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Article

Ursane-Type Triterpenoids from the Aerial Parts of *Potentilla discolor*

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Four ursane-type triterpenoids, ursolic acid (1), 23-hydroxyursolic acid (2), corosolic acid (3), and tormentic acid (4), and a phytosterol, β-sitosterol-3-*O*-β-D-glucoside, were isolated from an EtOAc-soluble extract of the aerial parts of *Potentilla discolor*. The structures of 1-4 were identified by spectroscopic methods, particularly by extensive NMR studies. This is the first report on the isolation of compounds 1-4 from this plant.

Key words: *Potentilla discolor, Rosaceae, triterpenoid, ursolic acid*

The genus *Potentilla* belongs to the Rosaceae family, comprising of more than 200 species, and is widely distributed in the Northern temperate zone. *Potentilla discolor* Bunge (Rosaceae) is a perennial herb native in Korea, China, and Japan which can be distinguished distinctly by the densely pubscent on the both sides of leaflets and the divided thick spindle-shape roots from the other related species such as *P. fragarioides* var. *major* Maxim. and *P. yokusaiana* Makino.[1)] Its dried roots are used as a traditional Chinese medicine for the treatment of diarrhea and hemorrhage.[2)] The whole plants are used mainly in the treatment of amebic dysentery, trichomonas vaginitis, and tuberculosis involving the lymph nodes of the neck in China. It also causes smooth muscle relaxation, especially of the bronchial and intestinal muscles.[3)] *P. discolor* is one of the botanical origins of Korean folk medicine "Jin Hae Cho Ip" which has been used as a remedy for neuralgia and as an invigorating drug after a childbirth.[4)] However, to the best of our knowledge, there are just two prior reports on secondary metabolites of *P. discolor*; on the isolations of phenolic acids and flavonoids from the whole plants[5)] and hydrolysable tannins from the roots.[2)] Therefore the present work was designed to study the phytochemical investigation of the aerial parts of *P. discolor*.

Materials and Methods

Plant materials. The aerial parts of *Potentilla discolor* Bunge (Rosaceae) were collected in Youngcheon, Kyungbuk, Korea, in May, 2005 and were identified by Prof. J.-H. Kim, Daejeon University. A voucher specimen (no. KIOM-P031) has been deposited at the Herbarium of Department of Herbal Pharmaceutical Development, Korea Institute of Oriental Medicine, Korea.

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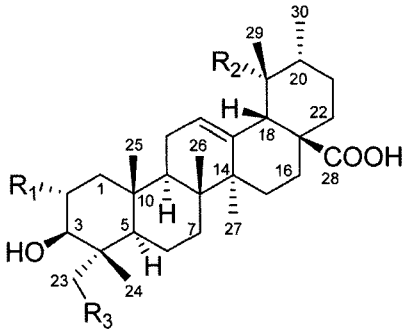
General experimental procedures. Melting points were measured on an IA9100 melting point apparatus (Barnstead International, USA) and were quoted uncorrected. LRESI was recorded on an Autospec (Micromass, UK). NMR experiments were conducted on a DRX-300 FT-NMR (Bruker, Germany), and the chemical shifts were referenced to the residual solvent signals. TLC analysis was performed on Kieselgel 60 F_{254} (Merck) plates (silica gel, 0.25 mm layer thickness); compounds were visualized by dipping plates into 10% (v/v) H_2SO_4 reagent (Aldrich) and then heat treated at 110°C for 5-10 min. Silica gel (Merck 60A, 70-230 or 230-400 mesh ASTM) and Sephadex LH-20 (Amersham Pharmacia Biotech) were used for column chromatography. All solvents used for the chromatographic separations were distilled before use.

Extraction and isolation. The fresh and cut plant material (750 g) was extracted with MeOH (3 × 7 *l*) by maceration. The extracts were combined and concentrated *in vacuo* at 40°C. The concentrated extract (87 g) was suspended in H_2O (1.0 *l*) and then partitioned with *n*-hexane (3 × 1.0 *l*) to afford a *n*-hexane-soluble fraction (67 g) on drying. Next, the aqueous partition was partitioned again with EtOAc (3 × 1.0 *l*) to give an EtOAc-soluble fraction (5.9 g) and an aqueous residue. The EtOAc-soluble fraction (5.8 g) was chromatographed over silica gel (ϕ 7.0 × 47 cm, 70-230 mesh) as the stationary phase using a $CHCl_3$–MeOH gradient (from 1 : 0 to 0 : 1 v/v) to yield 10 pooled fractions (fractions F01-F10). Compound **1** (99 mg) and β-sitosterol-3-*O*-β-D-glucoside (120 mg) were isolated from fractions F02 [eluted with $CHCl_3$-MeOH (99 : 1 v/v); 497 mg] and F05 [eluted with $CHCl_3$-MeOH (14 : 1 v/v); 445 mg], respectively by recrystallization (both in MeOH). Fraction F03 [eluted with $CHCl_3$-MeOH (49 : 1 v/v); 760 mg] was further chromatographed through silica gel (ϕ 4.6 × 36 cm, 230-400 mesh; *n*-hexane-acetone 3 : 1 v/v) to produce four subfractions (fractions F0301-0304). Compounds **2** (22 mg) and **3** (145 mg) were purified from fraction F0302 by reversed-phase column chromatography (ϕ 3.6 × 43 cm, 12 nm S-150 μm; MeOH-H_2O = 19 : 1). Fraction F0303 was



