d, 11.0) | 66.7 (t) | a 3.27 (d, 11.0)<br>b 3.51 (d, 11.0) |
| 24 | 13.7 (q) | 0.71 (s) | 13.8 (q) | 0.71 (s) |
| 25 | 16.6 (q) | 0.75 (s) | 17.9 (q) | 0.78 (s) |
| 26 | 17.6 (q) | 0.88 (s) | 17.7 (q) | 0.92 (s) |
| 27 | 24.7 (q) | 1.03 (s) | 25.0 (q) | 0.94 (s) |
| 28 | 178.6 (s) | – | 178.6 (s) | – |
| 29 | 27.1 (q) | 1.20 (s) | 28.6 (q) | 1.02 (s) |
| 30 | 17.5 (q) | 0.93 (d, 6.5) | 25.0 (q) | 1.32 (s) |
| 1′ | 95.8 (d) | 5.32 (d, 7.8) | 95.9 (d) | 5.37 (d, 7.8) |
| 2′ | 74.0 (d) | 3.30–3.31 (m) | 74.0 (d) | 3.30–3.31 (m) |
| 3′ | 78.6 (d) | 3.40–3.41 (m) | 78.7 (d) | 3.40–3.41 (m) |
| 4′ | 71.2 (d) | 3.67 (t, 9.5) | 71.3 (d) | 3.68 (t, 9.5) |
| 5′ | 78.4 (d) | 3.33–3.34 (m) | 78.6 (d) | 3.34–3.35 (m) |
| 6′ | 62.5 (t) | a 3.66 (dd, 12.0, 5.0)<br>b 3.80 (dd, 12.0, 2.0) | 62.6 (t) | a 3.67 (dd, 12.0, 5.0)<br>b 3.82 (dd, 12.0, 2.0) |



acid 28-*O*-β-D-glucopyranoside, and named incarvilloside A.

Compound **2**, a colorless gum, exhibited an $[M+Na]^+$ peak at *m/z* 689.3875 by positive HR-ESI-MS, which was consistent with the molecular formula $C_{36}H_{58}O_{11}$. Compound **2** also displayed positive Liebermann–Burchard and Molish tests. Its EI-MS showed characteristic peaks at *m/z* 504, 264, and 240 due to retro

Diels–Alder fission, which suggested the occurrence of three hydroxyl groups in the A/B rings, and one hydroxyl group and one carboxyl group in the D/E rings on the amyrin skeleton [12,13]. Compound **2** also afforded D-glucose on acid hydrolysis.

The $^1$H NMR spectrum of **2** (Table 1) indicated the presence of six tertiary methyl groups at $\delta$ 0.71, 0.78, 0.92, 0.94, 1.02, and 1.32, three oxymethine groups at $\delta$ 3.27 (br s, H-19), 3.32 (dd, $J = 12.0$, 4.5 Hz, H-3), and 3.35–3.39 (m, H-2), one oxymethylene group at $\delta$ 3.27 and 3.51 (AB, $J = 11.0$ Hz, $H_2$-23), one olefinic proton at $\delta$ 5.32 (br t, H-12), and one β-glucopyranose unit. The $^{13}$C NMR spectrum for the aglycone moiety of **2** exhibited the signals due to one carboxyl group at $\delta$ 178.6 (s), three oxymethine groups at $\delta$ 71.3 (d), 74.0 (d), and 82.6 (d), one oxymethylene group at $\delta$ 66.7 (t), and one C=C group at $\delta$ 124.9 (d) and 144.5 (s). Comparison of 1D and 2D NMR spectral data of **2** with those of ilexosapogenin A (**2a**) [15] and bayogenin (**2b**) [16] indicated that C-2, C-3, C-19, and C-23 of the aglycone moiety should be oxygenated and their orientations should be 2β, 3β, and 19α, respectively. The configurations of hydroxyl groups on C-3 and C-19 were also confirmed as 3β and 19α, respectively, according to the correlations between H-3 and H-23, and H-12 and H-19 in the NOESY spectrum (Figure 2). The β-D-glucose was also attached to C-28 of the aglycone, which was confirmed by the HMBC spectrum. Thus, the structure of **2** was identified as (2β,3β,19α)-2,3,19,23-tetrahydroxyolean-12-en-28-oic acid 28-*O*-β-D-glucopyranoside, and named incarvilloside B.

