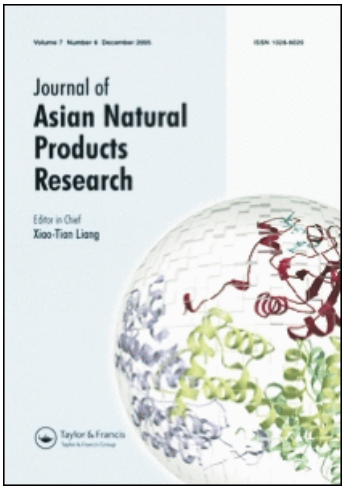
This article was downloaded by:
On: *22 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

A new triterpenoid saponin from the stem of *Akebia trifoliata* var. *australis*

Hui-Min Gao[a]; Zhi-Min Wang[a]; Wei-Hao Wang[a]
[a] Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing, China

To cite this Article Gao, Hui-Min , Wang, Zhi-Min and Wang, Wei-Hao(2009) 'A new triterpenoid saponin from the stem of *Akebia trifoliata* var. *australis*', Journal of Asian Natural Products Research, 11: 12, 1013 — 1018
To link to this Article: DOI: 10.1080/10286020903213439
URL: http://dx.doi.org/10.1080/10286020903213439

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

Journal of Asian Natural Products Research
Vol. 11, No. 12, December 2009, 1013–1018

Taylor & Francis
Taylor & Francis Group

A new triterpenoid saponin from the stem of *Akebia trifoliata* var. *australis*

Hui-Min Gao, Zhi-Min Wang* and Wei-Hao Wang

Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing 100700, China

(*Received 22 May 2009; final version received 26 July 2009*)

A new triterpene glycoside mutongsaponin F (**1**), together with five known saponins and two known lipids, was isolated from the 70% ethanol extract of the stems of *Akebia trifoliata* (Thunb.) Koidz. var. *australis* (Diels) Rehd. Their structures were elucidated on the basis of the spectroscopic analysis and physicochemical properties as 3-β-[(β-D-glucopyranosyl-(1 → 2)-*O*-[β-D-glucopyranosyl-(1 → 3)-*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 (**1**), 3-β-[(β-D-glucopyranosyl-(1 → 2)-*O*-[β-D-glucopyranosyl-(1 → 3)-*O*-]-α-L-arabinopyranosyl)oxy]-30-norolean-12-en-28-oic acid (**2**), leonticin E (**3**), collinsonidin (**4**), arjunolic acid 28-*O*-glucopyranoside (**5**), asiatic acid 28-*O*-glucopyranoside (**6**), soya-cerebroside I (**7**), and 1-*O*-α-L-galactosyl-(1 → 6)-*O*-β-D-galactosyl-3-*O*-hexadecanoyl-glycerol (**8**), respectively.

Keywords: *Akebia trifoliata* var. *australis*; mutongsaponin F; triterpenoid; lipids

Downloaded At: 18:32 22 January 2011

1. Introduction

The stems of *Akebia trifoliata* var. *australis,* together with the other two plants of the *Akebia* genus, *A. quinata* and *A. trifoliata*, were recorded in the Pharmacopoeia of the People's Republic of China as the original plant of *Caulis Akebiae* (Mutong in Chinese). It has been widely used in Chinese medicine as an antiphlogistic, a diuretic, and an analgesic [1]. A number of compounds including triterpenes, triterpenoid saponins, phenylethanoid glycosides, lignan glycosides, and coumarins have been isolated from the stems, fruits, or callus tissues [2–10] of the above three plants. We have previously reported five new triterpenoid saponins A–E from the stems of *A. trifoliata* var. *australis* [3]. As part of the continuous investigation of the chemical constituents of this species, a new triterpene glycoside, mutongsaponin F (**1**), was isolated and identified on the basis of spectroscopic analysis and chemical evidences, along with five known triterpenoid saponins (**2**–**6**) and two lipids (**7** and **8**) (Figure 1). In the present paper, the isolation and the structural elucidation of compound **1** are described.

