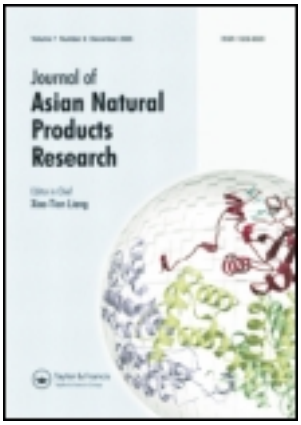


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# Three new flavone *C*-glycosides from the aerial parts of *Paraquilegia microphylla*

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Three new flavone *C*-glycosides, paraquinins A–C, were isolated from the aerial parts of *Paraquilegia microphylla* (Royle) Dromm. et Hutch, a Tibetan medicine distributed in the Qinghai-Tibet plateau. On the basis of 1D and 2D NMR evidence, their structures were elucidated as acacetin-6-*C*-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranoside (**1**), acacetin-6-*C*-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranoside (**2**), and acacetin-6-*C*-α-L-rhamnopyranosyl-(1 → 2)-(6‴-*O*-*E*-feruloyl)-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranoside (**3**).

**Keywords:** Ranunculaceae; *Paraquilegia microphylla*; flavone *C*-glycoside; paraquinins A–C



## 1. Introduction

*Paraquilegia microphylla* (Royle) Dromm. et Hutch (Ranunculaceae), a traditional Tibetan medicine called 'Yi Mao Dai Jin,' is distributed in the meadow at altitudes of 3000–5200 m in the Qinghai-Tibet plateau and used for the treatment of hemostasis, analgesia, detoxification, and dephlogistication [1,2]. Two triterpene saponins, paraquinosides A and B, were obtained from *P. microphylla* [3], whereas four alkaloids, (±)-fangchinoline, magnoflorine, paraquileginine, and *S*,*S*-dimethylcurine, and two diterpenes, paraquilgin and ent-kauran-16β,17-diol, were isolated from the same genus plant *P. anemonoides* [4–8]. With the aim to isolate structurally interesting and bioactive compounds, we investigated the constituents of the aerial parts of *P. microphylla*, and three new flavone *C*-glycosides, named paraquinins A–C (Figure 1), were isolated and identified by extensive NMR techniques.

## 2. Results and discussion

Compound **1** was isolated as a yellow powder, and the IR spectrum showed absorptions of OH and C=C groups at 3436 and 1655 $cm^{-1}$, respectively. The UV spectrum with absorption maxima at 284 nm (band II) and 355 nm (band I) indicated the presence of flavone. The ESI-MS of **1** exhibited the pseudo-molecular ion peak at *m/z* 609 $[M + H]^+$, and HR-ESI-MS established the molecular formula as $C_{28}H_{32}O_{15}$ at *m/z* 609.1807 $[M + H]^+$. On acid hydrolysis using HCl, glucose was detected in water solution. Comparing the optical rotation with that of glucose, it was

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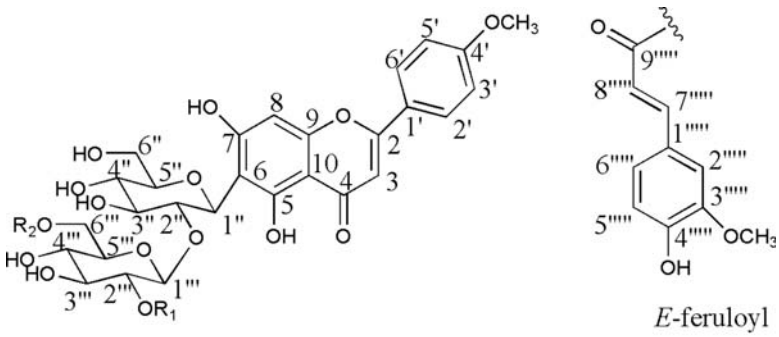


| | $R_1$ | $R_2$ |
|---|---|---|
| **1** | H | H |
| **2** | α-L-rhamnopyranosyl | H |
| **3** | α-L-rhamnopyranosyl | *E*-feruloyl |

Figure 1. Structures of compounds **1**–**3**.



proved as D-glucose. The tandem ESI-MS suggested the loss of glucosyl to produce the fragment ion at *m/z* 447 $[M + H - glc]^+$.

