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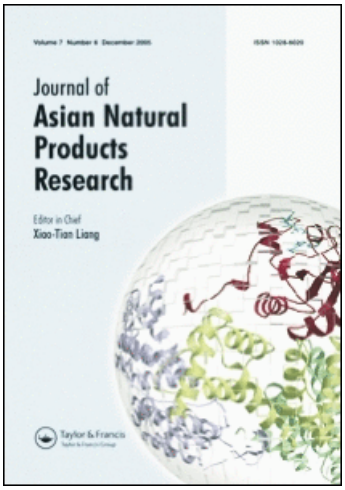

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Jian-Fu Xu[a]; Xiu-Ping Zheng[b]; Wei-Dong Liu[b]; Rui-Fang Du[b]; Li-Fu Bi[c]; Pei-Cheng Zhang[a]
[a] Key Laboratory of Bioactive Substances and Resources Utilization of Chinese Herbal Medicine (Ministry of Education), Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Bejing, China [b] Inner Mongolia Pharmaceutical Industrial Institute Co. Ltd, Hohhot, China [c] Inner Mongolia Medical College, Hohhot, China

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ORIGINAL ARTICLE

Flavonol glycosides and monoterpenoids from *Potentilla anserina*

Jian-Fu Xu[a], Xiu-Ping Zheng[b], Wei-Dong Liu[b], Rui-Fang Du[b], Li-Fu Bi[c]* and Pei-Cheng Zhang[a]*

[a]*Key Laboratory of Bioactive Substances and Resources Utilization of Chinese Herbal Medicine (Ministry of Education), Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Bejing 100050, China;* [b]*Inner Mongolia Pharmaceutical Industrial Institute Co. Ltd, Hohhot 010010, China;* [c]*Inner Mongolia Medical College, Hohhot 010059, China*

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Potentilin A (**1**), a rare diflavonol ester of μ-truxinic acid and a new normonoterpenoid, 2,6-dimethyl-2,3-dihydro-4-oxo-4*H*-pyran-2-acetic acid (**2**), was isolated from *Potentilla anserina*, together with 19 known flavonol glycosides (**3**–**21**) and 2 known monterpenoids (**22**,**23**). Their structures were elucidated by means of UV, IR, HR-ESI-MS, 1D and 2D NMR spectroscopic data.

Keywords: *Potentilla anserina*; flavonol glycoside; potentilin A; monterpenoid

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1. Introduction

Potentilla anserina L. (Rosaceae) is widely distributed in the north and southwest of China. The whole plant is used in traditional Chinese medicine for the treatment of hematemesis and diarrhea [1]. Recently, studies have found that the extracts of *P. anserina* showed significant anti-hepatitis B virus activities [2]. Previous phytochemical investigations on this species resulted in the isolation of terpenes, flavonoids, tannin, etc. [3–5]. In our further search for constituents from this plant, a rare diflavonol ester of μ-truxinic acid, named potentilin A (**1**), and a normonoterpenoid, 2,6-dimethyl-2,3-dihydro-4-oxo-4*H*-pyran-2-acetic acid (**2**), were isolated together with 19 known flavonol glycosides (**3**–**21**) and 2 known monterpenoids (**22**,**23**). Their structures were elucidated by means of spectroscopic methods.

2. Results and discussion

Compound **1** was obtained as a yellow amorphous powder, $[\alpha]_D^{25}$ +20.7 ($c = 0.05$, MeOH), and its UV spectrum showed the absorption characteristic of a flavonol skeleton at λ_{max} 350, 270sh, 224 nm. The IR spectrum of **1** exhibited the presence of hydroxyl (3425 cm^{-1}), carbonyl (1709 cm^{-1}), conjugated carbonyl groups (1657 cm^{-1}), and aromatic rings (1607 and 1510 cm^{-1}). Its ESI-MS showed an $[M + Na]^+$ peak at *m/z* 1211 and an $[M - H]^-$ peak at *m/z* 1187. The negative HR-ESI-MS data of **1** indicated an $[M - H]^-$ ion at *m/z* 1187.2663 $[M - H]^-$ corresponding to a molecular formula $C_{60}H_{52}O_{26}$. In the ^1H and ^{13}C NMR spectra of **1** (Table 1), two sets of similar signals existed in the flavone skeleton, sugar moiety and lignan units of **1**, suggesting that the structure of **1** was a dimer (Figure 1). The characteristic proton