1 $R_1 = R_2 = R_3 = H$
2 $R_1 = H, R_2 = H, R_3 = OH$
3 $R_1 = OH, R_2 = H, R_3 = H$
4 $R_1 = R_2 = OH, R_3 = H$

Fig. 1. Structures of Compounds 1-4 from the Aerial Parts of *Potentilla discolor*.

further fractionated by Sephadex LH-20 column chromatography (ϕ 4.6 × 65 cm, MeOH) to afford compound **4** (61 mg).

Ursolic acid (1); White powder (MeOH); mp 284-286°C; ESIMS *m/z* (rel. int.): 457 $[M+H]^+$; ^{1}H-NMR (pyridine-d_5, 300 MHz) δ 5.51 (1H, t-like, H-12), 3.47 (1H, dd, J = 9.0, 7.2 Hz, H-3), 2.65 (1H, d, J = 11.1 Hz, H-18), 1.26 (3H, s, H-23), 1.25 (3H, s, H-27), 1.07 (3H, s, H-26), 1.04 (3H, s, H-24), 1.03 (3H, d, J = 6.3 Hz, H-30), 0.98 (3H, d, J = 5.4 Hz, H-29), 0.91 (3H, s, H-25); ^{13}C-NMR (pyridine-d_5, 75 MHz) data, see Table 1.

23-Hydroxyursolic acid (2); White powder ($CHCl_3$-MeOH); mp 284-286°C; ESIMS *m/z*: 473 $[M+H]^+$; ^{1}H-NMR (pyridine-d_5, 300 MHz) δ 5.51 (1H, t-like, H-12), 4.23 (1H, overlapped, H-3), 4.19 (1H, d, J = 10.2 Hz, H-23a), 3.74 (1H, d, J = 10.2 Hz, H-23b), 2.65 (1H, d, J = 11.1 Hz, H-18), 1.20 (3H, s, H-27), 1.09 (3H, s, H-26), 1.07 (3H, s, H-24), 1.01 (3H, d, J = 5.4 Hz, H-29), 0.99 (3H, s, H-25), 0.95 (3H, d, J = 5.4 Hz, H-30); ^{13}C-NMR (pyridine-d_5, 75 MHz) data, see Table 1.

Corosolic acid (3); White powder; mp 252-254°C ($CHCl_3$-MeOH); EIMS *m/z*: 473 $[M+H]^+$; ^{1}H-NMR (pyridine-d_5, 300 MHz) δ 5.48 (1H, t-like, H-12), 4.09 (1H, ddd, J = 11.4, 9.3, 4.5 Hz, H-2), 3.40 (1H, d, J = 9.3 Hz, H-3), 2.64 (1H, d, J = 11.1 Hz, H-18), 1.29 (3H, s, H-23), 1.23 (3H, s, H-27), 1.09 (3H, s, H-24), 1.02 (3H, s, H-25), 1.01 (3H, d, J = 6.6 Hz, H-30), 1.00 (3H, s, H-26), 0.97 (3H, d, J = 6.0 Hz, H-29); ^{13}C-NMR (pyridine-d_5, 75 MHz) data, see Table 1.

Tormentic acid (4); White powder ($CHCl_3$-MeOH); mp 273-274°C; ESIMS *m/z*: 489 $[M+H]^+$; ^{1}H-NMR (pyridine-d_5, 300 MHz) δ 5.59 (1H, t-like, H-12), 4.88 (1H, br s, OH-19), 4.09 (1H, ddd, J = 11.1, 9.6, 4.2 Hz, H-2), 3.39 (1H, d, J = 9.6 Hz, H-3), 3.06 (1H, s, H-18), 3.12 (1H, dt, J = 12.9, 4.5 Hz, H-18a), 1.72 (3H, s, H-27), 1.44 (3H, s, H-29), 1.28 (3H, s, H-23), 1.13 (3H, d, J = 6.0 Hz, H-30), 1.12 (3H, s, H-26), 1.09 (3H, s, H-24), 1.03 (3H, s, H-25); ^{13}C-NMR (pyridine-d_5, 75 MHz) data, see Table 1.