The $^1$H NMR spectral data (Table 1) revealed a doubling of many of the signals and it was noted that the paired signals were in a nearly 2:1 ratio. The $^{13}$C NMR data (Table 1) also showed a similar duplication of signals. We attributed this to the presence of two rotamers which was caused by an energy barrier about the *C*-glycosidic bond sufficiently high to prevent fast exchange between the two conformers at room temperature [9]. In the following structure resolution of compounds **1**–**3**, we adopted the spectral data of the preferential conformation.

The $^1$H NMR spectral data showed that the singlet at about $\delta$ 13.5 implied the presence of a 5-OH substituent. A pair of AA′BB′ doublets in the aromatic region at $\delta$ 8.05 and 7.12 indicated the presence of a 4′-oxysubstituted B ring. The singlet at $\delta$ 6.95 was assigned to H-3 and the signal at $\delta$ 6.92 to H-8 of a flavone skeleton.

The $^{13}$C NMR spectrum exhibited 28 signals, among which 15 were attributed to a flavone skeleton, 12 to 2 hexose units, and 1 to the methoxy group. Combining the $^1$H NMR, $^{13}$C NMR, and HMBC spectra, we proved that the aglycone skeleton of **1** was acacetin [10]. According to the coupling constants of the anomeric hydrogens at $\delta$ 4.69 (1H, d, $J = 9.0$ Hz) and 4.68 (1H, d, $J = 8.4$ Hz), the two glucose units were both β configurated. The cross peak between H-1″ ($\delta$ 4.69) and C-5 ($\delta$ 159.8), C-6 ($\delta$ 111.1), C-7 ($\delta$ 163.0) in the HMBC spectrum revealed that the internal glucose was connected to C-6 of aglycone, whereas the cross peak between H-1‴ ($\delta$ 4.68) and C-2″ ($\delta$ 79.4) suggested that the external glucose was linked to C-2′ of internal glucose (Figure 2). Therefore, the structure of compound **1** was characterized as acacetin-6-C-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranoside, namely paraquinin A.

Compound **2** was isolated as a yellow powder, and the IR spectrum showed absorptions of OH and C=C groups at 3435 and 1659 $cm^{-1}$, respectively. The UV spectrum with absorption maxima at 285 nm (band II) and 356 nm (band I) indicated the presence of flavone. The ESI-MS of **2** exhibited the pseudo-molecular ion peak at *m/z* 777 $[M + Na]^+$, and HR-ESI-MS