*Corresponding authors. Email: yujiandandan@126.com; pczhang@imm.ac.cn

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Table 1. ^{1}H, ^{13}C NMR data and HMBC correlations of **1** in DMSO-d_6, δ in ppm, J in Hz[a].

No.	δ_H	δ_C	Correlated carbons in HMBC
Kaempferol moiety			
2		156.2, 156.3	
3		133.2, 133.0	
4		177.3, 177.3	
5		161.1, 161.1	
6	6.14, 6.18 (1H each, br s)	98.6, 98.6	C-8, C-10
7		164.1, 164.1	
8	6.32, 6.36 (1H each, br s)	93.6, 93.5	C-6, C-10
9		156.4, 156.3	
10		103.8, 103.8	
1′		120.7, 120.7	
2′	7.98 (1H, d, $J = 8.0$ Hz), 7.97 (1H, d, $J = 8.0$ Hz)	130.7, 130.7	C-3′, C-4′
3′	6.86 (1H, d, $J = 8.0$ Hz), 6.82 (1H, d, $J = 8.0$ Hz)	115.0, 114.9	C-1′
4′		159.9, 159.9	
5′	6.86 (1H, d, $J = 8.0$ Hz), 6.82 (1H, d, $J = 8.0$ Hz)	115.0, 114.9	C-1′
6′	7.98 (1H, d, $J = 8.0$ Hz), 7.97 (1H, d, $J = 8.0$ Hz)	130.7, 130.7	C-4′, C-5′
5-OH	12.59, 12.54 (1H each, br s)		
Glucoside moiety			
1″	5.43 (1H, d, $J = 7.0$ Hz), 5.31 (1H, d, $J = 7.0$ Hz)	101.2, 100.5	C-3
2″	3.18 (2H, m)	74.0, 74.0	
3″	3.31, 3.14 (1H each, m)	76.2, 76.0	
4″	3.00 (2H, m)	70.1, 69.8	
5″	3.07, 2.94 (1H each, m)	74.2, 74.0	
6″	4.21 (1H, d, $J = 11.0$ Hz), 3.56 (1H, dd, $J = 7.0$, 11.0 Hz)/3.82 (1H, d, $J = 11.5$ Hz), 3.36 (1H, m)	63.4, 63.4	
μ-Truxinyl moiety			
1(1′)		128.5, 128.3	
2(2′)	6.66 (1H, d, $J = 8.5$ Hz)/6.63 (1H, d, $J = 8.5$ Hz)	128.0, 127.9	C-7 (C-7′), C-4 (C-4′)
3(3′)	6.51 (1H, d, $J = 8.5$ Hz)/6.50 (1H, d, $J = 8.5$ Hz)	114.8, 114.7	C-1 (C-1′), C-5 (C-5′)
4(4′)		155.9, 155.8	
5(5′)	6.51 (1H, d, $J = 8.5$ Hz)/6.50 (1H, d, $J = 8.5$ Hz)	114.8, 114.7	C-1 (C-1′), C-3 (C-3′)
6(6′)	6.66 (1H, d, $J = 8.5$ Hz)/6.63 (1H, d, $J = 8.5$ Hz)	128.0, 127.9	C-7 (C-7′), C-4 (C-4′)
7	3.75 (1H, m)	38.9	C-2, C-6, C-8′, C-9
7′	3.71 (1H, m)	38.9	C-2′, C-6′, C-8, C-9′
8	3.43 (1H, m)	45.7	C-1, C-7′, C-9′
8′	3.36 (1H, m)	45.6	C-1′, C-7, C-9
9(9′)		171.0, 170.8	

Note: [a]^{1}H and ^{13}C NMR spectroscopic data were measured at 500 and 125 MHz, respectively.

