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Lingyun Jia [a], Jing Wang [a], Chongning Lv [a], Tanye Xu [a], Lihua He [b], Yan Dong [a] & Jincai Lu [a]

[a] School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Box 79, Wenhua Road 103, Shenhe District, Shenyang, Liaoning Province, 110016, PR China

[b] School of Medical Devices, Shenyang Pharmaceutical University, Shenyang, Liaoning Province, 110016, PR China

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Two new compounds from *Potentilla multicaulis* Bunge

Lingyun Jia[a], Jing Wang[a], Chongning Lv[a], Tanye Xu[a], Lihua He[b], Yan Dong[a] and Jincai Lu[a]*

[a]*School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Box 79, Wenhua Road 103, Shenhe District, Shenyang, Liaoning Province 110016, PR China;* [b]*School of Medical Devices, Shenyang Pharmaceutical University, Shenyang, Liaoning Province 110016, PR China*

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Two new compounds (triterpenoid saponin and heterocyclic compound), 2α,3β, 19α,23,30-pentahydroxyurs-12-en-28-oic acid-28-*O*-β-D-glucopyranosyl ester (**1**) and N-hydroxyl-hexahydroazepin-2,4-diones (**2**), with 11 known compounds, picein (**3**), (7*S*,8*R*)-dihydrodehydrodiconiferyl alcohol-9′-*O*-β-D-glucopyranoside (**4**), (+)-1-hydroxy-2-epipinoresinol-1-β-D-glucoside (**5**), (+)-1-hydroxypinoresinol-1-β-D-glucoside (**6**), (+)-1-hydroxypinoresinol-4′-β-D-glucoside (**7**), schaftside (6-C-β-D-glucopyranosyl-8-C-α-L-arabinosyl apigenin) (**8**), isoschaftside (6-C-α-L-arabinosyl-8-C-β-D-glucopyranosyl apigenin) (**9**), isorhamnetin-3-*O*-β-D-glucopyranoside (**10**), quercetin-3-*O*-β-glucuronide (**11**), 8-*O*-methylherbacetin-3-*O*-sophoroside (**12**) and kaempferol (**13**), were isolated from *Potentilla multicaulis* Bunge. The structure of the compounds was elucidated by chemical and spectral evidence.

Keywords: *Potentilla multicaulis* Bunge; triterpenoid saponin; heterocyclic compound; structural elucidation

1. Introduction

Potentilla multicaulis Bunge is widely distributed in the northeast province of China (Institute of Botany, 1972). The whole plant of *P. multicaulis* Bunge has been used as a Chinese folk medicine to treat diabetes as a substitute of *Potentilla discolor* Bunge, which is a well-known treatment for this disease in China (Hong, Li, & Pu, 2007; Liu, Yan, & Xu, 2003; Ma & Wen, 2002). The genus *Potentilla* L., which belongs to the Rosaceae family, includes more than 200 species of plants widely distributed in the north temperate zone, boreal and alpine areas, and a few species near the equator. The aim of our work was to carry out the chemical study of *P. multicaulis* Bunge that has never been reported before to provide the chemical basis for *P. multicaulis* Bunge as a substitute of *P. discolor* Bunge. We had reported the phytochemical studies of this plant before (He, Hua, Zhang, & Lu, 2009). This study has also focused on investigating the remaining constituents of this herb medicine. Here, we report our recent investigation resulting in isolation and structural characterisation of two new compounds (**1** and **2**).

*Corresponding author. Email: jincailu@yahoo.com.cn

2. Results and discussion

The chemical studies of 70% ethanol extracts of *P. multicaulis* Bunge, by different chromatographic techniques, afforded compounds **1** and **2**. Compounds **1** and **2** were new compound and were identified by 1D, 2D NMR spectroscopy (Figure 1) and high-resolution (HR)-ESI-MS analysis, whereas the other compounds are known compounds identified by comparison of their NMR data with those reported in the literature as picein (**3**), (7*S*,8*R*)-dihydrodehydrodiconiferyl alcohol-9′-*O*-β-D-glucopyranoside (**4**), (+)-1-hydroxy-2-epipinoresinol-1-β-D-glucoside (**5**), (+)-1-hydroxypinoresinol-1-β-D-glucoside (**6**), (+)-1-hydroxypinoresinol-4′-β-D-glucoside (**7**), schaftside (6-C-β-D-glucopyranosyl-8-C-α-L-arabinosyl apigenin) (8), isoschaftside (6-C-α-L-arabinosyl-8-C-β-D-glucopyranosyl apigenin) (**9**), isorhamnetin-3-*O*-β-D-glucopyranoside (**10**), quercetin-3-*O*-β-glucuronide (**11**), 8-*O*-methyl-herbacetin-3-*O*-sophoroside (**12**) and kaempferol (**13**).