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

| | **1**[a] | | **2**[a] | | **3**[b] | |
|---|---|---|---|---|---|---|
| No. | δ(H) | δ(C) | δ(H) | δ(C) | δ(H) | δ(C) |
| 2 | | 164.2 (s) | | 164.2 (s) | | 164.4 (s) |
| | | 164.0 (s) | | 164.1 (s) | | 164.3 (s) |
| 3 | 6.95 (s) | 104.3 (d) | 6.95 (s) | 104.1 (d) | 6.95 (s) | 104.7 (d) |
| | 6.94 (s) | 104.2 (d) | 6.93 (s) | 103.9 (d) | 6.96 (s) | 104.5 (d) |
| 4 | | 182.6 (s) | | 182.5 (s) | | 183.1 (s) |
| | | 182.9 (s) | | 182.9 (s) | | 183.3 (s) |
| 5 | | 159.8 (s) | | 159.7 (s) | | 160.9 (s) |
| | | 160.8 (s) | | 160.9 (s) | | 160.9 (s) |
| 6 | | 111.1 (s) | | 111.0 (s) | | 111.0 (s) |
| | | 111.5 (s) | | 111.6 (s) | | 111.2 (s) |
| 7 | | 163.0 (s) | | 163.1 (s) | | 163.9 (s) |
| | | 162.0 (s) | | 161.7 (s) | | 163.8 (s) |
| 8 | 6.92 (s) | 94.3 (d) | 6.88 (s) | 94.8 (d) | 7.41 (s) | 94.9 (d) |
| | 6.90 (s) | 94.0 (d) | 6.90 (s) | 94.6 (d) | 7.51 (s) | 95.1 (d) |
| 9 | | 156.9 (s) | | 157.1 (s) | | 157.8 (s) |
| | | 156.7 (s) | | 156.7 (s) | | 157.5 (s) |
| 10 | | 105.4 (s) | | 105.5 (s) | | 106.4 (s) |
| | | 105.9 (s) | | 106.0 (s) | | 106.1 (s) |
| 1′ | | 123.0 (s) | | 122.9 (s) | | 123.1 (s) |
| 2′, 6′ | 8.05 (d, $J = 8.6$) | 128.9 (d) | 8.04 (d, $J = 8.8$) | 128.8 (d) | 7.83 (d, $J = 8.8$) | 128.3 (d) |
| 3′, 5′ | 7.12 (d, $J = 8.6$) | 115.1 (d) | 7.10 (d, $J = 8.8$) | 115.1 (d) | 6.91 (d, $J = 8.8$) | 116.5 (d) |
| 4′ | | 162.9 (s) | | 162.9 (s) | | 162.8 (s) |
| 4′-$OCH_3$ | 3.85 (s) | 56.0 (q) | 3.83 (s) | 56.0 (q) | 3.70 (s) | 55.6 (q) |
| 5-OH | 13.47 (s) | | 13.45 (s) | | 14.33 (s) | |
| | 13.48 (s) | | 13.46 (s) | | 14.35 (s) | |
| Glc-1‴ | 4.69 (d, $J = 9.0$) | 71.3 (d) | 4.72 (d, $J = 8.8$) | 71.0 (d) | 5.78 (d, $J = 8.0$) | 71.3 (d) |
| | 4.74 (d, $J = 9.0$) | 71.4 (d) | 4.84 (d, $J = 8.8$) | 70.9 (d) | 5.87 (d, $J = 8.0$) | 71.2 (d) |
| Glc–2″ | 4.90 (m) | 79.4 (d) | 4.78 (m) | 79.9 (d) | 5.35 (t, $J = 8.0$) | 82.1 (d) |
| | 4.85 (m) | 79.3 (d) | 4.75 (m) | 79.7 (d) | 5.54 (t, $J = 8.0$) | 81.4 (d) |
| Glc–3″ | 3.53 (m) | 77.7 (d) | 3.52 (m) | 77.7 (d) | 4.59 (m) | 75.7 (d) |
| | 3.49 (m) | 77.6 (d) | 3.50 (m) | 77.7 (d) | 4.58 (m) | 76.8 (d) |
| Glc–4″ | 3.62 (m) | 70.1 (d) | 3.72 (m) | 70.2 (m) | 4.28 (m) | 70.9 (d) |
| | | 70.8 (d) | 3.70 (m) | 70.5 (m) | 4.30 (m) | 71.2 (d) |
| Glc–5″ | 3.48 (m) | 81.4 (d) | 3.48 (m) | 81.2 (d) | 4.41 (m) | 82.1 (d) |

