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Triterpenoid saponins and phenylethanoid glycosides from stem of *Akebia trifoliata* var. *australis*

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Abstract

A detailed phytochemical study on the 70% aqueous ethanol extract of stems of *Akebia trifoliata* (Thunb.) Koidz. var. *australis* (Diels) Rehd led to isolation of five compounds, together with 12 known triterpenoid saponins and three known phenylethanoid glycosides. The structures of the five compounds were elucidated on the basis of analysis of spectroscopic data and physicochemical properties as: 2α, 3β, 23-trihydroxy-30-norolean-12-en-28-oic acid β-D-glucopyranosyl ester (**1**), 2α, 3β, 23-trihydroxy-30-norolean-12-en-28-oic acid β-D-xylopyranosyl-(1 → 3)-*O*-α-D-rhamnopyranosyl-(1 → 4)-*O*-β-D-glucopyranosyl-(1 → 6)-*O*-β-D-glucopyranosyl ester (**2**), 2α, 3β, 23-trihydroxyurs-12-en-28-oic acid β-D-xylopyranosyl-(1 → 3)-*O*-α-L-rhamnopyranosyl-(1 → 4)-*O*-β-D-glucopyranosyl-(1 → 6)-*O*-β-D-gluco-pyranosyl ester (**3**), 3-β-[(β-D-glucopyranosyl-(1 → 3)-*O*-α-L-arabinopyranosyl)oxy]-23-hydroxy-30-norolean-12-en-28-oic acid α-L-rhamnopyranosyl-(1 → 4)-*O*-β-D-glucopyranosyl-(1 → 6)-*O*-β-D-glucopyranosyl ester (**4**) and 3-β-[(α-L-xylopyranosyl-(1 → 2)-*O*-α-L-arabinopyranosyl)oxy]-30-norolean-12-en-28-oic acid α-L-rhamnopyranosyl-(1 → 4)-*O*-β-D-glucopyranosyl-(1 → 6)-*O*-β-D-glucopyranosyl ester (**5**), named mutongsaponin A, B, C, D and E, respectively.

Keywords: *Akebia trifoliata* var. *australis*; Lardizabalaceae; Stem; Triterpenoid saponins; Phenylethanoid glycosides

1. Introduction

Akebia trifoliata (Thunb.) Koidz. var. *australis* (Diels) Rehd, commonly called ''Bai Mu Tong'', is a liana distributed in China. It is used as a diuretic and an antiphlogistic in traditional Chinese medicine. According to the Chinese Pharmacopoeia (Committee for the Pharmacopoeia of P.R. China, 2005), the origin of *Caulis Akebiae* is legally used from the stems of *Akebia quinata* (Houtt.) Decne., *A. trifoliata* (Thunb.) Koidz. or *A. trifoliata* (Thunb.) Koidz. var. *australis* (Diels) Rehd (Lardizabalaceae). Although phytochemical analyses were extensively investigated on the stems (Fujita et al., 1974a,b,c; Mimaki et al., 2003; WangYe et al., 2004), pericarps (Kawasaki and Higuchi, 1976a,b), seeds (Kawasaki et al., 1972; Kawasaki and Higuchi, 1972) and callus tissues (Ikuta and Itokawa, 1986, 1995; Itokawa and Ikuta, 1988, 1989) of *A. quinata* or *A. trifoliata*, only a few chemical papers were published on *A. trifoliata* var. *australis*, except for studies on triterpenoid saponins from its fruits (Ma et al., 1993, 1994a,b). To investigate further the chemical differences among the three plants above, a detailed phytochemical investigation was carried out on the stems of *A. trifoliata* var. *australis*, which resulted in isolation of five new triterpenoid saponins (**1**–**5**), named mutongsaponin A, B, C, D and E, respectively, together with twelve known triterpenoid saponins and three known phenylethanoid glycosides. The identification of the isolated compounds has been established on the basis of spectroscopic analyses, including that of two-dimensional NMR spectroscopic data and the results of acid hydrolysis.

2. Result and discussion

The EtOAc-soluble and *n*-BuOH-soluble fractions of the ethanol –H_2O (7:3) extract of the stems of *A. trifoliata* var.

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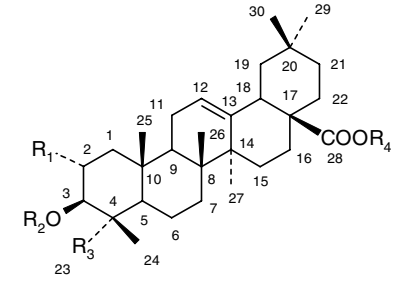
australis were subjected to column chromatographic separations on silica gel, Sephadex LH-20, macroporous resin HPD_{100} and octadecylsilanized (ODS) silica gel, and to reversed phase preparative HPLC, giving compounds **1**–**20**. Compounds **6**–**20** were identified as leonticin E (**6**) (Chen et al., 1997), aradecoside D (**7**) (Lin and Yang, 2004), ciwujianoside A_1 (**8**), ciwujianoside A_2 (**9**) (Shao et al., 1989), saponin P_{J1} (**10**) (Mimaki et al., 2003), scheffoleoside A (**11**) (Maeda et al., 1994; Mimaki et al., 2003), asiaticoside (**12**) (Mahato et al., 1987; Mimaki et al., 2003), saponin P_H (**13**) (Mimaki et al., 2003), 3-β-[(β-D-glucopyranosyl-(1 → 2)-*O*-[β-D-glucopyranosyl-(1 → 3)-*O*-]-α-L-arabinopyranosyl)oxy]-olean-12-en-28-oic acid (**14**), 3-β-[(β-D-glucopyranosyl-(1 → 2)-*O*-α-L-arabinopyranosyl)oxy]-30-norolean-12-en-28-oic acid (**15**), saponin P_E (**16**) (Zhong et al., 2001), guaianin N (**17**) (Itokawa and Ikuta, 1989), calceolarioside B (**18**)(Damtoft and Jensen, 1994), (3,4-dihydroxyphenyl)-ethyl-6-*O*-(E)-feruloyl-β-D-glucopyranoside (**19**), (4-hydroxyphenyl)-ethyl-6-*O*-(E)-caffeoyl-β-D-glucopyranoside (**20**) (Shimomura et al., 1987), respectively.