Compound **1** was obtained as white needles. The molecular formula was established as $C_{36}H_{58}O_{12}$ by the positive-ion HR-ESI-MS at *m/z*: 705.3751 $[M + Na]^+$ (calcd. 705.3820). The IR spectrum also released the absorption of hydroxyl (3422 cm^{-1}), carbonyl (1732 cm^{-1}) and double band (1636 cm^{-1}). The ^{13}C NMR spectrum showed 36 carbon signals, of which 30 were assigned to the triterpenoid aglycone and 6 to the sugar moieties. The five sp^3 carbons at δ 14.4, 17.6, 17.6, 24.1 and 27.4, and the two sp^2 carbons at δ 128.7 and 138.8, coupled with information from the ^1H NMR (five methyl proton singlets at δ 1.08, 1.14, 1.23, 1.55 and 1.60, and a broad single vinyl proton signal at δ 5.54), indicated that the aglycone had an urs-12-ene skeleton. Furthermore, the ^{13}C NMR spectrum showed a carbonyl signal at δ 177.0 (C-28). These together with comparison of chemical shifts for the aglycone region of nigaichigoside F_1 (Zhang, Peng, & Wang, 2001), only chemical shifts were observed at C-19 (+1.3 ppm), C-20 (+6 ppm), C-21 (+4.4 ppm) and C-30 (+48.1 ppm), which enabled the identification of the aglycone in 1 as 2,3,19,23, 30-pentahydroxy-12-ursen. After 1 was hydrolysed in acidic condition, D-glucose was isolated from the water layer by preparation layer chromatography (PTLC). The absolute configuration of the sugar was affirmed by optical rotation. The ^1H and ^{13}C NMR spectra of **1** revealed signals assignable to β-glucopyranosyl moiety with the anomeric proton signals at δ 6.30 (1H, d, $J = 8.4$ Hz, Glc-H-1), and the

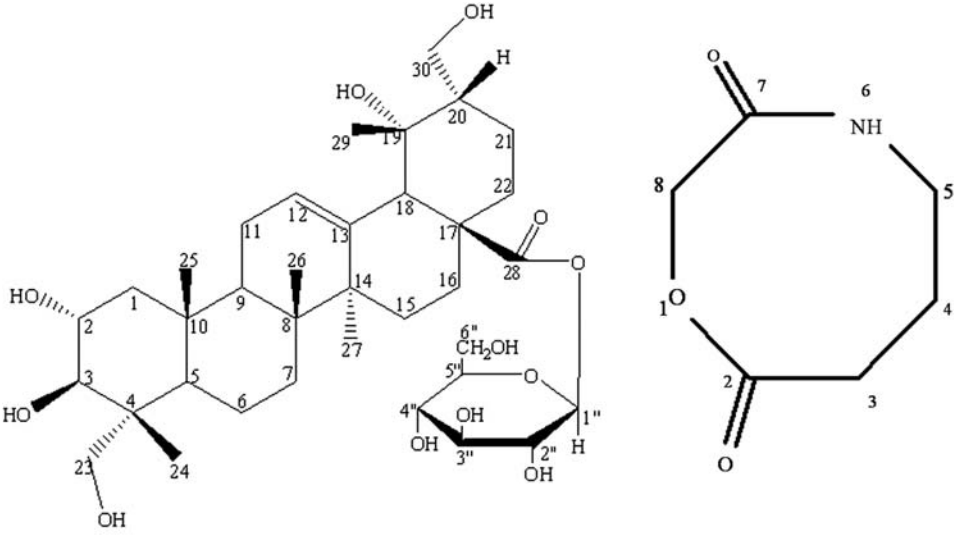

Figure 1. Structures of compounds isolated from *P. multicaulis* Bunge.

