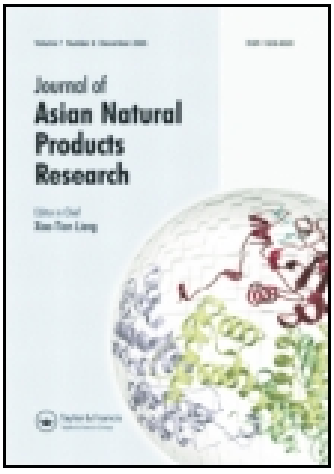

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Two new flavonol glycosides from the Tibetan medicinal plant *Aconitum tanguticum*

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Two new flavonol glycosides characterized as quercetin 3-O-α-L-rhamnopyranosyl-(1 → 2)-[β-D-glucopyranosyl-(1 → 3)-α-L-(4-O-*trans-p*-coumaroylrhamnopyranosyl)-(1 → 6)]-β-D-galactopyranoside-7-O-α-L-rhamnopyranoside (**1**) and kaempferol 3-O-α-L-rhamnopyranosyl-(1 → 2)-[β-D-glucopyranosyl-(1 → 3)-α-L-(4-O-*trans-p*-coumaroyl rhamnopyranosyl)-(1 → 6)]-β-D-galactopyranoside-7-O-α-L-rhamnopyranoside (**2**), together with two known flavonol glycosides quercetin 3-O-α-L-rhamnopyranosyl-(1 → 2)- [α-L-rhamnopyranosyl-(1 → 6)]-β-D-galactopyranoside-7-O-α-L-rhamnopyranoside (**3**) and kaempferol 3-O-α-L-rhamnopyranosyl-(1 → 2)-[α-L-rhamnopyranosyl-(1 → 6)]-β-D-galactopyranoside-7-O-α-L-rhamnopyranoside (**4**), were isolated from the whole plant of *Aconitum tanguticum* (Maxim.) Stapf. The structures of the new compounds were elucidated by spectroscopic methods, and the total ^{1}H and ^{13}C NMR chemical shifts were assigned.

Keywords: *Aconitum tanguticum*; Ranunculaceae; flavonol glycosides

1. Introduction

Aconitum tanguticum (Maxim.) Stapf. (Ranunculaceae) is a perennial herb distributed around an altitude of 3200–4800 m in the alpine meadows of Tibet Autonomous Region, Qinghai Province, Gansu Province, Sichuan Province, and Yunnan Province [1]. The whole plant, commonly called Ponka, has been used in traditional Tibetan medicine for the treatment of gastricism, hepatitis, nephritis, and other diseases for thousands of years [2]. Although the chemical constituents of plants of the genus *Aconitum* have been extensively studied [3], there are few reports on the chemical constituents and biological activities of *A. tanguticum* [4–8]. In order to find biologically active components from Chinese medicinal plants, we obtained from the ethanol extract of the whole plant of *A. tanguticum*, two new flavonol glycosides **1** and **2** as well as two known flavonol glycosides **3** and **4** (Figure 1). The structures of the new compounds were established mainly on the basis of HR-ESI-MS, ^{1}H, ^{13}C and 2D NMR spectroscopic methods.

2. Results and discussion

The 30% ethanol fraction was subjected to repeated column chromatography (CC) over silica gel and octadecylsilane (ODS) to obtain two new compounds **1** and **2** (Figure 1), along with two known compounds **3** and **4**. Compounds **3** and **4** were isolated from *A. tanguticum* for the first

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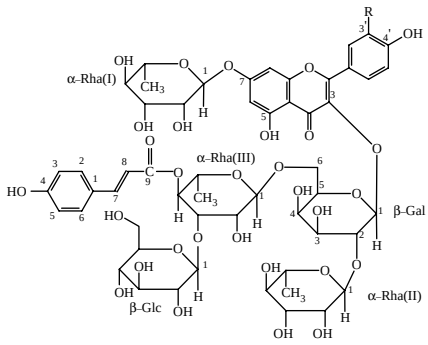

1 R=OH
2 R=H

Figure 1. The structures of compounds **1** and **2**.

time. Their structures were identified by means of spectroscopic methods and compared with the reported data.