Mutongsaponin A (**1**) was deduced to have the molecular formula $C_{35}H_{54}O_{10}$, as indicated from positive-ion HR-FAB-MS (*m/z* 657.3649 $[M + Na]^+$) and the positive-ion ESI-MS (*m/z* 673 $[M + K]^+$, 657 $[M + Na]^+$) data. The ^1H NMR spectrum of **1** was typical of a triterpene monoglycoside, showing signals for four tertiary methyl groups at δ 1.12, 1.11, 1.07, 1.05 (each s), an exomethylene group at δ 4.72 and 4.66 (each s), an olefinic proton at δ 5.40 (br.s) and an anomeric proton at δ 6.28 (1H, d, $J = 8.0$ Hz). Acid hydrolysis of **1** with trifluoroacetic acid gave 2α, 3β, 23-trihydroxy-30-norolean-12-en-28-oic acid as the aglycone and D-glucose as the carbohydrate moiety. The monosaccharide was identified by comparison of both the R_f value with that of D-glucose on TLC and of its ^{13}C NMR spectroscopic data. In addition, the β configuration of the glucose was determined from the coupling constant($J = 8.0$ Hz) of the anomeric proton and the chemical shift of the anomeric carbon. From the above NMR spectroscopic and chemical data, together with the information from the positive HR-FAB-MS and positive ESI-MS, **1** was deduced to be 2α, 3β, 23-trihydroxy-30-norolean-12-en-28-oic acid monoglycoside. Analysis of the ^1H and ^{13}C NMR spectra of **1** allowed us to conclude that D-glucose was involved in a linkage at C-28 of the aglycone. This was ascertained by determination of the HMBC correlations from H-1 of glc at δ 6.28 to C-28 of the aglycone at δ 175.7. Accordingly, the structure of **1** was assigned as 2α, 3β, 23-trihydroxy-30-norolean-12-en-28-oic acid β-D-glucopyranosyl ester (see Fig. 1).

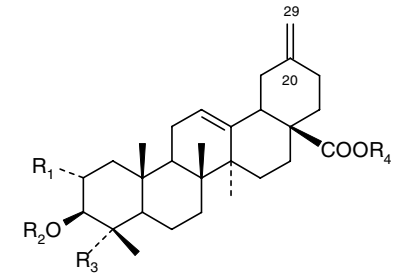
The molecular formula of mutongsaponin B (**2**) was determined by the positive-ion HR-ESI-MS(*m/z* 1097.5139 $[M + Na]^+$) and positive-ion ESI-MS(*m/z* 1097 $[M + Na]^+$) to be $C_{52}H_{82}O_{23}$. The ^1H NMR spectrum of **2** exhibited signals for four anomeric protons at δ 6.18 (1H, d, $J = 8.0$ Hz), 5.86(1H, br.s), 5.22 (1H, d, $J = 7.5$ Hz) and 4.91(1H, d, $J = 7.5$ Hz), as well as signals for four methyl groups at δ 1.10, 1.09, 1.07, 1.04 (each s), an exomethylene group at δ 4.69, 4.63 (each s) and an olefinic proton at δ 5.39 (1H, br.s). Acid hydrolysis of **2** with trifluoroacetic acid at 110 °C for 6 h provided the same aglycone as that of **1** but with the sugar moiety composed of L-rhamnose, D-xylose and D-glucose. The spectroscopic and chemical data showed that compound **2** was a tetraglycoside with an ester linkage to C-28 of the aglycone based on norarjunolic acid, which was confirmed by determination of key HMBC correlations from H-1 of the terminal β-D-xylopyranosyl unit at δ 5.22 to C-3 of the inner α-L-rhamnopyranosyl unit (rham) at δ 83.2, H-1 of rham at δ 5.86 to C-4 of the inner β-D-glucopyranosyl unit (glc′) at δ 77.3, H-1 of glc′ at δ 4.91 to C-6 of the additional inner β-D-glucopyranosyl unit (glc) at δ 69.2, and from H-1 of glc at δ 6.18 to C-28 of the aglycone at δ 175.7. Furthermore, the ^1H and ^{13}C NMR spectroscopic assignments of each monosaccharide were established by the interpretation of the HMQC and HMBC data. Thus the structure of **2** was identified as 2α, 3β, 23-trihydroxy-30-norolean-12-en-28-oic acid β-D-xylopyranosyl-(1 → 3)-*O*-α-L-rhamnopyranosyl-(1 → 4)-*O*-β-D-glucopyranosyl-(1 → 6)-*O*-β-D-glucopyranosyl ester.