2. Results and discussion

The molecular formula of mutongsaponin F (**1**) was established as $C_{64}H_{102}O_{31}$ by a positive HR-ESI-MS experiment at *m/z* 1389.6271 $[M+Na]^+$. Owing to the whole profiles of ^1H and ^{13}C NMR, **1** was deduced as a 30-norolean-type triterpene

*Corresponding author. Email: zhmw123@263.net

ISSN 1028-6020 print/ISSN 1477-2213 online
© 2009 Taylor & Francis
DOI: 10.1080/10286020903213439
http://www.informaworld.com

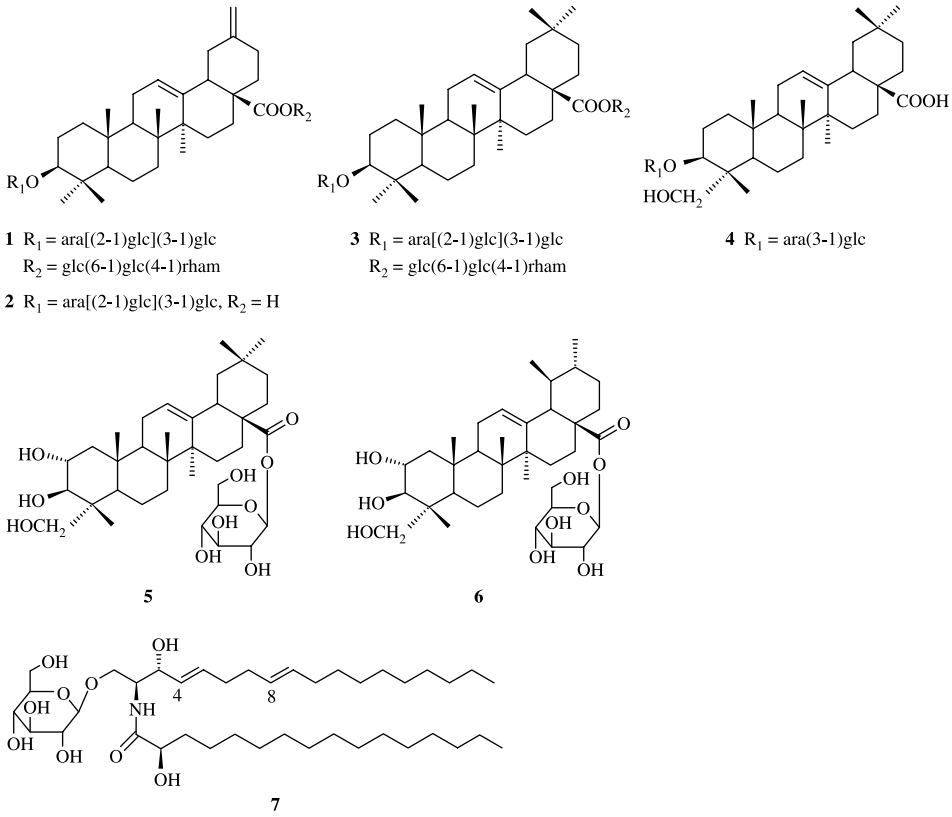


Figure 1. Structures of compounds **1**–**7**.

saponin. The ^{1}H NMR spectrum exhibited six anomeric protons at δ 6.21 (1H, d, $J = 8.0$ Hz), 5.86 (1H, br s), 5.52 (1H, d, $J = 8.0$ Hz), 5.33 (1H, d, $J = 8.0$ Hz), 4.96 (1H, d, $J = 7.5$ Hz), and 4.78 (1H, d, $J = 6.0$ Hz), an exomethylene group at δ 4.73 and 4.68 (each s), an olefinic proton at δ 5.41 (1H, br s), as well as five tertiary methyl signals at δ 1.25 (3H, s), 1.18 (3H, s), 1.07 (3H, s), 1.05 (3H, s), and 0.82 (3H, s). The ^{13}C NMR spectrum displayed six anomeric carbons at δ 105.5, 104.9, 104.8, 104.4, 102.7, and 95.7, two pairs of olefinic carbons at δ 123.1 and 143.4, as well as δ 148.4 and 107.4, along with an ester carbonyl carbon at δ 175.8 and an oxygenated carbon at δ 88.9 of the aglycone. In addition, acid hydrolysis of **1** confirmed that the sugar moieties are composed of glucose, rhamnose, and arabinose, which were identified by co-chromatographic examinations on thin layer plates with authentic samples and by the comparison of the NMR spectral data of the sugar moieties with those of the literature [3,11]. According to the above spectroscopic and chemical information, compound **1** was deduced to be a bisdesmosidic triterpenoid saponin with four glucopyranosyl units, one arabinopyranosyl, and one rhamnopyranosyl unit, disubstituted at positions C-3 and C-28 of the aglycone, respectively. A detailed comparison of the ^{1}H and ^{13}C NMR spectral data of **1** with those of **2** and **3** indicates that compound **1** has the same aglycone as that of **2** and the same sugar moieties as well as the linkage sequence as that of **3** (see Table 1). Thus, the structure of **1** was preliminarily established as the

Downloaded At: 18:32 22 January 2011

Downloaded At: 18:32 22 January 2011

Table 1. ^{1}H and ^{13}C NMR spectral data for compounds **1**–**3** in pyridine-d_5.