Compound **1** was isolated as a yellow amorphous powder. The HR-ESI-MS spectrum showed a positive ion at *m/z* 1211.3663 $[M + H]^+$, indicating a molecular formula of $C_{54}H_{66}O_{31}$. The IR spectrum exhibited the absorptions of hydroxyl (3402 cm^{-1}), carbonyl (1653.8 cm^{-1}), and aromatic rings (1603 and 1515 cm^{-1}). The UV spectrum exhibited absorption maxima characteristic for flavonols at 256 and 315 nm. The ^1H NMR spectrum showed the typical signal pattern for a quercetin derivative (Table 1). A pair of doublets (δ_H 7.50, 6.33) with a coupling constant of 15.6 Hz revealed the presence of a trans olefinic double bond. Two ortho-coupled doublets at δ_H 7.53 ($J = 8.4$ Hz) and 6.82 ($J = 8.4$ Hz), each integrating for two proton signals, showed a further o, p-disubstituted aromatic ring, indicating the presence of a coumaric acid residue. The anomeric signals of five sugar units were observed at δ_H 4.25 ($J = 7.8$ Hz), 4.53 (brs), 5.06 (brs), 5.54 (brs), and 5.63 ($J = 7.8$ Hz). The HMQC and TOCSY data, in combination with the literature data for similar compounds [9,10], showed that the sugars appeared as one β-D-glucopyranose unit, one β-D-galactopyranose, and three α-L-rhamnopyranose units. This suggestion was further supported by chemical means. Acid hydrolysis of **1** liberated D-galactopyranose, D-glucopyranose, and L-rhamnopyranose, which were identified by gas chromatography (GC) analysis of their thiazolidine derivatives. An HMBC correlation was

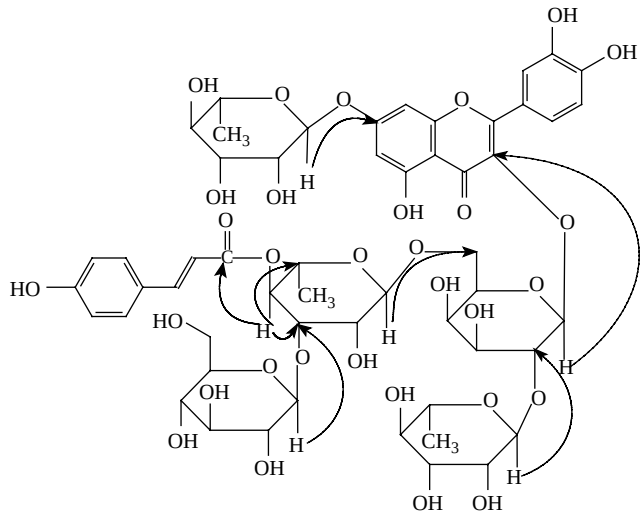

Figure 2. Key HMBC correlations (H → C) in compound **1**.

Table 1. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR spectroscopic data of compounds **1** and **2** (DMSO-d_6).

	1		**2**	
Position	δ_H	δ_C	δ_H	δ_C
2		157.2		157.2
3		133.6		133.4
4		177.8		177.9
5		161.4		161.4
6	6.43 (1H, d, 1.8 Hz)	99.8	6.44 (1H, d, 1.8 Hz)	99.8
7		162.1		162.1
8	6.74 (1H, d, 1.8 Hz)	94.8	6.77 (1H, d, 1.8 Hz)	95.1
9		156.3		156.3
10		106		106
1′		121.4		121.1
2′	7.53 (1H, d, 1.2 Hz)	116.3	8.10 (1H, d, 9.0 Hz)	131.4
3′		145	6.87 (1H, d, 9.0 Hz)	115.6
4′		149.1		160.6
5′	6.82 (1H, d, 8.4 Hz)	115.6	6.87 (1H, d, 9.0 Hz)	115.6
6′	7.72 (1H,dd, 8.4,1.8 Hz)	122.7	8.10 (1H, d, 9.0 Hz)	131.4
7-O-Rha (I)				
1	5.54 (1H, brs)	98.9	5.54 (1H, brs)	98.9
2	3.85 (1H, m)	70.1	3.85 (1H, m)	70.1
3	3.64 (1H, m)	70.5	3.64 (1H, m)	70.5
4	3.30 (1H, m)	72.1	3.30 (1H, m)	72.1
5	3.42 (1H, m)	70.3	3.42 (1H, m)	70.3
6	1.13 (3H, d, 6.0 Hz)	18.4	1.12 (3H, d, 6.0 Hz)	18.4
3-O-Gal				
1	5.63 (1H, d, 7.8 Hz)	99.5	5.62 (1H, d, 7.8 Hz)	99.4
2	3.83 (1H, m)	75.4	3.84 (1H, m)	75.4
3	3.65 (1H, m)	74.3	3.64 (1H, m)	74.2
4	3.76 (1H, m)	71.1	3.75 (1H, m)	71.1
5	3.65 (1H, m)	73.9	3.64 (1H, m)	73.8
6	3.65 (1H, m), 3.75 (1H, m)	68.7	3.64 (1H, m), 3.75(1H, m)	68.7
2^{Gal}-O-Rha (II)				
1	5.06 (1H, brs)	101.1	5.05 (1H, brs)	101.1
2	3.73 (1H, m)	70.7	3.75 (1H, m)	70.7
3	3.49 (1H, m)	71.1	3.43 (1H, m)	71.1
4	3.13 (1H, m)	72.4	3.30 (1H, m)	72.3
5	3.77 (1H, m)	68.8	3.79 (1H, m)	68.8
6	0.79 (3H, d, 6.6 Hz)	17.7	0.76 (3H, d, 6.0 Hz)	17.7
6^{Gal}-O-Rha(III)				
1	4.53 (1H, brs)	100.2	4.51 (1H, brs)	100.2
2	3.13 (1H, m)	72.4	3.13 (1H, m)	72.4
3	3.74 (1H, m)	76.8	3.75 (1H, m)	76.7
4	4.99 (1H, m)	72.6	4.98 (1H, m)	72.5
5	3.68 (1H, m)	66.6	3.64 (1H, m)	66.6
6	0.96 (3H, d, 6.0 Hz)	18	0.96 (3H, d, 6.6 Hz)	18
3^{Rha} (III) −O-Glc				
1	4.25 (1H, d, 7.8 Hz)	103.8	4.25 (1H, d, 7.8 Hz)	103.8
2	2.88 (1H, m)	73.4	2.88 (1H, m)	73.4
3	3.04 (1H, m)	76.9	3.04 (1H, m)	76.9
4	3.63 (1H, m)	70	3.64 (1H, m)	69.9
5	3.80 (1H, m)	77	3.80 (1H, m)	77
6	3.43 (1H, m), 3.58 (1H, m)	61.2	3.44 (1H, m), 3.57 (1H, m)	61.2