Mutongsaponin C (**3**) was deduced as $C_{53}H_{86}O_{23}$ from the positive-ion HR-ESI-MS (*m/z* 1113.5463 $[M + Na]^+$) and ^{13}C NMR spectrum (53 carbon signals). The ^1H NMR spectrum of **3** contained four three-proton singlet signals at δ 1.17, 1.11, 1.06, 1.06 (each s) and two three-proton doublets at δ 0.89, 0.85 (each d), an olefinic proton signal at δ 5.42 (1H, br.s) and four anomeric proton signals at δ 6.20 (d, $J = 8.0$ Hz), 5.88(br. s), 5.24 (d, $J = 8.0$ Hz), 4.92 (1H, d, $J = 6.0$ Hz). When the ^{13}C NMR spectrum of **3** was compared with that of **2** and **10**, the data of the sugar moiety and a majority of the aglycone were the same except for differences at C-12, C-13 and the E-ring part of the aglycone. Additionally, combining the ^1H and ^{13}C NMR spectra for **3** indicated that it was a pentacyclic triterpene tetraglycoside based on hydroxyurs-12-en-28-oic acid. Acid hydrolysis of **3** with trifluoroacetic acid yielded 2α, 3β, 23-trihydroxyurs-12-en-28-oic acid, L-rhamnose, D-xylose and D-glucose, according to its ^{13}C chemical shift data and co-TLC analysis with the authentic compound. The HMBC spectrum further validated the sugar sequences of the tetraglycoside moiety and linkage position of compound **3**. Therefore, the structure of **3** was determined as 2α, 3β, 23-trihydroxyurs-12-en-28-oic acid β-D-xylopyranosyl-(1 → 3)-*O*-α-L-rhamnopyranosyl-(1 → 4)-*O*-β-D-glucopyranosyl-(1 → 6)-*O*-β-D-glucopyranosyl ester.

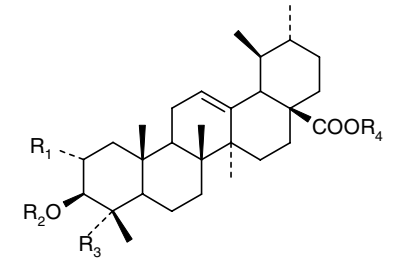
Mutongsaponin D (**4**) had the molecular formula of $C_{58}H_{92}O_{27}$ as indicated from its positive ion HR-ESI-MS (*m/z* 1243.5719 $[M + Na]^+$), which was the same as that of **8** as well as having one more oxygen than **9**. The ^1H NMR spectrum indicated the presence of five anomeric protons at δ 6.19 (d, $J = 8.0$ Hz), 5.84 (br.s), 5.31 (d, $J = 8.0$ Hz), 4.98 (d, $J = 6.0$ Hz), 4.94 (d, $J = 7.5$ Hz), four methyl groups at δ 1.13 (3H, s), 1.08 (3H, s), 0.94 (3H, s), 0.93 (3H, s), an exomethylene group at δ 4.72, 4.66 (each s) and an olefinic proton at δ 5.42 (br.s). By comparison with



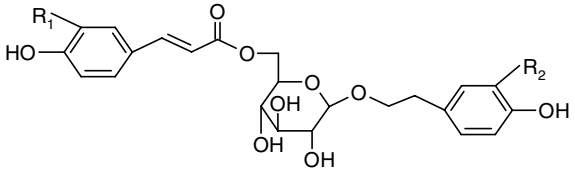
6 R_1=H, R_2=ara[(2-1)glc](3-1)glc, R_3=CH_3,R_4=glc(6-1)glc(4-1)rham
7 R_1=H, R_2=ara[(2-1)glc](3-1)glc, R_3=CH_3, R_4=glc(6-1)glc
8 R_1=H, R_2=ara(2-1)glc, R_3=CH_3, R_4=glc(6-1)glc(4-1)rham
10 R_1=OH, R_2=H, R_3=CH_2OH, R_4=glc(6-1)glc(4-1)rham(3-1)xyl
11 R_1=OH, R_2=H, R_3=CH_2OH, R_4=glc(6-1)glc(4-1)rham
14 R_1=H, R_2=ara[(2-1)glc](3-1)glc, R_3=CH_3, R_4=H
16 R_1=H, R_2=ara(2-1)glc, R_3=CH_3, R_4=H
17 R_1=H, R_2=ara(3-1)glc, R_3=CH_3, R_4=H



1 R_1=OH, R_2=H, R_3=CH_2OH, R_4=glc
2 R_1=OH, R_2=H, R_3=CH_2OH, R_4=glc(6-1)glc(4-1)rham(3-1)xyl
4 R_1=H, R_2=ara(3-1)glc, R_3=CH_2OH, R_4=glc(6-1)glc(4-1)rham
5 R_1=H, R_2=ara(2-1)xyl, R_3=CH_3, R_4=glc(6-1)glc(4-1)rham
9 R_1=H, R_2=ara(2-1)glc, R_3=CH_3, R_4=glc(6-1)glc(4-1)rham
13 R_1=OH, R_2=H, R_3=CH_2OH, R_4=glc(6-1)glc(4-1)rham
15 R_1=H, R_2=ara(2-1)glc, R_3=CH_3, R_4=H



3 R_1=OH, R_2=H, R_3=CH_2OH, R_4=glc(6-1)glc(4-1)rham(3-1)xyl
12 R_1=OH, R_2=H, R_3=CH_2OH, R_4=glc(6-1)glc(4-1)rham



18 R_1=OH, R_2=OH
19 R_1=OMe, R_2=OH
20 R_1=OH, R_2=H

Fig. 1. Structures of the compounds **1**–**20**.

its ^{1}H and ^{13}C NMR spectra of compounds **4** and **9**, **4** was assigned to be a 3, 28-bisdesmoside with five monosaccharide units based on 23-hydroxy-30-norolean-12-en-28-oic acid, containing the same linkage sequence of the sugar moiety and the same ester-glycoside chain as compound **9**, which was further confirmed by acid hydrolysis and the key HMBC correlation. The linkage of glc(1 → 3)ara involved in C-3 of the aglycone was shown by a HMBC correlation from H-1 of the terminal β-D-glucopyranosyl unit at δ 5.31 to C-3 of the inner α-L-arabinopyranosyl unit at δ 84.3. From the above information, the structure of **4** was elucidated as 3-β-[(β-D-glucopyranosyl-(1 → 3)-*O*-α- L-arabinopyranosyl)oxy]-23-hydroxy-30-norolean-12-en-28-oic acid α-L-rhamnopyranosyl-(1 → 4)-*O*-β-D-glucopyranosyl-(1 → 6)- O-β-D-glucopyranosyl ester.