No.	δ_C (**1**)	δ_H (**1**) (*J*, Hz)	HMBC (**1**)	δ_C (**2**)	δ_C (**3**)	No.	δ_C (**1**)	δ_H (**1**) (*J*, Hz)	HMBC (**1**)	δ_C (**2**)	δ_C (**3**)
1	38.8			38.8	38.9	3-Ara-1	105.5	4.78 (1H, d, $J = 6.0$)	Aglycone-C-3	105.5	105.4
2	26.6			26.6	26.6	2	77.4	4.74	Glc″-C-1	77.4	77.4
3	88.9	3.24 (1H, dd, $J = 11.5, 4.0$)		88.9	89.0	3	83.2	4.29		83.3	83.3
4	39.7			39.8	39.9	4	68.7	4.49		68.8	68.7
5	55.9	0.74 (1H, d, $J = 11.5$)		55.9	55.9	5	65.9	4.14, 3.63 (each 1H)		65.9	65.9
6	18.5			18.5	18.6	Glc-1″	104.9	5.33 (1H, d, $J = 8.0$)	Ara-C-3	105.0	104.9
7	33.1			33.1	33.1	2″	75.3	3.98		75.3	75.3
8	39.9			39.7	39.7	3″	78.6	4.19		78.6	78.6
9	48.0	1.55		47.9	48.1	4″	72.4	4.19		72.4	72.5
10	37.0			37.0	37.1	5″	77.5	3.69		77.5	77.5
11	23.7			23.9	23.8	6″	63.2	4.35, 4.27	Glc″-C-4	63.3	63.3
12	123.1	5.41 (1H, br s)		123.1	122.9	Glc-1‴	104.4	5.52 (1H, d, $J = 8.0$)	Ara-C-2	104.4	104.4
13	143.4			144.0	144.1	2‴	76.1	4.07	Glc‴-C-1	76.2	76.6
14	42.1			42.1	42.1	3‴	78.0	4.07		78.4	78.3
15	28.2			28.3	28.3	4‴	71.5	4.21		71.6	71.0
16	23.5			23.8	23.4	5‴	78.5	3.94		78.7	78.6
17	47.3			47.1	47.1	6‴	62.5	4.49, 4.29		62.6	62.6
18	47.5	3.09 (1H, dd, $J = 12.0, 3.5$)	C-12, 13, 17	48.1	41.7	28-Glc-1	95.7	6.21 (1H, d, $J = 8.0$)	Aglycone-C-28		95.7
19	41.7	2.55, 2.15	C-17, 18, 20	42.0	46.3	2	73.9	4.11	Glc-C-1		73.9
20	148.4			149.2	30.5	3	78.3	4.21			78.5
21	30.1			30.4	34.1	4	71.0	4.27			71.6
22	37.6			38.4	32.6	5	78.7	4.19	Glc-C-4		78.8
23	28.1	1.25 (3H, s)	C-4, 5, 24	28.1	28.1	6	69.4	4.63, 4.27			69.4
24	16.7	1.07 (3H, s)	C-4, 5	16.8	16.8	Glc-1′	104.8	4.96 (1H, d, $J = 7.5$)	Glc-C-6		104.8
25	15.6	0.82 (3H, s)	C-1, 5, 9, 10	15.5	15.6	2′	75.3	3.94	Glc′-C-1		75.3
26	17.5	1.05 (3H, s)	C-8, 9	17.4	17.5	3′	76.6	4.14			76.1
27	26.0	1.18 (3H, s)	C-8, 13, 14	26.1	26.1	4′	78.2	4.42	Rham-C-1		78.1
28	175.8			175.8	176.5	5′	77.2	3.63			77.2
29	107.4	4.73, 4.68 (each 1H, br s)		107.1	33.1	6′	61.5	4.21, 4.07			61.4
30					23.7	Rham-1	102.7	5.86 (1H, br s)	Glc′-C-4		102.8