Table 1 – *continued*

Position	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
4^{Rha} (III) -O-*trans*−coumaroyl				
1		125.7		125.7
2,6	7.53 (2H, d, 8.4 Hz)	130.7	7.53 (2H, d, 9.0 Hz)	130.7
3,5	6.82 (2H, d, 8.4 Hz)	116.2	6.81 (2H, d, 9.0 Hz)	116.2
4		160.2		160.2
7	7.50 (1H, d, 15.6 Hz)	145.3	7.50 (1H, d, 15.6 Hz)	145
8	6.33 (1H, d, 15.6 Hz)	114.9	6.33 (1H, d, 16.2 Hz)	114.9
9		166.9		166.6

observed between the anomeric proton signal of Rha I (brs, 5.54) and the carbon resonance at δ_C 162.1 (C-7). These data revealed the linkage between the Rha I and the C-7 of the quercetin unit. The fact that a long-range correlation was observed between the proton signal at δ_H 5.63 ($J = 7.8$ Hz, H-1 of Gal) and the carbon resonance at δ_C 133.6 (C-3) suggested that the second position of glycosidation was C-3 of quercetin unit. The HMBC correlations between the H-1 of Rha II (δ_H 5.06) and the C-2 of Gal (δ_C 75.4), between the H-1 of Rha III (δ_H 4.53) and the C-6 of Gal (δ_C 68.7), and between the H-1 of Glc (δ_H 4.25) and the C-3 of Rha III (δ_C 76.8) established that the residue was linked to position C-3 as a β-D-glucopyranosyl-(1 → 3)-α-L-rhamnopyranosyl-(1 → 6)-[α-L-rhamnopyranosyl-(1 → 2)]-β-D-galactopyranosyl unit. The HMBC correlation between the H-4 of Rha III (δ_H 4.99) and the carbon resonance at δ_C 166.9 (C-9″) revealed esterification of the coumaric acid at position 4 of this third α-L-rhamnopyranose unit. The main correlations between H and C in the HMBC spectrum are shown in Figure 2. Thus, compound **1** was characterized as quercetin 3-O-α-L-rhamnopyranosyl-(1 → 2)-[β-D-glucopyranosyl-(1 → 3)-α-L-(4-O-*trans-p*-coumaroylrhamnopyranosyl)-(1 → 6)]-β-D-galactopyranoside-7-O-α-L-rhamnopyranoside.