Mutongsaponin E (**5**) had the molecular formula of $C_{57}H_{90}O_{25}$ as indicated from its positive ion HR-ESI-MS (*m*/*z* 1197.5627 $[M + Na]^+$) and positive ion FAB-MS (*m*/*z* 1197 $[M + Na]^+$ and 1175 $[M + H]^+$). The ^{1}H NMR spectrum suggested signals for five anomeric protons at δ 6.21 (d, $J = 8.5$ Hz), 5.87 (br.s), 5.08 (d, $J = 4.0$ Hz), 4.97 (d, $J = 7.5$ Hz), 4.88 (d, $J = 5.0$ Hz), five methyl groups at δ 1.25 (3H, s), 1.20 (3H, s), 1.07 (6H, s, Me × 2), 0.88 (3H, s), an exomethylene group at δ 4.74, 4.68 (each s) and an olefinic proton at δ 5.42 (br.s). Its ^{1}H and ^{13}C NMR spectra showed that **5** had the same aglycone, 30-norolean-12-en-28-oic acid, and ester-glycoside linkage as compound **9**, but differed in the oligosaccharide part related to C-3 of the aglycone. On acid hydrolysis, **5** gave the aglycone of 30-norolean-12-en-28-oic acid and a sugar moiety composed of L-arabinose, L-rhamnose, L-xylose and D-glucose. The α or β configuration of the monosaccharides was estimated due to the coupling constant (*J* value) of the anomeric proton signal and the chemical shifts of ^{1}H and ^{13}C NMR spectra. The HMBC correlations from H-1 of the xylopyranosyl unit at δ 5.08 to C-2 of arabinopyranosyl unit at δ 81.7, as the H-1 of arabinopyranosyl unit at δ 4.88 to C-3 of the aglycone at δ 88.9, revealed that **5** had a linkage of xyl(1 → 2)ara involved in C-3 of the aglycone. Thus the structure of **5** was identified as 3-β-[(α-L-xylopyranosyl-

(1 → 2)-*O*-α-L-arabinopyranosyl) oxy]-30-norolean-12-en-28-oic acid α-L-rhamnopyranosyl-(1 → 4)-*O*-β-D-glucopyranosyl-(1 → 6)-*O*-β-D-glucopyranosyl ester.

3. Concluding remarks

The current study, to the best our knowledge, is the first chemical investigation of the stems of *A. trifoliata* var. *australis*. Compounds **1**–**5** are new triterpene saponins based on an olean-12-en-28-oic acid skeleton. Moreover, compounds **3** and **10** were confirmed to be prominent compounds in the stems of *A. trifoliata* and *A. trifoliata* var. *australia*. By contrast, they were rarely found in the stems of *A. quinata* upon comparison of the HPLC chromatograms of the MeOH extracts of the stems from the three plant species. Accordingly, these two compounds could be regarded as chemical markers in distinguishing *A. trifoliata* or *A. trifoliata* var. *australia* from *A. quinata.*

Compounds **18**, **19** and **20**, due to the phenylethanoid glycosides, were obtained from the genus *Akebia* for the first time. The conventional TLC examinations and HPLC profiles of the samples collected from different growing areas showed that the phenylethanoid glycosides were widely distributed in the roots, stems, leafs or fruits of *A. trifoliata*, *A. trifoliata* var. *Australia* and *A. quinata*. The occurrence of phenylethanoid glycosides in the plants of the genus *Akebia* not only increases the chemical diversity but also has chemotaxonomic significance, revealing the certainly close relationship between *Akebia* and *Sargentodoxa*. Lardizabalaceae is a relatively small family with about 50 species of nine genera, namely, *Decaisnea, Akebia, Archakebia, Holboellia, Stauntonia, Sinofranchetia, Sargentodoxa, Lardizabala* and *Boquila* (Chinese Academy of Sciences, 2001). The chemotaxonomy of Lardizabalaceae has been settled according to previous chemical analyses and phytotaxonomic characteristics (Zheng and Yang, 2001). However, there has been some debate on the phylogenetic classification status of *Sargentodoxa*. Most investigations have suggested moving *Sargentodoxa* to a separate family, i.e., Sargentodoxaceae (Sheng and Liu, 2003; Xia and Peng, 1989; Xia and Kong, 1991), owing to the absence of phenolic components in the other genera of Lardizabalaceae and enriching in *Sargentodoxa* (Zheng and Yang, 2001), on the other hand, some experts insists that *Sargentodoxa* should be attached to Lardizabalaceae (Loconte et al., 1995; Wu and Kubitzli, 1997). Isolation of phenylethanoid glycosides from the genus *Akebia* provides new evidence on retaining both genera in a single family.

4. Experimental

4.1. General

NMR spectra were recorded on a Teol AL-500 (JEOL, 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR) spectrometer, with chemical shifts given as δ values with reference to tetramethylsilane (TMS) as an internal standard. FAB-MS, ESI-MS or HR-ESI-MS spectra were recorded on a Jabstec (Micromass) mass spectrometer and EI spectra on a Trace-MS spectrometer (Finigan). Sephadex LH-20 (Japan) and ODS silica gel (Fuji Silysia Chemical, Japan) were used for column chromatography. A Kromasil C_{18} column (250 mm × 10 mm, 10 μm) was used for preparative HPLC experiments, whereas a Phenomenex Luna C_{18} column (250 mm × 4.6 mm, 5 μm) was used for the HPLC analysis. A mixture of MeOH–H_2O–H_3PO_4 (35:65:0.05) for phenylethanoid glycosides, or MeOH–MeCN–H_2O–H_3PO_4 (20:20:60:0.1) for triterpene saponins, as the mobile phase was delivered by a Agillent HP1100 pump at a flow rate of 1.0 mL min^{-1}. MeCN and MeOH for analysis were of chromatographic grade, ethanol used for extraction was of chemical grade and other reagents were of analytical grade.