Table 1 – *continued*

| No. | **1**[a] δ(H) | **1**[a] δ(C) | **2**[a] δ(H) | **2**[a] δ(C) | **3**[b] δ(H) | **3**[b] δ(C) |
|---|---|---|---|---|---|---|
| | 3.49 (m) | 81.9 (d) | 3.46 (m) | 81.4 (d) | 4.43 (m) | 82.4 (d) |
| Glc–6″ | 3.95, 3.74 (dd, $J = 13.0, 8.8$) | 61.2 (t) | 4.11, 3.78 (t, $J = 9.0$) | 61.3 (t) | 4.67, 4.53 (dd, $J = 11.8, 8.5$) | 61.5 (t) |
| | 4.07, 3.80 (dd, $J = 13.0, 8.8$) | 62.3 (t) | 4.35, 3.80 (t, $J = 9.0$) | 62.1 (t) | 4.63, 4.58 (dd, $J = 11.8, 8.5$) | 61.3 (t) |
| Glc-1‴ | 4.68 (d, $J = 8.4$) | 101.6 (d) | 4.75 (d,$J = 7.2$) | 102.1 (d) | 5.48 (d, $J = 8.0$) | 101.4 (d) |
| | | 101.4 (d) | 4.82 (d,$J = 7.2$) | 101.3 (d) | 5.46 (d, $J = 8.0$) | 102.4 (d) |
| Glc-2‴ | 3.17 (m) | 73.1 (d) | 3.50 (m) | 77.3 (d) | 4.13 (t, $J = 8.0$) | 77.7 (d) |
| | 3.20 (m) | 73.4 (d) | 3.38 (m) | 75.4 (d) | 4.19 (t, $J = 8.0$) | 78.2 (d) |
| Glc-3‴ | 3.25[c] | 74.2 (d) | 3.40[c] | 74.1 (d) | 4.02[c] | 74.7 (d) |
| | 3.24[c] | 73.8 (d) | 3.36[c] | 73.6 (d) | 4.00[c] | 74.6 (d) |
| Glc-4‴ | 3.20[c] | 70.0 (d) | 3.24[c] | 70.1 (d) | 4.31[c] | 72.9 (d) |
| | | 69.9 (d) | 3.22[c] | 70.0 (d) | 4.26[c] | 72.8 (d) |
| Glc-5‴ | 3.12 (m) | 76.2 (d) | 3.16 (m) | 76.2 (d) | 4.36[c] | 75.6 (d) |
| | | 76.5 (d) | 3.13 (m) | 76.7 (d) | 4.34[c] | 75.4 (d) |
| Glc-6‴ | 3.33[c], 3.20[c] | 60.8 (t) | 3.48[c], 3.40[c] | 60.7 (t) | 3.98[c], 3.76[c] | 64.6 (t) |
| | 3.22[c], 3.14[c] | 61.0 (t) | 3.20[c], 3.16[c] | 61.0 (t) | 3.96[c], 3.72[c] | 64.4 (t) |
| Rha-1⁗ | | | 5.03 (br s) | 101.1 (d) | 6.55 (br s) | 102.9 (d) |
| | | | 4.94 (br s) | 100.8 (d) | 6.38 (br s) | 102.5 (d) |
| Rha-2⁗ | | | 3.54[c] | 71.6 (d) | 4.36[c] | 72.5 (d) |
| | | | 3.52[c] | 71.5 (d) | 4.37[c] | 72.1 (d) |
| Rha-2⁗’ | | | 3.24[c] | 70.7 (d) | 3.94 (m) | 72.7 (d) |
| | | | 3.22[c] | 70.9 (d) | 3.92 (m) | 72.3 (d) |
| Rha-4⁗ | | | 3.15 (m) | 72.0 (d) | 3.71[c] | 73.5 (d) |
| | | | 2.90 (m) | 72.4 (d) | 3.66[c] | 73.1 (d) |
| Rha-5″ | | | 2.33 (m) | 68.8 (d) | 3.23 (m) | 69.7 (d) |
| | | | 2.25 (m) | 68.5 (d) | 3.50 (m) | 69.6 (d) |
| Rha-6⁗ | | | 0.58 (d, $J = 6.1$) | 17.9 (q) | 1.63 (d, $J = 6.1$) | 18.7 (q) |
| | | | 0.48 (d, $J = 6.1$) | 17.9 (q) | 1.25 (d, $J = 6.1$) | 18.6 (q) |