Compound **2** was also isolated as a yellow amorphous powder. Its molecular formula was deduced to be $C_{54}H_{66}O_{30}$ by a positive HR-ESI-MS at *m/z* 1195.37293$[M + H]^+$. The UV spectrum showed absorption maxima characteristic for flavonols at 266 and 320 nm. The IR spectrum exhibited the absorptions of hydroxyl (3417 cm^{-1}), carbonyl (1654.6 cm^{-1}), and aromatic rings (1603 and 1514 cm^{-1}). Comparison of the NMR spectroscopic data of **2** with those of **1** indicated that the structure of **2** was very close to that of **1**, except that the aglycone quercetin in **1** was substituted by kaempferol in **2** (Table 1). Moreover, 2D experiments resulted in the assignments of all signals and the linkage of structural units of **2**. Consequently, compound **2** was established as kaempferol 3-O-α-L-rhamnopyranosyl-(1 → 2)-[β-D-glucopyranosyl-(1 → 3)-α-L-(4-O-*trans-p*-coumaroylrhamnopyranosyl)-(1 → 6)]-β-D-galactopyranoside-7-O-α-L-rhamnopyranoside.

In addition, the known compounds were identified as quercetin 3-O-α-L-rhamnopyranosyl-(1 → 2)- [α-L-rhamnopyranosyl-(1 → 6)]-β-D-galactopyranoside-7-O-α-L-rhamnopyranoside (**3**) and kaempferol 3-O-α-L-rhamnopyranosyl-(1 → 2)-[α-L-rhamnopyranosyl-(1 → 6)]-β-D-galactopyranoside-7-O-α-L-rhamnopyranoside (**4**) by comparison of their NMR spectroscopic data with those reported in literatures [9,10].

3. Experimental

3.1 General experimental procedures

Optical rotation was taken on a RUDOLPH Autopol Vplus polarimeter (Rudolph, Research Flanders, NJ, USA; Part No of Polarization tube (10 mm): 40T-5.0-10-0.2). UV spectra were measured on T6 New Century UV–vis spectrophotometer (Pgeneral, Beijing, China). IR spectra were recorded on a Nicolet 5700 FT-IR spectrometer (Thermo Nicolet Corporation, Madison, WI, USA). HR-ESI-MS data were recorded on an Agilent Technologies 6250 Accurate-Mass Q-TOF LC/MS spectrometer (Agilent, Santa Clara, CA, USA). ESI-MS were measured on an Agilent 6130SQ-MSD (Agilent). NMR spectra were recorded on a Bruker Advance 600 spectrometer (Bruker, Faellanden, Switzerland). GC data were recorded on an Agilent 7890A instrument with a Flame Ionization Detector (FID) (Agilent 6130SQ-MSD). Preparative HPLC was performed on an LC3000 instrument (Beijing Chuangxintongheng Science and Technology Co., Ltd, Beijing, China) connected to a UV 3000 detector, using an Intersil-ODS column (250 × 20 mm, 10 μm; GL Sciences, Inc., Tokyo, Japan). A TLC plate precoated with silica gel GF_{254} (20 × 20 cm) was produced by Merck (Darmstadt, Germany). Sephadex LH-20 (TOSOH Corporation, Tokyo, Japan), ODS (Octadecylsilyl, 50 μm, YMC Company, Kyoto, Japan), silica gel (200–300 mesh, Qingdao Marine Chemical Industry, Qingdao, China), and AB-8 macroporous adsorption resin (The Chemical Plant of Nankai University, Tianjin, China) were used for CC. Spots were visualized under UV light or by spraying with 10% H_2SO_4 in EtOH–H_2O (95:5, v/v) followed by heating. Solvents [petroleum ether (60–90°C), $CHCl_3$, EtOAc, MeOH, CH_2Cl_2, and EtOH] were of analytical grade and purchased from Beijing Chemical Company, Beijing, China.

3.2 Plant material

The whole plant of *A. tanguticum* (collected from Qinghai Province, China, in August 2010) was bought from Xining San Jiangbao Trade Co., Ltd and identified by Researcher Xue-feng Feng of the Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences. The specimen (No. 20100813) was deposited in the Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences.

3.3 Extraction and isolation

Dried and powdered plants of *A. tanguticum* (10 kg) were extracted under reflux with 95% EtOH under reflux, and the EtOH extract was concentrated to dryness. The residue (1.8 kg) was suspended in H_2O and then extracted with petroleum ether and EtOAc. The residual aqueous solution was chromatographed over macroporous resin AB-8 with H_2O containing increasing amounts of EtOH as eluent to afford H_2O eluate 605 g (A), 30% EtOH eluate 407.9 g (B), 60% EtOH eluate 107.0 g (C), and 95% EtOH eluate 2.3 g (D). The fraction D was chromatographed on silica gel and eluted with gradient $CHCl_3$–MeOH–H_2O (8:2:0.125/7:3:0.5/6:4:1/5:5:1) to give fractions 1 ~ X. Fr. X (31.5 g) was repeatedly purified on silica gel CC using gradient $CHCl_3$–MeOH–H_2O (7:3:0.5/65:35:10/6:4:1/5:5:1) followed by ODS CC with gradient MeOH–H_2O (2:8/3:7/10:0) to afford compounds **1** (878 mg), **2** (114 mg), **3** (522 mg) and **4** (50 mg).