4.2. Plant materials

The stems of *A. trifoliata* var. *australis* were collected in the fields of E'mei mountain, Sichuan province, China, in June 2004. The plant was identified by Professor Xirong HE (*Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing, China*) and the voucher specimens (No. BMT-200406) have been deposited in our laboratory. The plant materials used for qualitative analysis were collected in the following places: *A. trifoliata* var. *australis*: Sichuan, Guiyang; *A. trifoliata*: Sichuan, Anhui, Jiangsu, Jiangxi; *A. quinata*: Anhui, Jiangsu, Jiangxi.

4.3. Extraction and isolation

A. trifoliata var. *australia* stems (dried weight, 3.0 kg) were extracted with hot EtOH-H_2O(7:3) by refluxing. The EtOH–H_2O (7:3) extract was concentrated under reduced pressure and partitioned using EtOAc and *n*-BuOH, respectively. The EtOAc-soluble fraction (57 g) was subjected to silica gel CC eluted with petroleum ether–EtOAc (1:1), $CHCl_3$–EtOAc (1:1), $CHCl_3$–MeOH (9:1, 3:1) and MeOH, respectively, to give six fractions (Frs. I–VI). Fr. IV was subjected to Sephadex LH-20 CC eluted with MeOH–H_2O (3:7) to give **18** (1400 mg), **19** (100 mg) and **20** (20 mg). Fr. V was separated by ODS silica gel CC eluted with MeOH–H_2O (6:4) to yield **1** (22 mg), **15** (20 mg), **16**(7.5 mg) and **17** (10 mg). The n-BuOH-soluble fraction (136 g) was passed through a macroporous resin (HPD_{100}) column eluted with H_2O and EtOH–H_2O (3:7, 5:5, 95:5), whereas the EtOH–H_2O (3:7) eluted portion was subjected to silica gel CC eluted with $CHCl_3$–MeOH–H_2O (15:8:1) and further purified by ODS silica gel CC eluted with MeOH–H_2O (5:5) or preparative HPLC using MeOH–H_2O (6:4) to give compounds **2** (200 mg), **3** (220 mg), **4** (23 mg), **6** (180 mg), **7** (10 mg), **8** (246 mg), **9** (18 mg), **10** (1000 mg) and **13** (12 mg). The EtOH–H_2O (5:5) eluted portion was subjected to silica gel CC eluted with $CHCl_3$–

MeOH–H_2O (18:8:1) and further purified by ODS silica gel CC, combining the crystallization to yield compounds **5** (13 mg), **11** (12 mg), **12** (10 mg) and **14** (27 mg).

4.3.1. Sample preparation for analysis

Each sample (2.0 g) of *A. quinata*, *A. trifoliata* and *A. trifoliata* var. *australis* were extracted with MeOH at room temperature. The MeOH extract was partitioned between H_2O and EtOAc, followed by *n*-BuOH. The EtOAc extract was used for the TLC examination or HPLC analysis of phenylethanoid glycosides, whereas the *n*-BuOH extract, in which the triterpene saponins were enriched, was used for the HPLC analysis of compounds **3** and **10**.

*4.3.2. Compound **1***

Amorphous solid. $[\alpha]_D^{27}$ +44.1 (MeOH; c0.2675). ESI-MS (positive mode): 673 $[M+K]^+$, 657 $[M+Na]^+$. FAB MS (positive mode): *m/z* 657 $[M+Na]^+$. HR-FAB-MS (positive mode): *m/z* 657.3649 $[M+Na]^+$(Calc.: 657.3614). ^1H NMR (C_5D_5N) δ: 6.28(1H, *d*, $J = 8.0$ Hz, glc-H-1), 5.40(1H, *br.s*, H-12), 4.72, 4.66(each 1H, *s*, H-29), 4.21, 3.70(each 1H, *d*, H-23), 3.09(1H, *dd*, H-18), 2.52(1H, *t*, $J = 13.5$ Hz), 2.13(1H, *dd*, $J = 14.5$, 4.0 Hz, H-19), 4.18(1H, glc-H-2), 4.26(1H, like-*t*, glc-H-3), 4.32(1H, *d*, $J = 9.5$ Hz, glc-H-4), 4.00(1H, *d*, $J = 8.5$ Hz, glc-H-5), 4.45(1H, *d*, $J = 11.5$ Hz), 4.38(glc-H-6), 1.12(3H, *s*, H-27), 1.11(3H, *s*, H-26), 1.07(3H, *s*, H-25), 1.05(3H, *s*, H-24). For ^{13}C NMR spectroscopic data, see Tables 1 and 2.

*4.3.2.1. Acid hydrolysis of **1**.* A solution of **1** (3.0 mg) in 2.0 M trifluoroacetic acid (2 mL) was heated at 110 °C for 6 h (kept sealed) and the reaction solution became cloudy. After cooling, the reaction mixture was extracted with EtOAc saturated with H_2O (3 mL × 2). The EtOAc extract and authentic sample (2α,3β,23-trihydroxy-30-norolean-12-en-28-oic acid) were detected by TLC and had the same R_f value, which indicated that the aglycone of **1** was 2α,3β,23-trihydroxy-30-norolean-12-en-28-oic acid. The H_2O residue was concentrated under reduced pressure and the identification of D-glucose was carried out by analysis of the chemical shifts of the ^1H and ^{13}C NMR spectra as well as comparison of the R_f value with that of the authentic sample (see Table 3).