Notes: [a] Measured in DMSO-$d_6$.
[b] Measured in pyridine-$d_5$.
[c] Represents ‘overlapped’ signals. (*E*)-feruloyl: δ(H) 3.68 (3H, s, 3⁗′-$OCH_3$), 6.70, 6.68 (1H, d, $J = 15.8$ Hz, H-8⁗′), 6.92, 6.91 (1H, d, $J = 8.1$ Hz, H-5⁗′), 6.98, 6.99 (1H, br s, H-2⁗′), 7.08, 7.09 (1H, d, $J = 8.1$ Hz, H-6⁗′), 8.04, 7.95 (1H, d, $J = 15.8$ Hz, H-7⁗′). δ(C) 55.4 (3⁗′-$OCH_3$), 111.0, 111.2 (C-2⁗′), 114.6, 114.7 (C-5⁗′), 114.9, 114.8 (C-8⁗′), 123.7, 123.9 (C-6⁗′), 126.3, 126.2 (C-1⁗′), 145.9, 146.0 (C-7⁗′), 148.5, 148.6 (C-3⁗′), 150.8, 150.9 (C-4⁗′), 167.3, 167.4 (C-9⁗′).

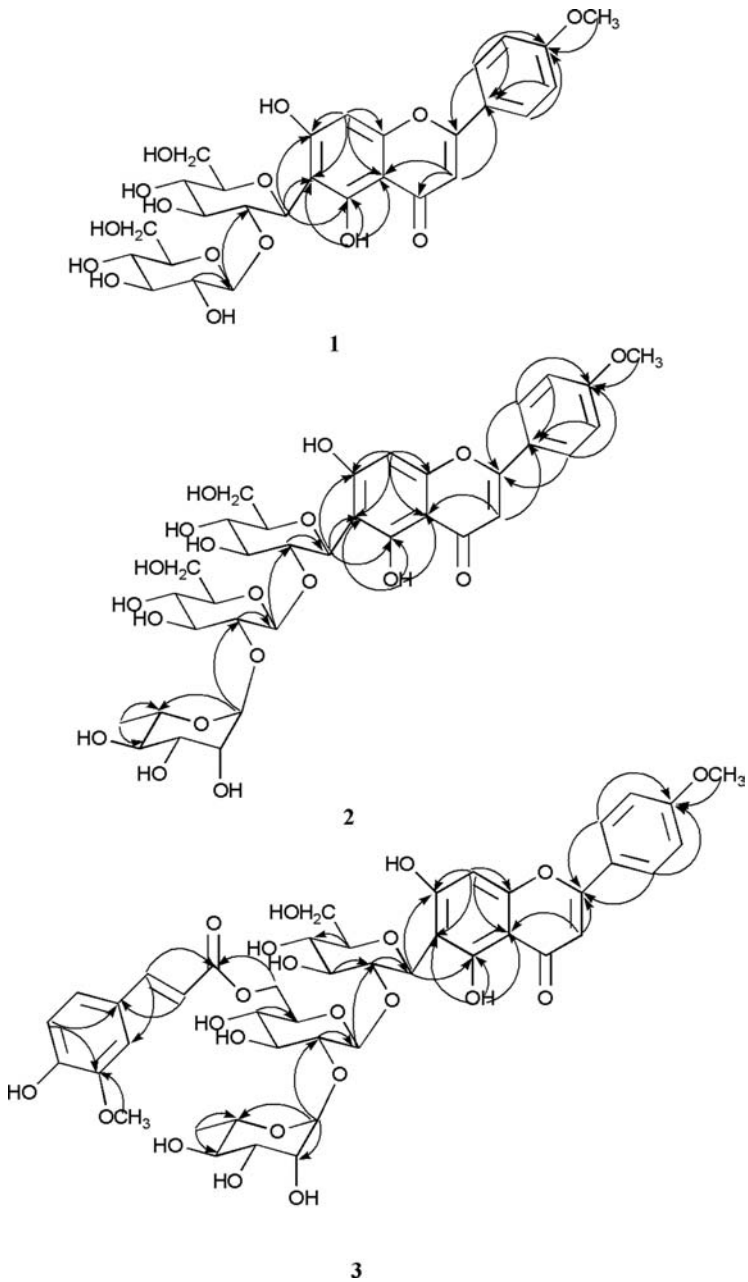


Figure 2. Key HMBC correlations of compounds **1**–**3**.