3.3.1 *Quercetin 3-O-α-L-rhamnopyranosyl-(1 → 2)-[β-D-glucopyranosyl-(1 → 3)-α-L-(4″-O-trans-p-coumaroylrhamnopyranosyl)-(1 → 6)]-β-D-galactopyranoside-7-O-α-L-rhamnopyranoside (**1**)*

Yellow amorphous powder; $[\alpha]_D$ − 85.0 ($c = 0.02$, MeOH); UV (MeOH) λ_{max}: 256, 315 nm; IR (KBr) ν_{max}: 3402, 2935, 1654, 1603, 1515, 1170, 1072 cm^{-1}. ^1H

NMR (600 MHz) and ^{13}C NMR (150 MHz) spectral data, see Table 1. (-) ESI-MS: *m/z* 1209 $[M-H]^-$; LC-ESI-$MS^{(n)}$: *m/z* 1209 $[M-H]^-$, 1063 [M−H-rha]$^-$, 917 [M−H-rha-rha]$^-$, 755 [M−H-rha-rha-glc]$^-$, 753 [M−H-rha-rha-*p*-coumaroyl]$^-$; HR-ESI-MS: *m/z* 1211.3663 $[M+H]^+$ (calcd for $C_{54}H_{67}O_{31}$, 1211.3666).

*3.3.2 Kaempferol 3-O-α-L-rhamnopyranosyl-(1 → 2)-[β-D-glucopyranosyl-(1 → 3)-α-L-(4″-O-trans-p-coumaroylrhamnopyranosyl)-(1 → 6)]-β-D-galactopyranoside-7-O-α-L-rhamnopyranoside (**2**)*

Yellow amorphous powder; $[\alpha]_D$ − 30.0 ($c = 0.1$, MeOH); UV (MeOH) λ_{max}: 266, 320 nm; IR (KBr) ν_{max}: 3417, 2933, 1655, 1603, 1514, 1174, 1069 cm^{-1}. ^1H NMR (600 MHz) and ^{13}C NMR (150 MHz) spectral data, see Table 1. (-) ESI-MS: *m/z* 1193 $[M-H]^-$; HR-ESI-MS: *m/z* 1195.3729 $[M+H]^+$ (calcd for $C_{54}H_{67}O_{30}$, 1195.3717).

3.4 Determination of the absolute configuration of the sugar moieties

According to the reported method [11], each (2 mg) of the compounds **1** and **2** was hydrolyzed by 2 M HCl–H_2O (2 ml) at 90°C for 4 h. After removal of HCl by evaporation and extraction with $CHCl_3$, the H_2O extract was evaporated and dried *in vacuo* to give the monosaccharide residue. The residue was treated with L-cysteine methyl ester hydrochloride (2 mg) in pyridine (1.0 ml) at 60°C for 2 h, then evaporated under N_2 stream and dried *in vacuo*. The residue was dissolved in 0.2 ml of *N*-trimethylsilylimidazole and heated at 60°C for 1 h. The reaction mixture was partitioned between *n*-hexane and H_2O (2 ml each), and the *n*-hexane extract was analyzed by GC (Agilent 7890A) under the following conditions: capillary column HP-5 (30 m × 0.32 mm × 0.25 μm); detector FID; carrier gas N_2, flow rate 1 ml/min; detector temperature 280°C; injection temperature: 250°C; oven temperature gradient: 100°C for 2 min, 100°C → 280°C (10°C/min), and 280°C for 5 min. The same procedure was applied to authentic samples. By comparing with the retention time of authentic sample ($t_{R\text{-D-galactose}}$ 20.034 min, $t_{R\text{-L-galactose}}$ 20.037 min, $t_{R\text{-D-glucose}}$ 19.849 min, $t_{R\text{-L-glucose}}$ 19.822 min, $t_{R\text{-L-rhamnose}}$ 18.697 min, $t_{R\text{-D-rhamnose}}$ 18.706 min), D-galactose, D-glucose, and L-rhamnose were identified in the hydrolysate of **1** and **2**.

Acknowledgements

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