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signals in pairs at δ 6.14, 6.18 (1H each, br s), 6.32, 6.36 (1H each, br s), 7.98 (2H, d, $J = 8.0$ Hz), 7.97 (2H, d, $J = 8.0$ Hz), 6.86 (2H, d, $J = 8.0$ Hz), 6.82 (2H, d, $J = 8.0$ Hz), and 12.59, 12.54 (1H each, br s) revealed the existence of two 5,7,4′-triihydroxy flavonol skeletons. This was also confirmed by the ^{13}C NMR spectrum of **1** and MS fragmentations indicating a successive loss of kaempferol (*m/z* 1211.3 $[M + Na]^+$, 925.2 $[M + Na - 286]^+$, and 639.2 $[M + Na - 2 \times 286]^+$). The HMBC correlations of flavonol C-3 carbons at δ 133.2 and 133.0 respective with anomeric protons at δ 5.43 (1H, d, $J = 7.0$ Hz) and 5.31 (1H, d, $J = 7.0$ Hz) indicated that the sugar moieties were located at C-3 carbons of the flavonol skeletons. Furthermore, the saccharide was identified as D-glucose based on the ^{13}C NMR spectroscopic data

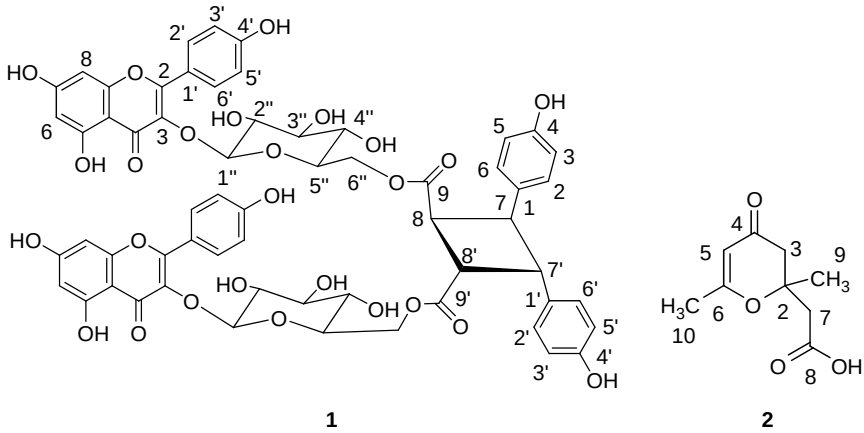

Figure 1. Structures of compounds **1** and **2**.

of the sugar moiety in **1** and the isolation of some known kaempferol-3-*O*-β-D-glucopyranoside derivatives from this plant. The coupling constant ($J = 7.0$ Hz) of the anomeric proton of the glucosyl moiety demonstrated that the sugar moiety had β-anomeric configurations. Except for the NMR signals of two sets of kaempferol-3-*O*-β-D-glucopyranosides, the ^{1}H NMR spectrum of **1** (Table 1) showed two AB-type aromatic proton signals and four methine proton signals, and the ^{13}C NMR spectrum of **1** (Table 1) indicated 18 carbon signals including 12 aromatic carbons, 4 methine carbons, and 2 carbonyl carbons. Taking account of the residual formula $C_{18}H_{14}O_4$, a diacyl moiety with a cyclobutane ring bearing two 4-hydroxyphenyl should exist in **1**. From a biosynthetic pathway perspective, such acyl moiety is possibly formed by a [2 + 2] cycloaddition reaction of two *p*-coumaric acids in either main μ-truxinyl-type (7.7′, 8.8′) or α-truxillyl-type (7.8′, 8.7′). The substitution pattern of two 4-hydroxyphenyl and two ester acyl groups in a cyclobutane ring was determined as μ-truxinyl-type rather than α-truxillic-type based on the chemical shifts and coupling patterns of the cyclobutyl protons [6,7] as well as HMBC and NOESY spectra. In the NOESY spectrum there was a clear correlation between H-8 and H-7 (Figure 2), but no correlation between H-8 and H-8′ was observed, while the HMBC spectrum