established the molecular formula as $C_{34}H_{42}O_{19}$ at *m/z* 777.2214 $[M + Na]^+$. On acid hydrolysis using HCl, glucose and rhamnose were detected in water solution. Comparing the optical rotation that of glucose and rhamnose, the two sugars were proved to be D-glucose and L-rhamnose, respectively. The tandem ESI-MS suggested the subsequent loss of rhamnosyl and glucosyl to produce the fragment ions at *m/z* 631 $[M + Na - rha]^+$ and 469 $[M + Na - rha - glc]^+$, respectively.

The $^{1}H$ and $^{13}C$ NMR spectral data of **2** (Table 1) were similar to those of **1**. The difference between the two compounds was that **2** possessed an extra α-L-rhamnopyranosyl moiety compared with **1**. According to the coupling constants of the anomeric hydrogens at δ 4.72 (1H, d, $J = 8.8$ Hz) and 4.75 (1H, d, $J = 7.2$ Hz), the two glucose units were both β configurated. According to the anomeric hydrogen at δ 5.03 (1H, br s), the rhamnose unit was α configurated. The cross peaks between H-1$''''$ (δ 5.03) and C-2$'''$ (δ 77.3) in the HMBC spectrum revealed that the extra rhamnose was connected to the C-2$'''$ of the intermediate glucose. Therefore, the structure of compound **2** was determined as acacetin-6-*C*-α-L-rhamnopyranosyl-(1 → 2) -β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranoside, namely paraquinin B.

Compound **3** was isolated as a yellow powder, and the IR spectrum showed absorptions of OH and C═C groups at 3447 and 1655 $cm^{-1}$, respectively. The UV spectrum with absorption maxima at 285 nm (band II) and 356 nm (band I) indicated the presence of flavone, whereas the absorption maxima at 255 nm implied the presence of independent benzene ring. The ESI-MS of **3** exhibited the pseudo-molecular ion peak at *m/z* 953 $[M + Na]^+$, and HR-ESI-MS established the molecular formula as $C_{44}H_{50}O_{22}$ at *m/z* 953.2675 $[M + Na]^+$. The tandem ESI-MS suggested the subsequent loss of rhamnosyl and (6$'''$-*O*-*E*-feruloyl)-glucosyl at *m/z* 807 $[M + Na-rha]^+$ and 469 $[M + Na-rha-glc-C_{10}H_8O_3]^+$.

The $^{1}H$ and $^{13}C$ NMR spectral data of **3** (Table 1) were similar to those of **2**, indicating that they have the same aglycone. The difference between compounds **2** and **3** was that **3** possessed an extra (*E*)-feruloyl moiety compared with **2**. According to the coupling constants of the anomeric hydrogens at δ 5.78 (1H, d, $J = 8.0$ Hz) and 5.48 (1H, d, $J = 8.0$ Hz), the two glucose units were both β configurated. According to the anomeric hydrogen at δ 6.55 (1H, br s), the rhamnose unit was α configurated. The $^{1}H$ NMR spectral data of **3** showed the typical signals of an (*E*)-feruloyl moiety as follows [11]: three aromatic H-atoms forming an ABX system [δ (H) 7.08 (br d, $J = 8.1$ Hz, 1H); 6.92 (br d, $J = 8.1$ Hz, 1H); 6.98 (br s, H-2$'''''$, 1H)]; two H-atoms of an (*E*)-configured C═C double bond [δ (H) 8.04 (d, $J = 15.8$ Hz, 1H); 6.70 (d, $J = 15.8$ Hz, 1H)]; and an aromatic MeO group at δ 3.68 (s, 3H). The cross peak between H-6$'''$ (δ 3.98, 3.76) and C-9$'''''$ (δ 167.3) in the HMBC spectrum revealed that the extra (*E*)-feruloyl moiety was connected to the C-6$'''$ of the intermediate glucose. Therefore, the structure of compound **3** was elucidated as acacetin-6-*C*-α-L-rhamnopyranosyl-(1 → 2) -(6$'''$-*O*-*E*-feruloyl)-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranoside, namely paraquinin C.

