Triterpenoid Saponins from the Fruits of *Akebiae quinata*

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Three new triterpenoid saponins together with eight known compounds have been isolated from the fruits of *Akebiae quinata*. On the basis of the spectroscopic and physiochemical evidence, the new compounds were elucidated as 3-*O*-β-D-xylopyranosyl-(1→2)-α-L-arabinopyranosyl gypsogenin, 3-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl gypsogenin and 3-*O*-β-D-xylopyranosyl-(1→2)-α-L-arabinopyranosyl-28-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl gypsogenin.

Key words *Akebiae quinata*; triterpenoid saponin; gypsogenin

Akebiae quinata (THUNB.) DECNE. a well-known Chinese medicinal plant, is widely distributed in China, the fruits of the plant named "Bayuezha" is popularly used as an antineoplastic, diuretic agent in traditional Chinese medicine. Previous investigations revealed the genus *Akebiae* possessing rich triterpenoid saponins. Up to date, more than thirty triterpenoid saponins have been isolated from the fruits, leaves and roots of the plant of this genus.[1)] As a continuous investigation on the chemical constituents of the fruits of *Akebiae quinata*, eleven triterpenoid saponins including three new ones have been afforded. Herein we describe the isolation and structure elucidation of the new compounds.

Results and Discussion

The dried fruits of *Akebiae quinata* (THUNB.) DECNE. were extracted with hot 95% ethanol, After removal of the solvent under reduced pressure at 60 °C, the residue was suspended in water and defatted with petroleum ether. The aqueous layer was further extracted with ethyl acetate and *n*-butanol successively. A portion of the *n*-butanol extract was subjected to D101 porous polymer resin, silica gel, ODS C18 column chromatography and purefied by preparative HPLC (PHPLC) to afford eleven triterpinoid saponins including three new compounds. By comparing their NMR data with literatures, the known compounds were elucidated as hederagenin 3-*O*-α-L-arabinopyranoside (**1**),[2)] oleanolic acid 3-*O*-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranoside (**4**),[3)] hederagenin 3-*O*-α-L-arabinopyranosyl-28-*O*-β-D-glucopyranosyl(1→6)-β-D-glucopyranoside (**5**),[4)] hederagenin 3-*O*-β-D-glucopyranosyl(1→3)-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranoside (**6**),[5)] hederagenin 3-*O*-{β-D-glucopyranosyl(1→4)-[α-L-rhamnopyranosyl(1→2)]}-α-L-arabinopyranoside (**7**),[6)] hederagenin 3-*O*-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranosyl-28-*O*-β-D-glucopyranosyl(1→6)-β-D-glucopyranoside (**8**),[7)] oleanolic acid 3-*O*-β-D-xylopyranosyl(1→2)-α-L-arabinopyranosyl-28-*O*-β-D-glucopyranosyl(1→6)-β-D-glucopyranoside (**10**)[8)] and hederagenin 3-*O*-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranosyl-28-*O*-α-L-rhamnopyranosyl(1→4)-β-D-glucopyranosyl(1→6)-β-D-glucopyranoside (**11**).[9)] The new compounds **2**, **3** and **9** were elucidated on the basis of their spectroscopic and physiochemical evidence.