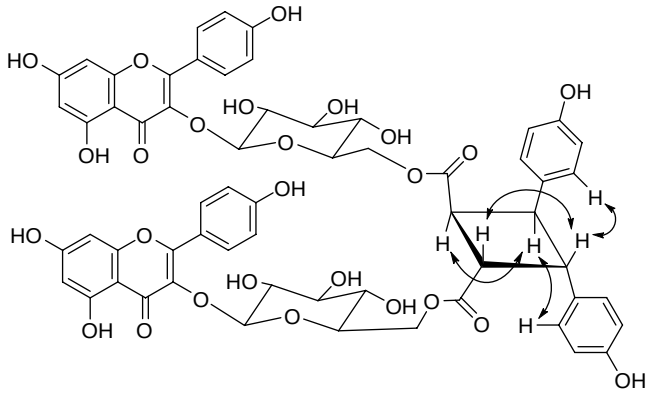

Figure 2. Selected NOESY correlations of compound **1**.

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(Table 1) revealed key long distance correlations of the proton [H-7 (H-7′)] and the carboxyl-bearing carbons [C-8′ (C-8)] (Table 1). Finally, the position of the acyl group was deduced to be C-6″ in the sugar moiety by the long distance correlations of carbonyl signal at δ 171.0 and 170.8, and the protons at δ 4.21 (1H, d, $J = 11.0$ Hz), 3.56 (1H, dd, $J = 11.0$, 7.0 Hz), 3.82 (1H, d, $J = 11.5$ Hz) and 3.36 (1H, m), respectively. This was also substantiated by the downfield shifts of C-6″ (Δ 2.5) and the upfield shifts of C-5″ (Δ 2.0) of glucopyranose compared with the corresponding signal of kaempferol-3-*O*-β-D-glucopyranoside (**17**). Thus, compound **1**, a rare diflavonol ester of μ-truxinic acid, was elucidated as depicted and named potentilin A. Only two diflavone glycoside truxinate esters, stachysetin [8] and monochaetin [9], were reported.

Compound **2** was obtained as a colorless oil, and the molecular formula was determined to be $C_9H_{12}O_4$ by HR-ESI-MS at *m/z* 207.0627 $[M + Na]^+$. The ^{1}H NMR spectrum showed an olefinic proton, 6 methyl protons and 4 methylene protons. The ^{13}C NMR spectrum showed nine carbon signals, which were observed as a ketone carbon, a carboxyl carbon, two olefinic carbons, a quaternary carbon substituted by oxygen, two methylene carbons, and two methyl carbons. The ^{1}H and ^{13}C NMR spectra were similar to those of 2,2,6-trimethyl-2,3-dihydro-4*H*-pyran-4-one [10]. The difference was that the C-2 methyl in 2,2,6-trimethyl-2,3-dihydro-4*H*-pyran-4-one was replaced by a carboxymethyl group in **2**. The proton and carbon signals of **2** were assigned by ^{1}H, ^{13}C NMR, HSQC, and HMBC spectra (Figure 3). Thus, the structure of **2** was characterized as 2,6-dimethyl-2,3-dihydro-4-oxo-4*H*-pyran-2-acetic acid, a new normonoterpenoid.

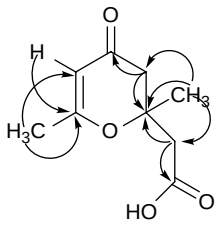

Figure 3. Selected HMBC correlations of compound **2**.