## 3. Experimental

### 3.1 General experimental procedures

Optical rotations were determined using a Perkin-Elmer 341 polarimeter. The IR spectra were recorded on a Perkin-Elmer Spectrum-One FT-IR spectrometer. UV spectra were measured in DMSO using a Perkin-Elmer Lambda 35 spectrophotometer. ESI-MS were recorded on a Finnigan $LCQ^{DECA}$ mass spectrometer. HR-ESI-MS were recorded using a Bruker Bio TOF Q mass spectrometer. The NMR spectra were recorded on a Bruker Avance 600 spectrometer (600 MHz for $^{1}H$ and 150 MHz for $^{13}C$), with TMS as an internal standard. Column chromatographies were performed on a commercial silica gel (160–200 and 200–300 mesh, Qingdao Marine Chemical Co. Ltd., Qingdao, China) and ODS (Nacalai, Tokyo, Japan).

### 3.2 Plant material

The aerial parts of *P. microphylla* were collected from the Maizhokunggar County of Tibet by Prof. Gesang Suolang, the

deputy director of the Tibet Autonomous Region Institute for Food and Drug Control. A voucher specimen (NO. JLD-20080701) has been deposited in the Tibet Autonomous Region Institute for Food and Drug Control.

### 3.3 Extraction and isolation

The air-dried and powered plant material (11.5 kg) was extracted exhaustively with 90% $CH_3OH$ three times at room temperature. The extract was concentrated and partitioned with petroleum ether, EtOAc, and *n*-BuOH, successively. The *n*-BuOH extract (400 g) was applied to silica gel column chromatography (8 cm i.d. × 130 cm) with gradient $CHCl_3$–MeOH (10:0 to 0:10, 6 L) to obtain fractions 1–9. Compound **1** (6.2 g) was educed from fraction 8 (40 g). Fraction 9 (103 g) was subjected to silica gel column chromatography (6 cm i.d. × 120 cm) with gradient $CHCl_3$–MeOH (10:1–1:1, 4 L) to obtain fractions A–H. Fraction H (40 g) was applied to reverse-phase silica gel column chromatography (5 cm i.d. × 44 cm) with gradient MeOH–$H_2O$ (5–80%) to obtain fractions a–d. Both fraction a (20 g) and fraction b (10 g) were subjected to silica gel column chromatography (4 cm i.d. × 80 cm) with gradient $CHCl_3$–MeOH (5:1 to 1:1, 2 L) to afford compounds **2** (100 mg) and **3** (10 mg), respectively.

#### 3.3.1 *Acacetin-6-C-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranoside (**1**)*

A yellow amorphous powder, $[\alpha]_D^{20}$ −48.0 ($c = 0.5$, DMSO). UV (DMSO) $\lambda_{max}$: 284, 355 nm. IR (KBr) $\nu_{max}$ ($cm^{-1}$): 3436, 2996, 2913, 1655, 1437, 1407, 1312, 1047, and 954. $^1H$ and $^{13}C$ NMR spectral data are given in Table 1. ESI-MS *m/z*: 609 $[M + H]^+$, 631 $[M + Na]^+$, and 643 $[M + Cl]^-$. HR-ESI-MS *m/z*: 609.1807 $[M + H]^+$ (calcd for $C_{28}H_{33}O_{15}$, 609.1814).