Compound **2** was obtained as a white amorphous powder. The positive mode HR-FAB-MS showed a $[M+Na]^+$ ion peak at *m/z* 757.4157, in accordance with an empirical molecular formula of $C_{40}H_{62}O_{12}$, which was supported by the ^{13}C-NMR spectrum and various DEPT data. The ^{13}C-NMR spectrum showed 40 carbons, of which 30 of them were assigned to the aglycone, 10 of them to the sugar moieties. The ^{1}H-NMR spectrum showed six tertiary methyl signals at δ 0.83, 0.93, 0.93, 0.98, 1.25 and 1.37 respectively, an olefinic proton signal at δ 5.44, an aldehyde proton signal at δ 9.79 and an oxymethine proton signal at δ 4.00. The ^{13}C-NMR spectrum indicated two olefinic carbon signals (δ 122.2, 144.8), two carbonyl carbon signals (δ 180.2, 208.4) and an oxymethine carbon signal (δ 82.7). Detailed analysis of the NMR data established the aglycone to be gypsogenin.[10)] Acid hydrolysis of compound **2** with 2 N CF_3COOH afforded gypsogenin and monosaccharides L-Ara and D-Xyl. The chemical shifts δ 82.7 (C-3) and 180.2 (C-28) suggested compound **2** was a monodesmosidic glycoside and the sugar moieties were linked to C-3. The ^{1}H-NMR spectrum showed two anomeric proton signals at δ 4.77 (d, J=6.5 Hz) and 4.97 (d, J=6.5 Hz), the corresponding carbon signals in the ^{13}C-NMR spectrum of compound **2** were presented at δ 103.1 and 106.6 respectively. The coupling constants suggested α-anomeric configuration for the arabinosyl unit and a β-anomeric configuration for the xylosyl unit. All protons and carbons were unambiguously assigned by ^{1}H–^{1}H COSY, TOCSY, HSQC and HMBC experiments. The linkage of the sugars and the sugars with aglycone were established by HMBC experiment, in the HMBC spectrum, H-1 of arabinose showed long-range correlation with C-3 of the aglycone, confirming the arabinose was linked to C-3 of the aglycone; H-1 of xylose showed interaction signal with C-2 of arabinose, suggesting the xylose was linked to C-2 of the ara-

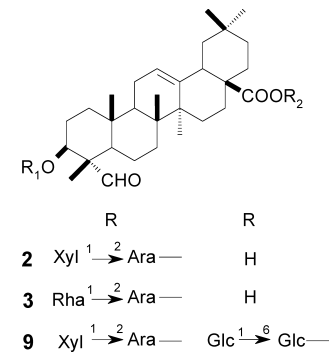

Fig. 1. Structures of Compounds **2**, **3**, and **9**

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binose. From the above evidence, the structure of compound **2** was identified as 3-*O*-*β*-D-xylopyranosyl-(1→2)-*α*-L-arabinopyranosyl gypsogenin.

Compound **3** was obtained as a white amorphous powder. Its molecular formula was determined as $C_{41}H_{64}O_{12}$ by the positive-ion HR-FAB-MS. The ^{1}H- and ^{13}C-NMR spectra data suggested compound **3** was a monodesidic glycoside with the same aglycone (gypsogenin) as that in compound **2**. Acid hydrolysis of compound **3** with 2 N CF_3COOH afforded gypsogenin and monosaccharides L-Ara and L-Rha. In the ^{1}H-NMR spectrum showed two anomeric proton signals at δ 4.70 (d, J=5.5 Hz) and 6.07 (br s) respectively, The coupling constant (J=5.5 Hz) suggested α-anomeric configuration for the arabinose. Starting from the anomeric protons of each sugar unit, all the hydrogens within each spin system were assigned using ^{1}H–^{1}H COSY and TOCSY experiments, while the carbons were assigned by HSQC, DEPT and further confirmed by HMBC experiments. The linkage of the sugars and the sugars with aglycone were established from the following HMBC correlations: H-1 of arabinose with C-3 of aglycone; H-1 of rhamnose with C-2 of arabinose. Accordingly, the structure of compound **3** was identified as 3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*α*-L-arabinopyranosyl gypsogenin.