Downloaded At: 18:32 22 January 2011

Table 1 – *continued*

No.	δ_C (**1**)	δ_H (**1**) (*J*, Hz)	HMBC (**1**)	δ_C (**2**)	δ_C (**3**)
2	72.6	4.63			72.6
3	72.8	4.56			72.8
4	74.0	4.29	Rham-C-5		74.0
5	70.3	4.96			70.3
6	18.5	1.69 (3H, d, $J = 6.0$)	Rham-C-4, 5		18.5

derivative of **3**, with the absence of 30-CH_3 of the aglycone. Starting from the anomeric proton of each glucopyranosyl and arabinopyranosyl unit, all the hydrogens within each spin system were assigned by 2D TOCSY experiments. In 2D TOCSY, H-1 of rhamnopyranosyl had only the correlations with H-2 and H-3; moreover, the correlation between H-1 and H-3 was weak, which indicated the characteristic of rhamnose. The 2D COSY spectrum displayed the correlations between H-6 (the methyl group) and H-5, H-5 and H-4, respectively, and then, the protons of rhamnopyranosyl were assigned. All the carbons of the sugar units were assigned by HSQC, DEPT and further confirmed by HMBC experiments. The linkage sequences and linkage position of the sugar moieties were further ascertained by the following key HMBC correlations: from H-1‴ of glucopyranosyl at δ 5.52 to C-2 of arabinopyranosyl at δ 77.4, from H-1″ of glucopyranosyl at δ 5.33 to C-3 of arabinopyranosyl at δ 83.2, from H-1 of arabinopyranosyl at δ 4.78 to C-3 of the aglycone at δ 88.9, from H-1 of rhamnopyranosyl at δ 5.86 to C-4′ of glucopyranosyl at δ 78.2, H-1′ of glucopyranosyl at δ 4.96 to C-6 of the inner glucopyranosyl at δ 69.4, H-1 of the inner glucopyranosyl at δ 6.21 to C-28 of the aglycone at δ 175.8 (see Table 1). Accordingly, the structure of **1** was elucidated as 3-β-[(β-D-glucopyranosyl-(1 → 2)-*O*-[β-D-glucopyranosyl-(1 → 3)-*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.

Compounds **2**–**8** were unambiguously identified by the comparison of their spectroscopic data with those reported in the literatures as 3-β-[(β-D-glucopyranosyl-(1 → 2)-*O*-[β-D-glucopyranosyl-(1 → 3)-*O*-]-α-L-arabinopyranosyl)oxy]-30-norolean-12-en-28-oic acid (**2**) [12], leonticin E (**3**) [11], collinsonidin (**4**) [13], arjunolic acid 28-*O*-glucopyranoside (**5**), asiatic acid

28-*O*-glucopyranoside (**6**) [14], soya-cerebroside I (**7**) [15], and 1-*O*-α-L-galactosyl-(1 → 6)-*O*-β-D-galactosyl-3-*O*-hexadecanoyl-glycerol (**8**) [16]. Compound **2** was previously obtained as an artifact derived from guaiacin E by alkaline hydrolysis. This is the first report on isolation from natural sources.

3. Experimental

3.1 General experimental procedures

NMR spectra were recorded at 500 and 125 MHz for ^{1}H and ^{13}C, respectively, on a JEOL Teol 500 MHz NMR spectrometer in pyridine-d_5 with TMS as the internal standard. HR-ESI-MS were recorded on a Jabstec (Micromass) mass spectrometer. Column chromatography was performed with silica gel (200–300 mesh; Qingdao Haiyang Chemical Co., Qingdao, China), Sephadex LH-20, and ODS silica gel (Fuji Silysia Chemical Ltd, Aichi, Japan). TLC was carried out with precoated silica gel plates (GF-254; Qingdao Haiyang Chemical Co.). The reference glucose, rhamnose, and arabinose were bought from Sigma Company (Poole, Dorset, UK). All solvents were of analytical grade.

Downloaded At: 18:32 22 January 2011

3.2 Plant material

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 Associate Prof. Xi-Rong 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.