#### 3.3.2 *Acacetin-6-C-α-L-rhamno pyranosyl-(1 → 2)-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranoside (**2**)*

A Yellow amorphous powder, $[\alpha]_D^{20}$ −53.3 ($c = 0.36$, DMSO). UV (DMSO) $\lambda_{max}$: 285, 356 nm. IR (KBr) $\nu_{max}$ ($cm^{-1}$): 3435, 2997, 2913, 1659, 1436, 1407, 1312, 1047, and 954. $^1H$ and $^{13}C$ NMR spectral data are given in Table 1. ESI-MS *m/z*: 777 $[M + Na]^+$, 789 $[M + Cl]^-$. HR-ESI-MS *m/z*: 777.2214 $[M + Na]^+$ (calcd for $C_{34}H_{42}O_{19}Na$, 777.2213).

#### 3.3.3 *Acacetin-6-C-α-L-rhamno pyranosyl-(1 → 2)-(6‴-O-E-feruloyl)-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyrano side (**3**)*

A Yellow amorphous powder, $[\alpha]_D^{20}$ −161.0 ($c = 0.028$, DMSO). UV (DMSO) $\lambda_{max}$: 255, 285, and 353 nm. IR (KBr) $\nu_{max}$ ($cm^{-1}$): 3447, 2998, 2914, 1655, 1437, 1407, 1311, 1055, and 953. $^1H$ and $^{13}C$ NMR spectral data are given in Table 1. ESI-MS *m/z*: 953 $[M + Na]^+$, 965 $[M + Cl]^-$. HR-ESI-MS *m/z*: 953.2675 $[M + Na]^+$ (calcd for $C_{44}H_{50}O_{22}Na$, 953.2686).

### 3.4 TLC hydrolysis of flavone C-glycosides

The thin-layer plates spotted with the sample of compounds **1**–**3** and authentic sugars (D-glucose and L-rhamnose) were set into a beaker containing 8 ml concentrated hydrochloric acid. And then, the beaker sealed with double filter paper and plastic film was put into the waterbath at 50–60°C for 30 min. When the hydrolysis completed, the thin-layer plate was submitted to TLC analysis ($CHCl_3$–MeOH–$H_2O$–$CH_3COOH$ = 2:1:0.5:1). The $R_f$ value of monosaccharide from compound **1** was the same as that of D-glucose, whereas the monosaccharides from compounds **2** and **3** were also accordant with D-glucose and L-rhamnose, respectively.

### *3.5 Acid hydrolysis of flavone* C*-glycosides and the identification of the resulting monosaccharide*

Paraquinin A (50 mg) dissolved in DMSO (10 ml) and paraquinin B (10 mg) dissolved in DMSO (5 ml) were heated with concentrated hydrochloric acid (5 ml) under reflux for 8 h, respectively. The reaction mixture was diluted with water (50 ml) and extracted with EtOAc ($5 \times 50$ ml). The $H_2O$ layer was neutralized with NaOH and subjected to TLC analysis with authentic sugars.

The configuration of sugars was determined as follows: the standard solution of D-glucose, L-rhamnose, and the mixed standard solution of D-glucose and L-rhamnose with ratio of 2:1 were prepared and their respective optical rotation was measured as $[\alpha]_D^{20}$ +79.6 ($c = 0.5$, $H_2O$), $[\alpha]_D^{20}$ +9.6 ($c = 0.5$, $H_2O$), and $[\alpha]_D^{20}$ +54.8 ($c = 0.5$, $H_2O$). The optical rotation of acid hydrolysis solution of paraquinin A and paraquinin B was $[\alpha]_D^{20}$ +77.6 ($c = 0.3$, $H_2O$) and $[\alpha]_D^{20}$ +53.4 ($c = 0.07$, $H_2O$), respectively. Therefore, the configuration of the two sugars should be D-glucose and L-rhamnose.

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