Compound **3** was obtained as a colorless oil. The positive HR-ESI-MS showed an $[M+Na]^+$ ion at *m/z* 359.2046, in accordance with a molecular formula of $C_{16}H_{32}O_7$, indicating one degree of

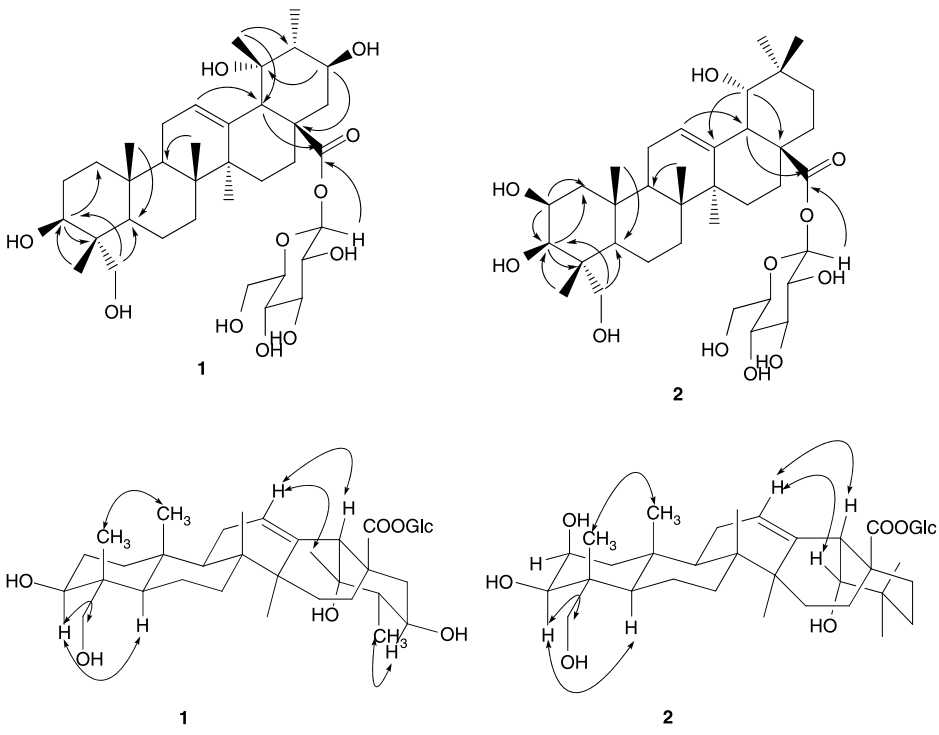


Figure 2. Key HMBC (H⟶C) and NOESY (H⟷H) correlations of compounds **1** and **2**.

unsaturation in its structure. Acid hydrolysis of **3** afforded aglycone and D-glucose by GC analysis.

The $^1$H and $^{13}$C NMR spectra of **3** (Table 2) showed the presence of two methyl groups ($\delta_H$ 0.93 (d, $J = 7.5$ Hz), 0.94 (d, $J = 7.5$ Hz); $\delta_C$ 17.5, 20.1), two oxymethylene groups ($\delta_H$ 3.39 (dd, $J = 10.5$, 5.9 Hz, H-1), 3.65–3.69 (m, $H_2$-8), 3.89 (dd, $J = 10.5$, 6.5 Hz, H-1); $\delta_C$ 61.2 (t) and 76.1 (t)), and the remaining four methylene groups ($\delta_C$ 25.3 (t), 34.6 (t), 37.7 (t), and 38.6 (t)), and two methine groups ($\delta_C$ 30.7 (d), 36.8 (d)) in the aglycone moiety. By comparing the NMR spectral data with those in the literatures [17,18], the aglycone was identified as (2*S*,6*R*)-2,6-dimethyl-1,8-octanediol. Its absolute configuration was further confirmed by comparing its optical rotation value $[\alpha]_D^{20}$ −5.8 ($c = 0.4$ $CHCl_3$) with the literature value $[\alpha]_D^{25}$ −6.3 ($c = 9.5$ $CHCl_3$), while its six-epimer's rotation value was $[\alpha]_D^{25}$ −15.5 ($c = 4.1$ $CHCl_3$) [18]. The $^1$H NMR spectrum of **3** showed an anomeric proton at $\delta_H$ 4.58 (d, $J = 7.2$ Hz), demonstrating the β-conformation of the glucose. The β-D-glucose was connected to C-1 of the aglycone, as indicated by a HMBC correlation of H-1 to the anomeric carbon of the glucose unit and of the anomeric proton to C-1. Accordingly, compound **3** was assigned as (2*S*,6*R*)-2,6-dimethyl-1,8-octanediol 1-*O*-β-D-glucopyranoside, and named incarvilloside C.