3.3 Extraction and isolation

The stems of *A. trifoliata* var. *australis* (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 column chromatography eluted with petroleum ether–EtOAc (1:1), $CHCl_3$–EtOAc (1:1), $CHCl_3$–MeOH (9:1–3:1), and MeOH, respectively, to afford six fractions (I–VI). Fraction IV was purified on Sephadex LH-20 column chromatography eluted with MeOH–H_2O (3:7) to give **7** (40 mg). Fraction V was purified by ODS silica gel column chromatography eluted with MeOH–H_2O (6:4) to yield **4** (10 mg), **5** (7.5 mg), **6** (8 mg), and **8** (50 mg). The *n*-BuOH-soluble fraction (136 g) was fractioned by macroporous resin HPD_{100} column chromatography eluted with H_2O and EtOH–H_2O (3:7, 5:5, 95:5). The EtOH–H_2O (3:7) fraction was separated on silica gel column chromatography ($CHCl_3$–MeOH–H_2O 15:8:1) and purified by ODS column chromatography eluted with MeOH–H_2O (5:5) to give **1** (18 mg) and **3** (180 mg). The EtOH–H_2O (5:5) fraction was subjected to silica gel column chromatography ($CHCl_3$–MeOH–H_2O 18:8:1) and purified by ODS silica gel and preparative HPLC using MeOH–H_2O (6:4) as the mobile phase, to afford **2** (10 mg), respectively.

Mutongsaponin F (**1**) was obtained as a white amorphous powder. ^{1}H and ^{13}C NMR spectral data, see Table 1. HR-ESI-MS (positive): *m/z* 1389.6271 $[M+Na]^+$ (calcd for $C_{64}H_{102}O_{31}Na$ 1389.6303).

3.4 Acid hydrolysis of mutongsaponin F (1)

A solution of **1** in 2.0 M trifluoroacetic acid (2 ml) was sealed in a tube and heated at 110°C for 6 h. The reaction mixture was extracted with EtOAc. The H_2O layer was concentrated under reduced pressure for the identification of glucose, arabinose, and rhamnose by comparison of the R_f value with that of the authentic sample on TLC [3].

Acknowledgements

The present study was supported by the National High Technology Research and Development Project of China (No. 2004AA223730-14).

References

[1] Committee for the Pharmacopoeia of P.R. China, *Pharmacopoeia of People's Republic of China. Part I* (Chemical Industry Press, Beijing, 2005), p. 43.
[2] G.Y. Liu, S.C. Ma, J. Zheng, Z.X. Yu, and R.C. Lin, *Chem. J. Chin. Univ.* **27**, 2120 (2006).
[3] H.M. Gao and Z.M. Wang, *Chin. Pharm. J.* **41**, 333 (2006).
[4] Y. Mimaki, S. Doi, M. Kuroda, and A. Yokosuka, *Chem. Pharm. Bull.* **55**, 1319 (2007).
[5] G.Y. Liu, S.C. Ma, J. Zheng, J. Zhang, and R.C. Lin, *J. Integr. Plant Biol.* **49**, 196 (2007).
[6] S.H. Guan, J.M. Xia, Z.Q. Lu, G.T. Chen, B.H. Jiang, X. Liu, and D.A. Guo, *Magn. Reson. Chem.* **46**, 186 (2008).
[7] D. Jiang, Q.P. Gao, S.P. Shi, and P.F. Tu, *Chem. Pharm. Bull.* **54**, 595 (2006).
[8] D. Jiang, S.P. Shi, J.J. Cao, Q.P. Gao, and P.F. Tu, *Biochem. Syst. Ecol.* **36**, 138 (2008).
[9] J.M. Wang, Z.M. Wang, and H.M. Gao, *Chin. Pharm. J.* **43**, 98 (2008).
[10] J. Kawata, M. Kameda, and M. Miyazawa, *J. Oleo. Sci.* **56**, 59 (2007).
[11] M. Chen, W.W. Wu, D. Nanz, and O. Sticher, *Phytochemistry* **44**, 497 (1997).
[12] V.U. Ahmad, S. Perveen, and S. Bano, *Phytochemistry* **29**, 3287 (1990).
[13] B.S. Joshi, K.M. Moore, and S.W. Pelletier, *J. Nat. Prod.* **55**, 1468 (1992).
[14] J.D. Djoukeng, E. Abou-Mansour, R. Tabacchi, A.L. Tapondjou, H. Bouda, and D. Lontsi, *J. Ethnopharmacol.* **101**, 283 (2005).
[15] H. Shibuya, K. Kawashima, M. Sakagami, H. Kawanishi, M. Shimomura, K. Ohashi, and I. Kitagawa, *Chem. Pharm. Bull.* **38**, 2933 (1990).
[16] L. Yang, M.Y. Wang, Y.Y. Zhao, and Y.Y. Tu, *Acta Pharm. Sin.* **40**, 252 (2005).

Downloaded At: 18:32 22 January 2011