*4.3.3. Compound **2***

Amorphous solid. $[\alpha]_D^{27}$ +5.4 (MeOH; c0.4508). ESI-MS (positive mode): 1097(100) $[M+Na]^+$, 561, 560(100), 418, 315, 304, 203. HR-ESI-MS (positive mode): *m/z* 1097.5139 $[M+Na]^+$(Calc.: 1097.5144). ^1H NMR(C_5D_5N) δ: 6.18(1H, *d*, $J = 8.0$ Hz, glc-H-1), 5.86(1H, *br.s*, rham-H-1), 5.22(1H, *d*, $J = 7.5$ Hz, xyl-H-1), 4.91(1H, *d*, $J = 7.5$ Hz, glc′-H-1), 5.39(1H, *br.s*, H-12), 4.69, 4.63(each

Table 1
^{13}C NMR spectroscopic data for the aglycone moiety of compounds **1**–**6** and **8**–**13** in pyridine-d_5

Carbon	1	13	2	9	5	4	10	3	11	12	8	6
1	47.6	47.5	47.4	38.9	38.9	38.9	47.7	48.1	47.8	48.0	38.7	39.9
2	68.8	68.8	68.8	26.5	26.6	26.1	68.8	68.9	68.9	68.9	26.5	26.6
3	78.1	78.3	78.0	88.9	88.9	81.9	78.1	78.2	78.1	78.2	88.8	89.0
4	43.6	43.6	43.6	39.6	39.6	43.6	43.6	43.6	43.6	43.6	39.9	39.9
5	48.1	47.8	47.7	55.9	55.9	47.5	47.8	47.9	48.1	47.7	55.8	55.9
6	18.5	18.5	18.5	18.5	18.5	18.2	18.5	18.5	18.6	18.5	18.5	18.6
7	32.7	32.7	32.7	33.1	33.3	32.9	33.9	33.2	34.0	33.1	30.5	30.5
8	40.0	40.0	40.0	39.9	39.8	40.0	39.9	40.2	40.1	40.2	39.5	39.7
9	47.8	48.1	48.1	47.6	47.5	47.6	48.1	48.2	48.3	48.1	48.0	48.1
10	38.4	38.3	38.3	37.0	36.9	36.9	38.3	38.3	38.4	38.3	36.9	37.0
11	23.9	23.9	23.9	23.8	23.8	23.9	23.9	23.8	24.0	23.8	23.4	23.4
12	123.1	123.1	123.1	123.1	123.1	123.0	122.7	126.0	122.9	126.0	122.9	122.9
13	143.4	143.4	143.5	143.5	143.4	143.5	144.1	138.5	144.2	138.5	144.1	144.1
14	42.1	42.1	42.1	42.1	42.1	42.2	42.1	42.6	42.3	42.5	42.1	42.1
15	28.1	28.1	28.1	28.3	28.2	28.3	28.2	28.7	28.3	28.7	28.3	28.3
16	23.5	23.4	23.4	23.6	23.5	23.6	23.3	24.6	23.4	24.5	23.8	23.8
17	47.8	47.8	47.7	47.4	47.3	47.4	46.9	48.3	47.1	48.3	46.2	46.3
18	47.2	47.3	47.2	48.1	48.0	48.2	41.6	53.2	41.7	53.2	41.7	41.7
19	41.6	41.6	41.6	41.7	41.7	41.7	46.1	39.3	46.2	39.3	47	47.1
20	148.5	148.3	148.3	148.3	148.4	148.4	30.7	39.1	30.8	39.1	30.8	30.8
21	30.1	30.0	30.0	30.5	30.5	30.5	32.7	36.7	32.9	36.8	33.9	34.1
22	37.6	37.6	37.6	37.7	37.6	37.7	32.4	30.8	32.6	30.8	32.5	32.6
23	66.3	66.3	66.2	28.3	27.9	64.4	66.3	66.5	66.8	66.5	28.2	28.1
24	14.4	14.4	14.3	16.7	16.4	13.7	14.3	14.4	14.3	14.4	16.7	16.8
25	17.5	17.5	17.4	15.6	15.6	16.2	17.4	17.7	17.5	17.7	15.6	15.6
26	17.4	17.6	17.5	17.5	17.5	17.6	17.5	17.8	17.7	17.8	17.5	17.5
27	26.0	25.9	25.9	26.0	25.9	26.2	25.9	23.7	26.1	23.7	26.0	26.1
28	175.7	175.8	175.7	175.8	175.7	175.8	176.5	176.3	176.5	176.3	176.5	176.5
29	107.3	107.3	107.3	107.4	107.4	107.3	33.0	17.4	33.1	17.3	33.1	33.1
30							23.6	21.3	23.7	21.2	23.6	23.7

Table 2
^{13}C NMR spectroscopic data for the sugar moiety of compounds **1**–**6** and **8**–**13** in pyridine-d_5