Compound **4** had the molecular formula of $C_{16}H_{32}O_7$, identical to that of **3**. The NMR spectral data of **4** were similar to those of **3**, except for the position of glucose attached to the aglycone. The glucose was attached to C-8 of the aglycone, which was deduced from the HMBC spectrum. The absolute configuration of **4** was also (2*S*,6*R*), for its aglycone had the same optical rotation value as that of **3**. Thus, compound **4** was determined to be (2*S*,6*R*)-2,6-dimethyl-1,8-octanediol

Table 2. $^1$H (600 MHz) and $^{13}$C NMR (150 MHz) spectral data of compounds **3** and **4** in $CD_3OD$ ($\delta$ in ppm, $J$ in Hz).

| | **3** | | **4** | |
|---|---|---|---|---|
| Position | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ |
| 1 | 76.1 (t) | a 3.39 (dd, 10.5, 5.9)<br>b 3.89 (dd, 10.5, 6.5) | 68.4 (t) | a 3.51 (dd, 10.5, 5.8)<br>b 4.05 (dd, 10.5, 6.5) |
| 2 | 36.8 (d) | 1.50–1.52 (m) | 34.6 (d) | 1.51–1.53 (m) |
| 3 | 34.6 (t) | a 1.16–1.18 (m)<br>b 1.83–1.85 (m) | 34.9 (t) | a 1.18–1.20 (m)<br>b 1.72–1.79 (m) |
| 4 | 25.3 (t) | 1.23–1.26 (m) | 25.4 (t) | 1.24–1.28 (m) |
| 5 | 37.7 (t) | a 1.34–1.36 (m)<br>b 1.52–1.54 (m) | 38.5 (t) | a 1.35–1.37 (m)<br>b 1.68–1.71 (m) |
| 6 | 30.7 (d) | 1.65–1.67 (m) | 30.9 (d) | 1.71–1.72 (m) |
| 7 | 38.6 (t) | a 1.35–1.37 (m)<br>b 1.74–1.77 (m) | 40.8 (t) | a 1.37–1.40 (m)<br>b 1.65–1.68 (m) |
| 8 | 61.2 (t) | 3.65–3.69 (m) | 68.9 (t) | 3.64–3.68 (m) |
| 2-Me | 17.5 (q) | 0.93 (d, 7.5) | 17.1 (q) | 0.94 (d, 7.5) |
| 6-Me | 20.1 (q) | 0.94 (d, 7.5) | 20.1 (q) | 0.95 (d, 7.5) |
| 1′ | 102.1 (d) | 4.58 (d, 7.8) | 101.6 (d) | 4.58 (d, 7.8) |
| 2′ | 75.4 (d) | 3.96 (t, 7.8) | 75.4 (d) | 3.97 (t, 7.8) |
| 3′ | 78.2 (d) | 3.84–3.87 (m) | 78.2 (d) | 3.83–3.86 (m) |
| 4′ | 72.5 (d) | 3.91 (t, 9.5) | 72.6 (d) | 3.91 (t, 9.5) |
| 5′ | 78.2 (d) | 3.29–3.31 (m) | 78.2 (d) | 3.28–3.30 (m) |
| 6′ | 62.9 (t) | a 3.79 (dd, 12.0, 5.0)<br>b 3.95 (dd, 12.0, 2.0) | 62.9 (t) | a 3.78 (dd, 12.0, 5.0)<br>b 3.95 (dd, 12.0, 2.0) |

8-*O*-β-D-glucopyranoside, and named incarvilloside D.

## 3. Experimental

### 3.1 General experimental procedures

Optical rotations were taken on a Perkin-Elmer 241MC polarimeter. IR spectra were recorded on a Perkin-Elmer 577 spectrometer. NMR spectra were measured on a Bruker AM-600 spectrometer using TMS as the internal standard. EI-MS data were obtained on a Finnigan-MAT 95 mass spectrometer; HR-ESI-MS was recorded on a Micromass LCT spectrometer. For column chromatography (CC), silica gel (Qingdao Haiyang Chemical Group Corporation, Qingdao, China), Sephadex LH-20 (Pharmacia Fine Chemicals, Piscataway, NJ, USA), and ODS gel (25–40 μm; Merck, Darmstadt, Germany) were used. GC analyses were performed on an Agilent 6890N gas chromatograph; capillary column (28 m × 0.32 mm i.d.; HP-5); FID detector, operated at 260°C (column temperature 180°C); $N_2$ as the carrier gas (40 ml/min).