corresponding anomeric carbon signals at δ 95.9 (Glc-C-1), which were also observed in the heteronuclear singular quantum correlation (HSQC) experiment. The glucopyranosyl moiety bond connected between δ_C 177.0 (C-28) and δ_H 6.30 (1H, d, $J = 8.4$ Hz, Glc-H-1), δ_H 2.93 (1H, s, H-18), δ_H 1.88 (1H, ddd, $J = 4.2$, 13.2 Hz, H-22). Other structural fragments were confirmed by ^{1}H NMR, ^{13}C NMR and 2D NMR (HSQC and heteronuclear multiple-bond correlation (HMBC)) as showed in Table 1. Thus, the structure of compound **1** was determined to be 2α,3β,19α, 23,30-pentahydroxyurs-12-en-28-oic acid-28-*O*-β-D-glucopyranosyl ester (Figure 2).

Compound **2** was obtained as colourless needles, the molecular formula of **2** was determined as $C_6H_9O_3N$ by the HR-ESI-MS at *m/z*: 144.0657 $[M + H]^+$ (calcd. 144.0655), as well as from its NMR spectroscopic data. The IR spectrum suggested the presence of a hydroxyl (3423 cm^{-1}) and two carbonyl groups (1716 and 1632 cm^{-1}). Eight protons emerged in the ^{1}H NMR spectrum at δ 2.10 (2H, m), 2.47 (2H, t, $J = 7.8$ Hz), 3.52 (2H, t, $J = 7.2$ Hz) and 4.09 (2H, s). Taking into consideration of the two carboxyl (δ 171.6 and 176.7) and other four carbon signals (δ 17.9, 30.4, 44.2 and 48.2) found in the ^{13}C NMR spectra, the molecule was believed to contain N—CH_2—CH_2—CH_2—CO— and —CO—CH_2—CO— fragments. Simultaneously, the proton signal at δ 8.45 (1H, br s), in addition to the IR spectrum at 3423 cm^{-1} (—OH), revealed the presence of one N—OH fragment. The complete molecule was further demonstrated by 2D NMR (HSQC and HMBC) experiments. With all the above evidence, the structure of **2** was determined to be N-hydroxyl-hexahydroazepin-2,4-diones (Figure 2).

3. Experimental

3.1. *General*

Melting points were measured on a General Experimental Procedures X-4 purchased from Beijing Tech Instrument Co. Ltd (Beijing, China), and are uncorrected. IR spectra were recorded using a Bruker IFS-55 IR spectrometer with KBr discs. HR-ESI-MS were recorded on a Bruker micrOTOF-Q MS spectrometer. NMR spectra were recorded on a Bruker ARX-300 and Bruker AV-600 NMR spectrometers, with tetramethylsilane (TMS) as the internal standard. Silica GF_{254} for thin layer chromatography (TLC) and silica gel (200–300 mesh) for column chromatography were obtained from Qingdao Marine Chemical Company (Qingdao, People's Republic of China). Semi-preparative high performance liquid chromatography (HPLC) was carried out on a server side include (SSI) instrument consisting of a series pump and a Model 500

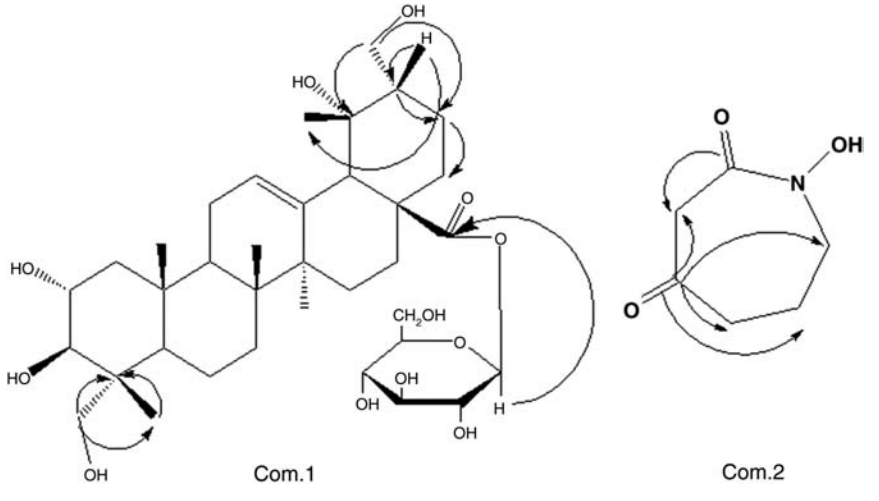

Figure 2. Structures and key HMBC correlations of compounds **1** and **2**.