Additionally, 21 known compounds (**3**–**23**), kaempferol-3-*O*-β-D-(6-*O*-*trans*-*p*-coumaroyl) glucopyranoside (**3**) [11], kaempferol-3-*O*-β-D-(2-*O*-*trans*-*p*-coumaroyl) glucopyranoside (**4**) [12], apigenin-6-*C*-(2″-*O*-α-L-rhamnopyranosyl-β-D-glucopyranoside) (**5**) [13], vicenin-II (**6**) [14], apigenin-6-*C*-β-D-glucopyranosyl-8-*C*-β-D-xylopyranoside (**7**) [14], schaftoside (**8**) [14], isovitexin (**9**) [14], rutin (**10**) [15], quercetin-3,7-di-*O*-β-D-glucopyranoside (**11**) [16], isoscutellarein-8-*O*-β-D-glucopyranoside (**12**) [17], luteolin-3′-*O*-β-D-glucopyranoside (**13**) [18], luteolin-7-*O*-β-D-glucuronide (**14**) [19], luteolin-7-*O*-β-D-glucopyranoside (**15**) [18], quercetin-7-*O*-β-D-glucopyranoside (**16**) [18,20], astragalin (**17**) [21], reynoutrin (**18**) [22], quercetin-3-*O*-β-D-glucuronide (**19**) [23], isoquercitrin (**20**) [21], quercetin-3-*O*-α-L-rhamnopyranoside (**21**) [22], loliolide (**22**) [24], and isololiolide (**23**) [24] were identified by comparing their spectroscopic data with references.

3. Experimental

3.1 General experimental procedures

The optical rotations were measured on a Jasco P-2000 polarimeter. The UV spectra were scanned by a Hitachi UV-240 spectrophotometer. IR spectra were run on an IMPACT 400 (KBr) spectrometer. ^{1}H NMR (500 MHz), ^{13}C NMR (125 MHz), and 2D NMR spectra were recorded on INOVA 500 MHz spectrometers. HR-ESI-MS were performed on a Finnigan LTQ FT mass spectrometer. The ESI mass spectra were recorded on an Agilent 1100 series LC/MSD Trap spectrometer from Agilent Technologies (Santa Clara, CA, USA). All solvents used

were of analytical grade (Beijing Chemical Industrial Factory, Beijing, China). Column chromatography (CC) was performed with macroporous resin (Diaion HP-20, Mitsubishi Chemical Corporation, Tokyo, Japan), Rp-18 (50 μm, YMC, Kyoto, Japan), Sephadex LH-20 (Pharmacia Fine Chemicals, Uppsala, Sweden), and silica gel (100–200, 200–300 mesh, Qingdao Marine Chemical Inc., Qingdao, China). Preparative HPLC was carried out on a Shimadzu LC-6AD instrument with an SPD-20A detector, using a YMC-Pack ODS-A column (250 mm × 20 mm, 5 μm).

3.2 Plant material

The dried rhizomes of *P. anserina* L. were collected in Anguo, Hebei province of China, and were identified by Professor Lin Ma (Institute of Materia Medica, Peking Union Medical College and Chinese Academy of Medical Sciences, Beijing, China). A voucher specimen is deposited in the Herbarium of the Institute of Materia Medica, Peking Union Medical College and Chinese Academy of Medical Sciences, Beijing, China.

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3.3 Extraction and isolation

Dried rhizomes (9 kg) of *P. anserina* L. were cut into pieces and refluxed three times with 90% EtOH for 2 h. The extract was concentrated under reduced pressure to give a residue (695 g). The residue was suspended in H_2O and partitioned successively with petroleum ether and EtOAc to yield petroleum ether (80 g), EtOAc (175 g) extracts, and aqueous residue (458 g).