Compound **9** was obtained as a white amorphous powder. Its positive mode HR-ESI-MS showed a quasi-molecular ion peak at *m*/*z* 1081.5215 ([M+Na]$^+$), corresponding to a molecular formula of $C_{52}H_{82}O_{22}$. Contrasting to compound **2** revealed compound **9** had the same aglycone (gypsogenin) as that in compound **2** and **3**. The chemical shifts δ 82.7 (C-3) and 176.4 (C-28) suggested compound **9** was a bidesmosidic glycoside. Acid hydrolysis of compound **9** afforded gypsogenin and monosaccharides L-Ara, D-Glu and D-Xyl. The ^{1}H- and ^{13}C-NMR spectra showed four anomeric proton signals at δ 4.36 (d, J=6.0 Hz), 4.95 (d, J=7.0 Hz), 5.02 (d, J=7.5 Hz) and 6.23 (d, J=8.0 Hz), the corresponding anomeric carbon signals at δ 103.1, 106.6, 105.2 and 95.9. The coupling constants 6.0—8.0 Hz suggested all the sugar units except arabinose were existed in a β-anomeric configuration. The ^{1}H- and ^{13}C-NMR chemical shift assignments were accomplished by a combination of ^{1}H–^{1}H COSY, TOCSY, DEPT, HSQC and HMBC experiments. Comparing the NMR data with compound **2** revealed that the sugar structure at C-3 was the same as that in compound **2**, which was also confirmed by HMBC experiment. The linkage of the remaining two sugars at C-28 was determined from the following HMBC correlations: H-1 (δ 5.02) of outer glucose with C-6 (δ 69.6) of inner glucose, H-1 (δ 6.23) of inner glucose with C-28 (δ 176.4) of aglycone. Therefore, the structure of compound **9** was elucidated as 3-*O*-*β*-D-xylopyranosyl-(1→2)-*α*-L-arabinopyranosyl-28-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl gypsogenin.

Experimental

General Experimental Methods Melting points were determined on X-4 digital micro melting point apparatus and were uncorrected. Optical rotations were recorded on a Perkin-Elmer 243B digital polarimeter. UV spectra were obtained from TU-1901 spectrometer. IR spectra were recorded on an AVATER-360 spectrometer. NMR spectra were recorded in pyridine-d_6 using Inova NMR spectrometer (500 Hz for ^{1}H and 125 Hz for ^{13}C) with tetramethylsilane as internal standard. HR-FAB-MS spectra were measured on an Autospec-UltimaETOF spectrometer in positive ion mode. HR-ESI-MS was recorded on a Q-T2ap LC/MS/MS System. PHPLC were carried on ODS column (Alltech 250×10 mm i.d., 5 μm) with an ELSD detector. GC analysis was carried out on an Agilent 6890N gas chromatogragh using a HP-5 capillary column (28 m×0.32 mm, i.d.); detection, FID; detector temperature, 260 °C; column temperature, 180 °C; carrier gas, N_2, flow rate, 1.0 ml/min. For CC, silica gel (200—300 mesh, Qingdao Mar. Chem. Ind. Co. Ltd.), D101 porous polymer resin (Tianjin Chem. Ind. Co. Ltd.).

Plant Material The fruits of *Akebiae quinata* (Thunb.) Decne. was purchased from Anguo market, Hebei province, People's Republic of China, and identified by one of the authors (Pengfei Tu). A voucher specimen is deposited in the herbarium of Peking University Modern Research Center for Traditional Chinese Medicine (A20030221).