Carbon	1	13	2	9	5	4	10	3	11	12	8	6
28-glc	95.8	95.7	95.7	95.8	95.7	95.8	95.6	95.6	95.7	95.6	95.6	95.7
2	74.1	73.8	73.7	73.9	74.0	73.9	73.8	73.8	74.0	73.8	73.9	73.9
3	78.8	77.9	78.3	78.5	77.9	78.4	78.6	78.8	78.8	78.7	78.1	78.3
4	71.1	70.8	70.9	71.0	70.9	71.0	70.9	71.1	71.1	71.0	70.9	71.0
5	79.3	78.7	77.8	78.7	78.7	78.7	77.9	77.8	78.5	77.9	78.8	78.8
6	62.2	69.3	69.2	69.4	69.3	69.3	69.1	69.6	69.4	69.4	69.2	69.4
glc		104.9	104.9	104.9	104.9	104.9	104.9	105.2	104.9	105.0	104.9	104.9
		75.3	75.3	75.4	75.3	75.3	75.4	75.5	75.3	75.3	75.4	75.3
		76.4	76.3	76.5	76.5	76.6	76.3	76.4	76.6	76.5	76.5	76.6
		78.1	77.3	78.2	78.2	77.9	77.3	77.2	78.4	78.2	78.2	78.1
		77.1	77.1	77.2	77.2	77.2	77.1	77.2	77.1	77.2	77.2	77.2
		61.3	61.1	61.4	61.3	61.5	61.1	61.2	61.4	61.3	61.3	61.4
rham		102.7	102.3	102.8	102.7	102.8	102.3	102.4	102.8	102.7	102.7	102.8
		72.6	72.1	72.6	72.6	72.6	72.0	72.1	72.6	72.6	72.6	72.6
		72.8	83.2	72.8	72.8	72.8	83.2	83.5	72.8	72.8	72.8	72.8
		73.9	72.9	74.0	73.9	74.0	72.9	73.0	73.9	74	74.0	74.0
		70.3	69.9	70.3	70.3	70.3	69.9	70.0	70.4	70.3	70.3	70.3
		18.5	18.4	18.5	18.5	18.5	18.4	18.4	18.5	18.5	18.4	18.5
xyl			107.3				107.3	107.5				
			75.6				75.6	75.7				
			78.6				78.3	78.4				
			70.7				70.7	71.1				
			67.2				67.2	67.3				
3-*O*-ara				104.8	105.1	106.5					104.8	104.8
				80.9	81.7	72.0					81.0	72.5
				73.4	73.8	84.3					73.4	83.3
				68.2	68.7	69.4					68.3	68.7
				64.9	65.7	67.0					64.9	65.9
glc				106.0		106.4					106.0	104.4
				76.4		75.8					76.4	75.3
				78.1		78.4					78.1	77.5
				71.7		71.7					71.6	77.4
				77.9		78.7					78.0	78.6
				62.7		62.8					62.6	63.3
xyl(glc)					106.7							105.4
					76.2							76.1
					78.3							78.5
					71.1							71.6
					67.4							78.5
												62.6

1H, *s*, H-29), 4.19, 3.69 (1H, *d*, $J = 10.5$ Hz, H-23), 3.08(1H, *dd*, $J = 13.0$, 4.5 Hz, H-18), 2.49(1H, *t*, $J = 13.5$ Hz, H-19), 2.11(1H, *dd*, $J = 13.5$, 4.0 Hz, H-19), 1.09(3H, *s*, H-27), 1.10 (3H, *s*, H-26), 1.07(3H, *s*, H-25), 1.04(3H, *s*, H-24), 1.65(3H, *d*, $J = 6.0$ Hz, rham-H-6). For ^{13}C NMR spectroscopic data, see Tables 1 and 2.

4.3.3.1. 2α,3β,23-Trihydroxy-30-norolean-12-en-28-oic acid

EI-MS(70 eV): 472$[M]^+$, 442$[M-CH_2O]^+$, 436$[M-2H_2O]^+$, 428$[M-CO_2]^+$, 391, 295, 232, 220, 205, 189.

4.3.3.2. Acid hydrolysis of ***2****.* A solution of **2** (34.0 mg) was hydrolysed as for **1**. The EtOAc extract was chromatographed on silica gel eluting with $CHCl_3$–MeOH (10:1) to afford the aglycone (1.5 mg). The identification of the sugar moiety was carried out as for **1**.

4.3.4. Compound ***3***

White crystal (MeOH). $[\alpha]_D^{27}$ −10.8 (MeOH; c0.1060). ESI-MS (positive mode): 1113 (100)$[M+Na]^+$, 981$[M+Na\text{-xyl}]^+$, 927, 814$[M+H\text{-rha-xyl}]^+$, 701, 569, 568(100), 418, 315, 304, 264, 219, 203. HR-ESI-MS (positive mode): *m/z* 1113.5463 $[M+Na]^+$(Calc.: 1113.5458). ^1H NMR(C_5D_5N) δ: 6.20(1H,*d*, $J = 8.0$ Hz, glc-H-1), 5.88(1H, *br.s*, rham-H-1), 5.24(1H, *d*, $J = 8.0$ Hz, xyl-H-1), 4.92(1H, *d*, $J = 7.6$ Hz, glc′-H-1), 5.42(1H, *br.s*, H-12), 1.17(3H, *s*, H-26), 1.11(3H, *s*, H-25), 1.06(3H, *s*, H-27), 1.06(3H, *s*, H-24), 0.89(3H, *d*, $J = 6.4$ Hz, H-29), 0.85(3H, *d*, $J = 5.6$ Hz, H-30), 1.67(3H, *d*, $J = 6.0$ Hz, rham-H-6). For ^{13}C NMR spectroscopic data, see Tables 1 and 2.

4.3.4.1. 2α,3β,23-Trihydroxyurs-12-en-28-oic acid. EI-MS(70 eV): 488$[M]^+$, 452$[M-2H_2O]^+$, 442$[M-HCOOH]^+$,

Table 3
^{1}H NMR spectroscopic data and key HMBC correlations for compounds **1**–**5**