The EtOAc extract (175 g) was subjected to silica gel CC eluted with $CHCl_3$–MeOH (100:1–0:1) to yield fractions A–H. Fraction A (5 g) was applied to Sephadex LH-20 (petroleum ether–$CHCl_3$–MeOH, 5:5:1) to yield fractions A1–A8. Fraction A2 (30 mg) was further separated by preparative ODS–HPLC using MeOH–H_2O to give **22** (6 mg). Fraction B (6 g) was subjected to Sephadex LH-20 (petroleum ether–$CHCl_3$–MeOH, 5:5:1) followed by preparative ODS–HPLC using MeOH–H_2O to obtain **23** (5 mg). Fraction C (3 g) was applied to Sephadex LH-20 (petroleum ether–$CHCl_3$–MeOH, 5:5:1) to yield fractions C1–C3. Fraction C1 (200 mg) was further separated by preparative ODS–HPLC using MeOH–H_2O to give **2** (5 mg). Fraction E (10 g) was applied to Sephadex LH-20 (petroleum ether–$CHCl_3$–MeOH, 5:5:1) to yield fractions E1–E8. Fraction E1 (500 mg) was further separated by preparative ODS–HPLC using MeOH–H_2O to give **4** (10 mg). Fraction F (1 g) was subjected to preparative ODS–HPLC using MeOH–H_2O to give **12** (8 mg) and **13** (7 mg). Fraction G (1 g) was subjected to preparative ODS–HPLC using MeOH–H_2O to give **21** (5 mg).

The aqueous residue (458 g) was subjected to macroporous resin chromatographic column eluting with H_2O, and 15, 30, 50, 70, and 95% EtOH. The 30% EtOH fraction was applied to Sephadex LH-20 (MeOH–H_2O) to yield fractions J1–J5. These yield fractions were applied to preparative ODS–HPLC using MeOH–H_2O to give **5** (8 mg), **6** (20 mg), **7** (10 mg), and **8** (25 mg) from fraction J1, **10** (25 mg) and **11** (15 mg) from fraction J2, **14** (8 mg) from fraction J3, **19** (10 mg) from fraction J4. The 50% EtOH fraction was subjected to Sephadex LH-20 (MeOH–H_2O) to yield fractions K1–K3. These yield fractions were applied to preparative ODS–HPLC using MeOH–H_2O to give **1** (20 mg), **3** (30 mg), and **9** (8 mg) from fraction K1, **15** (7 mg), **16** (8 mg), and **17** (20 mg) from fraction K2, **18** (25 mg) and **20** (35 mg) from fraction K3.

3.3.1 Potentilin A (*1*)

Yellow, amorphous powder; $[\alpha]_D^{25}$ +20.7 ($c = 0.05$, MeOH); UV λ_{max} nm (MeOH), 350, 270sh, 224; IR (KBr) ν_{max}: 3425,

1709, 1656, 1607, 1566, 1510, 1208, 1177, 1080, 1067, 886, 835, 812 cm^{-1}. ^1H and ^{13}C NMR spectral data: see Table 1; ESI-MS: *m/z* 1211 $[M + Na]^+$, 1187 $[M - H]^-$; HR-ESI-MS: *m/z* 1187.2663 $[M - H]^-$ (calcd for $C_{60}H_{51}O_{26}$ 1187.2669).

*3.3.2 2,6-Dimethyl-2,3-dihydro-4-oxo-4H-pyran-2-acetic acid (**2**)*

Colorless oil; ^1H NMR ($CDCl_3$, 500 MHz) δ 2.52 (1H, d, $J = 16.5$ Hz, H-3a), 2.75 (1H, d, $J = 16.5$ Hz, H-3b), 5.37 (1H, s, H-5), 2.80 (2H, s, H-7), 1.56 (3H, s, H-9), 1.98 (3H, s, H-10); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 80.5 (C-2), 45.0 (C-3), 191.8 (C-4), 103.8 (C-5), 172.1 (C-6), 42.7 (C-7), 173.9 (C-8), 21.3 (C-9), 24.3 (C-10); HR-ESI-MS: *m/z* 207.0627 $[M + Na]^+$ (calcd for $C_9H_{12}O_4Na$, 207.0633).

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