Extraction and Isolation The dried fruits (25 kg) of *Akebiae quinata* were extracted with hot 95% ethanol (3×150 l). After removal of the solvent under reduced pressure at 60 °C, the residue (3.0 kg) was suspended in water and defatted with petroleum ether. The aqueous layer was further extracted with ethyl acetate and *n*-butanol successively. A portion of *n*-butanol extract (300 g) was subjected to D101 porous polymer resin and eluted with 30% MeOH, 70% MeOH successively to afford fraction 1 (190 g) and fraction 2 (100 g). Fraction 2 was chromatographed on a silica gel column (2 kg, 100—200 mesh) and eluted with $CHCl_3$–MeOH in a gradient mode afforded four subfractions D-1—D-4. Subfraction D-1 was purified by PHPLC (MeOH–0.5% TFA, 80 : 20) to obtain compound **1** (60 mg). Subfraction D-2 was chromatographed on an ODS column and purified by PHPLC (MeOH–0.5% TFA, 75 : 25) to afford compounds **2** (10 mg), **3** (15 mg), **4** (30 mg) and **5** (50 mg). Subfraction D-3 was chromatographed on a silica gel column (eluted by $CHCl_3$–MeOH–H_2O, 8 : 5 : 2) and purified by PHPLC (MeOH–0.5% TFA, 77 : 23) to obtain compounds **6** (43 mg) and **7** (8 mg). Subfraction D-4 was chromatographed on a silica gel column (eluted by $CHCl_3$–MeOH–H_2O, 13 : 5 : 2) to afford subfraction D-4-1, D-4-2 and compound **8** (114 mg); subfraction D-4-1 was chromatographed on a PHPLC (MeOH–0.5% TFA, 69 : 31) to afford compounds **9** (16 mg) and **10** (2400 mg). Subfraction D-4-2 was purified by PHPLC (MeOH–0.5% TFA, 70 : 30) to afford compound **11** (58 mg).

Compound **2** obtained as a white amorphous powder, mp. 207 °C. UV λ_{max} (MeOH) nm: 199, 251, 256. IR (KBr) cm^{-1}: 3410, 2925, 2855, 1682, 1445, 1384, 1208, 1141, 1079, 1050. $[\alpha]_D^{20}$ +40.7° (c=1.80, MeOH). HR-FAB-MS *m*/*z*: 757.4157 ([M+Na]$^+$, Calcd for $C_{40}H_{62}O_{12}$: 757.4139). ^{1}H-

Table 1. ^{13}C-NMR Spectroscopic Data of Compounds **2**, **3** and **9** (in Pyridine-d_6)

No.	**2**	**3**	**9**	No.	**2**	**3**	**9**
1	38.1	38.2	38.1	Ara			
2	25.2	25.2	25.1	1	103.1	102.1	103.1
3	82.7	80.2	82.7	2	81.2	75.1	81.6
4	55.1	55.4	55.0	3	73.5	73.8	73.8
5	48.5	48.3	48.4	4	68.4	68.7	68.6
6	20.4	20.5	20.4	5	65.6	65.1	65.9
7	32.4	32.4	32.4	Xyl (Rha)	Xyl	Rha	Xyl
8	39.9	39.9	40.1	1	106.6	101.2	106.6
9	47.8	47.8	47.8	2	76.0	72.4	76.3
10	36.2	36.1	36.2	3	78.2	72.4	78.5
11	23.6	23.6	23.2	4	71.0	74.0	71.1
12	122.2	122.2	122.5	5	67.3	69.6	67.6
13	144.8	144.8	144.1	6		18.4	
14	41.9	41.9	41.6	Glc (inner)			
15	28.2	28.2	28.1	1			95.9
16	23.7	23.6	23.7	2			74.1
17	46.6	46.6	46.9	3			78.2
18	42.1	42.1	42.1	4			71.7
19	46.4	46.4	46.1	5			78.9
20	30.9	30.9	30.7	6			69.6
21	34.1	34.1	33.9	Glc (outer)			
22	33.1	33.1	32.4	1			105.2
23	208.4	207.5	208.4	2			75.4
24	10.6	10.6	10.6	3			78.6
25	15.6	15.5	15.7	4			71.2
26	17.3	17.3	17.4	5			78.6
27	26.1	26.1	26.0	6			62.8
28	180.2	180.1	176.4				
29	33.2	33.2	33.0				
30	23.7	23.7	23.6				

Table 2. ^{1}H-NMR Spectroscopic Data of Compounds **2**, **3** and **9** (in Pyridine-d_6)