Aglycone	H No.	1		2		3		4		5	
		δH	HMBC	δH	HMBC	δH	HMBC	δH	HMBC	δH	HMBC
	12	5.40(1H, br.s)		5.39(1H, br.s)		5.42(1H, br.s)		5.42(1H, br.s)		5.42(1H, br.s)	
	18	3.09(1H, dd)	C-12,13,28	3.08(1H, dd)	C-12,13,14,28	2.48(1H, dd)		3.10(1H, dd)		3.25(1H, dd)	C-16,17
	19	2.52, 2.13(each 1H, d)	C-20,29	2.49, 2.11(each 1H, d)	C-18,20,29			2.52, 2.14 (each 1H, d)		2.56, 2.17(each 1H)	C-20
	23	4.21, 3.70(each 1H, d)		4.19, 3.69(each 1H, d)						1.25(3H, s)	C-3,5
	24	1.05(3H, s, Me)		1.04(3H, s)	C-23	1.06(3H, s)		0.93(3H, s)		1.07(6H, s)	C-3,5
	25	1.07(3H, s, Me)		1.07(3H, s)		1.11(3H, s)		0.94(3H, s)		0.88(3H, s)	C-5,9
	26	1.11(3H, s, Me)		1.10(3H, s)		1.17(3H, s)		1.08(3H, s)		1.07(6H, s)	C-9
	27	1.12(3H, s, Me)		1.09(3H, s)		1.06(3H, s)		1.13(3H, s)		1.20(3H, s)	
	29	4.72, 4.66(each 1H, s)		4.69, 4.63(each 1H, s)	C-19,21	0.89(3H, d, $J = 6.4$ Hz)		4.72, 4.66 (each 1H, s)		4.74, 4.68(each 1H, s)	
	30					0.85(3H, d, $J = 5.6$ Hz)					
28-*O*-sugar (inner)	glc-1	6.28(1H, d, $J = 8.0$ Hz)	C-28	6.18(1H, d, $J = 8.0$ Hz)	C-28	6.20(1H, d, $J = 8.0$ Hz)	C-28	6.19(1H, d, $J = 8.0$ Hz)	C-28	6.21(1H, d, $J = 8.0$ Hz)	C-28
	glc′-1			4.91(1H, d, $J = 7.5$ Hz)	glc-C-6	4.92(1H, d, $J = 7.6$ Hz)	glc-C-6	4.98(1H, d, $J = 6.0$ Hz)	glc-C-6	4.97(1H, d, $J = 7.5$ Hz)	glc-C-6
	rham-1			5.86(1H, br.s)	glc′-C-4	5.88(1H, br.s)	glc′-C-4	5.84(1H, br.s)	glc′-C-4	5.87(1H, br.s)	glc′-C-4
	xly-1			5.22(1H, d, $J = 7.5$ Hz)	rham-C-3	5.24(1H, d, $J = 8.0$ Hz)	rham-C-3				
3-*O*-sugar (inner)	ara-1							4.94(1H, d, $J = 7.5$ Hz)	C-3	4.88(1H, d, $J = 5.0$ Hz)	C-3
	glc′′-1							5.31(1H, d, $J = 8.0$ Hz)	ara-C-3	5.08(1H, d, $J = 4.0$ Hz)	ara-C-2
	xyl-1										

407, 313, 248(100), 219, 203(95), 189. ^{13}C NMR(C_5D_5N) δ: 179.9,139.2, 125.7, 78.1, 68.8, 66.3, 53.1, 48.1, 47.9, 47.7, 46.9, 42.5, 42.1, 39.9, 39.8, 39.3, 38.2, 37.4, 33.2, 31.0, 28.5, 24.8, 23.8, 23.7, 21.3, 18.4, 17.5, 17.3, 17.2, 14.4.

*4.3.4.2. Acid Hydrolysis of **3**.* A solution of **3**(22.5 mg) was hydrolysed and analyzed as above for compound **2**.

*4.3.5. Compound **4***

Amorphous solid. $[\alpha]_D^{27}$ +13.5(MeOH; c0.1133). ESI-MS (positive mode): 1243$[M+Na]^+$, 927$[M+H\text{-ara-glc}]^+$, HR-ESI-MS (positive mode): *m/z* 1243.5719 $[M+Na]^+$ (Calc.: 1243.5724). ^{1}H NMR(C_5D_5N) δ: 6.19(1H, *d*, $J = 8.0$ Hz, glc-H-1), 5.84(1H, *br.s*, rham-H-1), 5.31(1H, *d*, $J = 8.0$ Hz, glc″-H-1), 4.98(1H, *d*, $J = 6.0$ Hz, glc′-H-1), 4.94(1H, *d*, $J = 7.5$ Hz, ara-H-1), 4.72,4.66(each *s*, H-29), 5.42(1H, *br.s*, H-12), 3.10(1H, *dd*, $J = 13.0$, 4.5 Hz, H-18), 1.69(3H, *d*, $J = 6.5$ Hz, rham-H-6), 1.13(3H, *s*, H-27), 1.08(3H, *s*, H-26), 0.94(3H, *s*, H-25), 0.93(3H, *s*, H-24). For ^{13}C NMR spectroscopic data, see Tables 1 and 2.

*4.3.5.1. Acid Hydrolysis of **4**.* A solution of **4** (5.5 mg) was hydrolysed and analyzed as for compound **2**.

*4.3.6. Compound **5***

Amorphous solid. ESI-MS (positive mode): 1197$[M+Na]^+$, 867$[M+H\text{-ram-glc}]^+$, FAB-MS(positive mode): 1197$[M+Na]^+$, 1175$[M+H]^+$, 1043$[M+H\text{-xyl}]^+$, 911$[M+H\text{-xyl-ara}]^+$, 765$[911\text{-rham}]^+$, 603$[765\text{-glc}]^+$, 423$[\text{aglycone-}H_2O]^+$, HR-ESI-MS (positive mode): *m/z* 1197.5627 $[M+Na]^+$(Calc.: 1197.5669). ^{1}H NMR(C_5D_5N) δ: 6.21(1H, *d*, $J = 8.0$ Hz, glc-H-1), 5.87(1H, *br.s*, rham-H-1), 5.08(1H, *d*, $J = 4.0$ Hz, xyl-H-1), 4.97(1H, *d*, $J = 7.5$ Hz, glc′-H-1),4.88(1H, *d*, $J = 5.0$ Hz, ara-H-1), 5.42(1H, *br.s*, H-12), 4.74, 4.68(each 1H, *s*, H-29, 3.25(1H, *dd*, H-18), 2.56, 2.17(each 1H, H-19), 1.25(3H, *s*, H-23), 1.20(3H, *s*, H-27), 1.07(6H, *s*, H-26, H-24), 0.88(3H, *s*, H-25). For ^{13}C NMR spectroscopic data, see Tables 1 and 2.

*4.3.6.1. Acid Hydrolysis of **5**.* A solution of **5** (3.0 mg) was hydrolysed and analyzed as for compound **2**.

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