UV detector. A YMC*Gel ODS-A was purchased from YMC Co. Ltd (YMC Karasume-Gojo Bldg., Japan). Reagents used were of analytical grade and purchased from Yuwang Group (Shandong, People's Republic of China).

3.2. *Plant material*

The whole herb of *P. multicaulis* Bunge was collected from Liaoning Province of China in August 2006 and was identified by Professor Qishi Sun (Department of Medicinal Plant, College of Traditional Chinese Medicine of Shenyang Pharmaceutical University). A voucher specimen (Voucher no.: 002155) has been deposited in the Herbarium of Shenyang Pharmaceutical University.

3.3. *Extraction and isolation*

The dry whole plant (10.8 kg) of *P. multicaulis* Bunge was extracted three times with 100 l of 70% ethanol for 1 h each time under reflux, and the solution was concentrated under reduced pressure. The resulting extract (1442 g) was subjected to a column (ϕ 15 × 200 cm) containing macroporous resin (AB8) and eluted with H_2O, 30%, 50%, 70%, 95% aqueous EtOH in six volumes of resin, successively. Evaporation of the solvent under reduced pressure delivered these fractions (frs). The viscous mass (35 g) of 95% aqueous EtOH was chromatographed on 350 g silica gel column (ϕ 10 × 120 cm), eluting with (P.E.)-EtOAc (50:1 → 0:1) (20:1, 15:1, 10:1, 5:1, 2:1, 1:1) to obtain 12 frs. Fr. 10 was purified by Sephadex LH-20 eluted with MeOH: H_2O (6:4) to afford a further 10 fractions (Fr. 10-1 to 10-10). Fr. 10-4 was further recrystallised to obtain compound **2** (30 mg). The concentrated viscous mass (292 g) of 70% aqueous EtOH was subjected to a column (ϕ 10 × 200 cm) containing silica gel 60, and then eluted with a linear gradient system of $CHCl_3$—MeOH (100:1 → 0:1) in 10 volumes of resin, and 500 mL of each fr. was collected to give 20 frs. Fr. 8 was further repeatedly subjected to Sephadex LH-20, modified decylsilyl silicion (MDS), octa decylsilyl silicion (ODS) chromatography and semi-preparative HPLC with various solvent systems to yield four known compounds **4** (125.4 mg), **5** (10 mg), **6** (23.3 mg), **7** (11 mg). Fr. 9 was repeatedly subjected to Sephadex LH-20, ODS chromatography with MeOH—H_2O (1:9–4:6) and further recrystallised to afford compound **1** (9 mg). Fr. 10 was also repeatedly subjected to Sephadex LH-20, MDS, ODS chromatography and preparative HPLC with various solvent systems to yield six known compounds **8** (13 mg), **9** (7 mg), **10** (10 mg), **11** (15 mg), **12** (23 mg), **13** (11 mg). The dissolved part of the concentrated viscous mass of 30% aqueous EtOH was isolated by Sephadex LH-20 and then eluted with gradient MeOH—H_2O (1:9–1:0) to give 11 frs. Fr. 4 was further purified by RP C-18 silica gel column (ϕ 3 × 60 cm), eluting with MeOH—H_2O (2:8) to obtain 10 frs. Fr. 5 was further recrystallised to obtain compound **3** (27 mg).

4. Acid hydrolysis of compound 1

An aqueous solution of 1 (5 mg) in 1 M HCl (2 mL) was heated at 80°C for 2 h. After the pH being modified to 7.0 with $NaHCO_3$ solution, the reaction mixture was extracted with $CHCl_3$ (3 × 4 mL). $CHCl_3$ part was evaporated and subjected to PTLC, using $CHCl_3$/CH_3OH (10:1) as eluent to yield an aglycone. The water layer was concentrated and subjected to PTLC (EtOAc/CH_3OH/H_2O = 7:3:0.4) to yield the sugar which were identified by TLC ($CHCl_3$/CH_3OH/H_2O = 16:9:1) with authentic samples and $[\alpha]_D$ as follows: D-glucose $[\alpha]_D^{25}$ + 9.6° (*c* 0.12, H_2O), spot was detected by spraying with EtOH—H_2SO_4–anisaldehyde (17:2:1) followed by heating, D-glucose were identified.