No.	**2**	**3**	**9**
3	4.00 (1H, br d, J=11.5 Hz)	4.08 (1H, dd, J=4.0, 11.5 Hz)	3.98 (1H, dd, J=3.5, 11.5 Hz)
12	5.44 (1H, br s)	5.44 (1H, br s)	5.38 (1H, br s)
18	3.28 (1H, dd, J=3.5, 13.5 Hz)	3.26 (1H, dd, J=3.5, 13.5 Hz)	3.17 (1H, dd, J=3.5, 14.0 Hz)
23	9.79 (1H, s)	9.67 (1H, s)	9.80 (1H, s)
24	1.37 (3H, s)	1.36 (3H, s)	1.37 (3H, s)
25	0.83 (3H, s)	0.82 (3H, s)	0.87 (3H, s)
26	0.93 (3H, s)	0.93 (3H, s)	1.04 (3H, s)
27	1.25 (3H, s)	1.25 (3H, s)	1.19 (3H, s)
29	0.93 (3H, s)	0.94 (3H, s)	0.85 (3H, s)
30	0.98 (3H, s)	0.99 (3H, s)	0.86 (3H, s)
Ara			
1	4.77 (1H, d, J=6.5 Hz)	4.70 (1H, d, J=5.5 Hz)	4.76 (1H, d, J=6.0 Hz)
2	4.40 (1H, dd, J=6.5, 7.5 Hz)	4.44 (1H, dd, J=5.5, 7.5 Hz)	4.37 (1H, dd, J=6.0, 7.5 Hz)
3	4.23 (1H, dd, J=3.3, 7.5 Hz)	4.18 (1H, dd, J=3.5, 7.5 Hz)	4.19 (1H, dd, J=3.1, 7.5 Hz)
4	4.29 (1H, br s)	4.21 (1H, br s)	4.18 (1H, br s)
5a	3.70 (1H, br d, J=12.0 Hz)	3.72 (1H, br d, J=11.0 Hz)	3.67 (1H, br d, J=10.5 Hz)
5b	4.26 (overlapped)	4.27 (1H, m)	4.22 (1H, m)
Xyl (Rha)	Xyl	Rha	Xyl
1	4.97 (1H, d, J=6.5 Hz)	6.07 (1H, br s)	4.95 (1H, d, J=7.0 Hz)
2	4.04 (overlapped)	4.62 (1H, br s)	4.05 (overlapped)
3	4.09 (overlapped)	4.61 (1H, dd, J=3.0, 9.5 Hz)	4.08 (overlapped)
4	4.18 (1H, m)	4.31 (1H, dd, J=9.5, 9.5 Hz)	4.27 (1H, m)
5a	3.62 (1H, t, J=10.5 Hz)	4.53 (1H, m)	3.62 (1H, t, J=10.5 Hz)
5b	4.36 (1H, br d, J=10.5 Hz)	—	4.36 (1H, dd, J=4.8, 10.5 Hz)
6		1.66 (3H, d, J=6.0 Hz)	
Glc (inner)			
1			6.23 (1H, d, J=8.0 Hz)
2			4.09 (1H, dd, J=8.0, 9.0 Hz)
3			4.06 (1H, dd, J=9.0, 9.0 Hz)
4			4.29 (overlapped)
5			4.16 (1H, m)
6a			4.36 (overlapped)
6b			4.70 (1H, br d, J=10.6 Hz)
Glc (outer)			
1			5.02 (1H, d, J=7.5 Hz)
2			3.98 (1H, dd, J=7.5, 9.0 Hz)
3			4.16 (1H, overlapped)
4			4.18 (1H, overlapped)
5			3.88 (1H, m)
6a			4.33 (1H, dd, J=5.0, 12.0 Hz)
6b			4.46 (1H, dd, J=2.5, 12.0 Hz)

and ^{13}C-NMR spectral data see Tables 1 and 2.