### 3.2 Plant material

The whole plants of *I. delavayi* were collected in Eryuan County, Yunnan Province, China, in July 2006, and identified by Prof. Li-Shan Xie of Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (No. 2006071003) has been deposited at the Herbarium of the School of Pharmacy, Second Military Medical University.

### 3.3 Extraction and isolation

The air-dried whole plants of *I. delavayi* (17 kg) were extracted with 80% EtOH (10 liters × 3) under reflux for 2 h each time. After concentration, the residue (790 g) was dissolved in 2% HCl and filtered. The filtrate was adjusted to pH 9–10 by adding 10% NaOH, and then extracted with $CHCl_3$ (2 liters × 3). The aqueous solution and the filter residue were collected together and adjusted to pH 7, then partitioned successively with AcOEt (2 liters × 3) and BuOH (2 liters × 3) after being defatted with petroleum ether (2 liters × 2). The BuOH extract (180 g) was subjected to CC ($SiO_2$; $CHCl_3$/$CH_3$-OH 20:1 → 1:1) to give fractions 1–6. Fraction 4 (16.7 g) was subjected to CC (ODS, 10, 30, 50, 70, 90% aqueous MeOH, respectively) and afforded five subfractions. Subfraction 3 (210 mg) was subjected to CC (Sephadex LH-20, MeOH; ODS, 47% aqueous MeOH) repeatedly to give compounds **1** (8 mg), **2** (10 mg), **3** (7 mg), and **4** (6 mg).

#### 3.3.1 Incarvilloside A (*1*)

Colorless gum, $[\alpha]_D^{20}$ +10.6 ($c = 0.50$, MeOH); IR (KBr) $\nu_{max}$ ($cm^{-1}$): 3421, 2925, 1740, 1553, 1076, 1064; $^1H$ and $^{13}C$ NMR spectral data, see Table 1; EI-MS: *m/z* 504 (1), 280 (100), 262 (87), 235 (38), 224 (51), 223 (16), 206 (29), 205 (23); ESI-MS (positive): *m/z* 689 $[M+Na]^+$; HR-ESI-MS (positive): *m/z* 689.3877 $[M+Na]^+$ (calcd for $C_{36}H_{58}O_{11}Na$, 689.3871).

#### 3.3.2 Incarvilloside B (*2*)

Colorless gum, $[\alpha]_D^{20}$ +27.3 ($c = 0.50$, MeOH); IR (KBr) $\nu_{max}$ ($cm^{-1}$): 3445, 2982, 1726, 1643, 1278, 1077; $^1H$ and $^{13}C$ NMR spectral data, see Table 1; EI-MS: *m/z* 504 (1), 264 (100), 246 (74), 240 (62), 239 (21), 224 (36), 222 (19), 221 (15), 219 (13); ESI-MS (positive): *m/z* 689 $[M+Na]^+$; HR-ESI-MS (positive): *m/z* 689.3875 $[M+Na]^+$ (calcd for $C_{36}H_{58}O_{11}Na$, 689.3871).

#### 3.3.3 Incarvilloside C (*3*)

Colorless oil, $[\alpha]_D^{20}$ −3.9 ($c = 0.4$, MeOH); IR (KBr) $\nu_{max}$ ($cm^{-1}$): 3638,

3625, 1027; $^{1}H$ and $^{13}C$ NMR spectral data, see Table 2; ESI-MS (positive): *m/z* 359 $[M+Na]^+$. HR-ESI-MS (positive): *m/z* 359.2046 $[M+Na]^+$ (calcd for $C_{16}H_{32}O_7Na$, 359.2040).

#### *3.3.4 Incarvilloside D (**4**)*

Colorless oil, $[\alpha]_D^{20}$ −2.1 ($c = 0.4$, MeOH); IR (KBr) $\nu_{max}$ ($cm^{-1}$): 3629, 3427, 1032. $^{1}H$ and $^{13}C$ NMR spectral data, see Table 2; ESI-MS (positive): *m/z* 359 $[M+Na]^+$. HR-ESI-MS (positive): *m/z* 359.2043 $[M+Na]^+$ (calcd for $C_{16}H_{32}O_7Na$, 359.2040).

### *3.4 Acid hydrolysis of 1–4*

Each compound (4 mg) was heated in 4 ml of 10% HCl/dioxane (1:1) at 80°C for 4 h. After the dioxane was removed, $H_2O$ (5 ml) was added and the solution was extracted with AcOEt (5 ml × 3). The aqueous fractions were evaporated and the residues were prepared as thiazolidine derivatives for GC analysis according to the methods described in the literature [19].



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