5. Data of compound 1

White needles; m.p. 214–215°C; $[\alpha]_D^{25}$ −11.7° (*c* 0.22, MeOH); IR ν_{max} (film on KBr): 3422, 2927, 1732, 1636, 1074, 619 cm^{-1}; showed a quasi-molecular ion peak at *m/z* 705.3751 $[M + Na]^+$ (calcd. for $C_{36}H_{58}O_{12}$ Na, 705.3820) in the HR-FAB-MS; ^{1}H NMR (600 MHz, in C_5D_5N), δ_H 1.38 (1H, m, H-1), 2.30 (1H, dd, 4.2, 12.0, H-1); 4.24 (1H, overlapped, H-2); 4.18 (1H, overlapped, H-3); 1.83 (1H, d, 11.4, H-5); 1.45, 1.69 (2H, m, H-6); 1.45 (1H, m, H-7), 1.75 (1H, ddd, 3.6, 11.2, H-7); 2.05 (1H, m, H-9); 1.92 (1H, m, H-10); 2.14, 1.58 (2H, m, H-11); 5.54 (1H, s, H-12); 1.18 (1H, dd, 3.6, 10.2, H-15), 2.45 (1H, m, H-15); 2.00 (1H, m, H-16), 3.13 (1H, ddd, 4.2, 13.2, H-16); 2.93 (1H, s, H-18); 1.45 (1H, m, H-20); 2.42, 1.38 (2H, m, H-21); 1.88 (1H, ddd, 4.2, 13.2, H-22), 2.14 (1H, m, H-22); 4.18 (1H, overlapped, H-23), 3.70 (1H, d,10.8, H-23); 1.08 (3H, s, H-24); 1.23 (3H, s, H-25); 1.23 (3H, s, H-26); 1.60 (3H, s, H-27); 1.55 (3H, s, H-29); 3.98 (1H, dd, 1.8, 10.8, H-30), 4.23 (1H, overlapped, H-30); 6.30 (1H, d, 8.4, H′-1); 4.22 (1H, m, H′-2); 4.05 (1H, m, H′-3); 4.36 (1H, t, 9.6, H′-4); 4.30 (1H, t, 9.6, H′-5); 4.40 (1H, dd, 4.2, 12.0, H′-6), 4.47 (1H, dd, 2.4, 12.0, H′-6). ^{13}C NMR (150 MHz, in C_5D_5N), δ_C 48.0 (C-1); 69.0 (C-2); 78.4 (C-3); 43.6 (C-4); 48.0 (C-5); 18.7 (C-6); 33.2 (C-7); 40.6 (C-8); 47.9 (C-9); 38.4 (C-10); 24.2 (C-11); 128.7 (C-12); 138.8 (C-13); 42.2 (C-14); 29.2 (C-15); 26.3 (C-16); 48.6 (C-17); 54.3 (C-18); 74.0 (C-19); 48.2 (C-20); 22.3 (C-21); 37.7 (C-22); 66.6 (C-23); 14.4 (C-24); 17.6 (C-25); 17.6 (C-26); 24.1 (C-27); 177.0 (C-28); 27.4 (C-29); 64.8 (C-30); 95.9 (C-1′); 74.1 (C-2′); 79.3 (C-3′); 71.2 (C-4′); 79.0 (C-5′); 62.3 (C-6′).

6. Data of compound 2

Colourless needles; m.p. 147–149°C; IR ν_{max} (film on KBr): 3423, 2964, 1716, 1660, 1478, 1397, 1342, 1264, 1002, 948, 873, 777, 658, 559, 511 cm^{-1}; showed a quasi-molecular ion peak at *m/z* 144.0657 $[M + H]^+$ (calcd. 144.0655) in the HR-FAB-MS; ^{1}H NMR (600 MHz, in DMSO-d_6), δ_H 8.45 (1H, br s, H-1); 4.09 (2H, s, H-3); 2.47 (2H, t, $J = 7.8$ Hz, H-5); 2.10 (2H, m, H-6); 3.52 (2H, t, $J = 7.2$ Hz, H-7). ^{13}C NMR (150 MHz, in DMSO-d_6), δ_C 171.6 (C-2); 44.2 (C-3); 176.7 (C-4); 30.4 (C-5); 17.9 (C-6); 48.2 (C-7).

Supplementary material

Spectral data relating to this article are available online.

Acknowledgements

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