Compound **3** obtained as a white amorphous powder, mp. 207 °C. UV λ_{max} (MeOH) nm: 199, 250, 256. IR (KBr) cm^{-1}: 3414, 2928, 2858, 1684, 1444,1384, 1206, 1139, 1053. $[\alpha]_D^{20}$ +19.4° (c=1.20, MeOH). HR-FAB-MS m/z: 771.4319 ([M+Na]$^+$, Calcd for $C_{41}H_{64}O_{12}$: 771.4295). ^{1}H- and ^{13}C-NMR spectral data see Tables 1 and 2.

Compound **9** obtained as a white amorphous powder, mp. 195 °C. UV λ_{max} (MeOH) nm: 199, 250, 256. IR (KBr) cm^{-1}: 3401, 2927, 2853, 1724, 1675, 1636, 1459, 1384, 1201, 1172, 1073. $[\alpha]_D^{20}$ +13.3° (c=1.50, MeOH). HR-ESI-MS m/z: 1081.5215 ([M+Na]$^+$, Calcd for $C_{52}H_{82}O_{22}$: 1081.5195). ^{1}H- and ^{13}C-NMR spectral data see Tables 1 and 2.

Acid Hydrolysis of Compounds 2, 3 and 9 Each compound (5 mg) was hydrolyzed with 2 N aqueous CF_3COOH (10 ml) at 110 °C for 8 h in a sealed tube. After this period, the reaction mixture was diluted with H_2O (20 ml) and extracted with EtOAc (3×10 ml). The combined EtOAc extracts was washed with H_2O and evaporated the solvent to afford gypsogenin. The aqueous layer was evaporated with MeOH repeatedly under vacuum to remove the solvent completely, The residue was dissolved in anhydrous pyridine (0.100 ml), then 1,1,1,3,3,3-hexamethyldisilazane (0.100 ml) and trimethylsilyl chloride (0.040 ml) were added, the reaction mixture was heated at 60 °C for 30 min, when the reaction was finished, the reaction mixture was kept under room temperature for 5 h, then filtrated by 0.45 μm membrane to remove the precipitation and analyzed by GC. D-Xylose (6.887 min) and L-arabinose (5.144 min) were detected from compound **2**; L-rhamnose (5.410 min) and L-arabinose (5.144 min) were detected from compound **3**; D-Glucose (12.866 min), L-arabinose (5.144 min) and D-xylose (6.887 min) were detected from compound **9** respectively.

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References

1) Liu G. Y., Wang H., Ma S. C., Lu J., Lin R. C., *Chin. Pharm. J.*, **39**, 330—332, 352 (2004).
2) Mao Q., Jia X. S., *Acta Pharmaceutica Sinica*, **24**, 269—274 (1989).
3) Shao C. J., Kasai R., Xu J. D., *Chem. Pharm. Bull.*, **36**, 601—608 (1988).
4) Kinjo J., Uemura H., Nakamura M., Nohara T., *Chem. Pharm. Bull.*, **42**, 1339—1341 (1994).
5) Saito S., Sumita S., Tamura N., Nakamura Y., Nishida K., Ito M., Ishiguro I., *Chem. Pharm. Bull.*, **38**, 411—414 (1990).
6) Opinya A. E., Norman R. F., *J. Nat. Prod.*, **59**, 431—435 (1996).
7) Kong J., Li X. C., Wei B. Y., Yang C. R., *Phytochemistry*, **33**, 427—430 (1993).
8) Ma S. C., Chen D. C., Zhao S. J., *Acta Pharmaceutica Sinica*, **29**, 285—289 (1994).
9) Shao C. J., Kasai R., Ohtani K., Tanaka O., Kohda H., *Chem. Pharm. Bull.*, **38**, 1087—1089 (1990).
10) Fu H. Z., Koike K., Li W., Nikaido T., Lin W. H., Guo D. A., *J. Nat. Prod.*, **68**, 754—758 (2005).