Table 1. ^{13}C-NMR chemical shifts of triterpenoids from the aerial parts of *Potentilla discolor* (75 MHz in pyridine-d_5)

Position	1	2	3	4
1	39.5	39.3	48.4	48.3
2	28.5	28.1	69.0	69.0
3	78.5	73.8	84.2	84.3
4	39.8	43.3	40.2	40.3
5	56.2	49.0	56.3	56.4
6	19.2	19.0	19.2	19.4
7	34.0	31.4	33.9	33.8
8	39.7	40.4	40.4	40.9
9	48.4	48.6	48.5	48.1
10	37.7	37.5	38.8	39.1
11	24.0	24.1	25.3	24.5
12	126.0	126.1	125.9	128.3
13	139.6	139.7	139.7	140.4
14	40.4	42.9	42.9	42.6
15	29.1	29.1	29.0	29.7
16	25.3	25.4	24.1	26.8
17	42.9	48.5	48.5	48.8
18	53.9	54.0	53.9	55.1
19	39.9	39.9	39.9	73.2
20	39.8	39.8	39.8	42.8
21	31.5	33.7	31.5	27.3
22	37.8	37.9	37.8	39.0
23	29.2	68.4	29.8	29.8
24	17.0	13.6	17.2	18.1
25	16.1	16.5	17.9	17.3
26	17.9	17.9	18.1	17.6
27	24.3	24.3	24.3	25.1
28	180.3	180.4	180.3	181.1
29	17.8	17.9	17.4	17.2
30	21.8	21.8	21.8	27.5

Results and Discussion

Four ursane-type triterpenoids (**1-4**) together with β-sitosterol-3-*O*-β-D-glucoside were isolated from the EtOAc-soluble extract of the aerial parts of *Potentilla discolor* through repeated silica gel (normal and reversed) and sephadex column chromatography.

Compound **1** was obtained as white amorphous powder and its (+)-ESIMS spectrum showed a molecular ion peak at *m/z* 457 $[M+H]^+$. All of the carbon signals of **1** are shown in Table 1, which reveals the presence of seven methyl, a carboxyl, an olefinic methine, an olefinic quaternary, and an oxygenated methine carbon. The ^{1}H-NMR spectrum of **1** showed resonances for five tertiary methyl singlet signals at δ 1.26 (3H, H-23), 1.25 (3H, H-27), 1.07 (3H, H-26), 1.04 (3H, H-24), and 0.91 (3H, H-25), two secondary methyl doublet signals at δ 1.03 (3H, d, J = 6.3 Hz, H-30) and 0.98 (3H, d, J = 5.4 Hz, H-29), an oxygenated methine signal at δ 3.47 (1H, dd, J = 9.0, 7.2 Hz, H-3), and a trisubstituted olefinic signals

at δ 5.51 (1H, t-like, H-12), suggesting that **1** is an 3β-hydroxy-urs-12-en type triterpenoid possessing a carboxyl group. Comparison of the above data with those in the literature[6] led to identification of **1** as ursolic acid (3β-hydroxy-urs-12-en-28-oic acid).

Compound **2** was obtained as white amorphous powder and its (+)-ESIMS spectrum showed a molecular ion peak at *m/z* 473 $[M+H]^+$, one more oxygen atom than **1**. The proton and carbon signals in the ^1H- and ^{13}C-NMR spectra of **2** were very similar to those of **1**, except for the signals of C-23. The resonances for a tertiary methyl group of **1** were missing in the ^1H- and ^{13}C-NMR spectra of **2** and an AB system appeared in the more down-field region at δ_H 4.19 and 3.74 (H_2-23, J= 10.2 Hz) and δ_C 68.4 (C-23), respectively. On the basis of the above result and by comparison with those in the literature,[7] compound **2** was identified as 23-hydroxyursolic acid (3β,23-dihydroxy-urs-12-en-28-oic acid).

Compound **3** was obtained as white amorphous powder and its (+)-ESIMS spectrum showed the same molecular ion peak at *m/z* 473 $[M+H]^+$ as **2**, one more oxygen atom than **1**. Careful comparison of NMR data of **1** (ursolic acid) and **3** showed very close similarities between two compounds. The major differences in the NMR spectra of **3** were the presence of an additional oxygenated methine signals at δ_H 4.09 (1H, ddd, J= 11.4, 9.3, 4.5 Hz, H-2) and δ_C 69.0 (C-2), suggesting that **3** is a 2,3-dihydroxy-ursane type triterpenoid. The 2α,3β-configuration of the two secondary hydroxyl groups of **3** were suggested by the coupling constant (J= 9.3 Hz) between H-2 and H-3. Thus, compound **3** was expected to be corosolic acid (2α,3β-dihydroxy-urs-12-en-28-oic acid) and was confirmed by comparison of its spectral data with those reported in the literature.[8]

The NMR spectral data of **4** were nearly identical to those of **3** except for an additional hydroxyl group at C-19 (δ_C 73.2). The ^1H NMR spectrum of **4** also showed resonances for two oxygenated methine signals at δ 4.09 (1H, ddd, J= 11.1, 9.6, 4.2 Hz, H-2) and 3.39 (1H, d, J= 9.3 Hz, H-3), and the stereochemistry of the two secondary hydroxyl groups were determined as 2α,3β-configuration according to the coupling constant (J= 9.6 Hz) between H-2 and H-3. Therefore, the structure of **4** was suggested to be tormentic acid (2α,3β,19α-trihydroxy-urs-12-en-28-oic acid) and its NMR spectral showed a good agreement with reported data.[9]

A literature survey indicated that triterpenoids and polyphenols are the main substances isolated from the *Potentilla* species and the most of triterpenoids have the urs-12-ene skeletone and hydroxyl substitutions at C-2, C-3, C-19.[10] All of the triterpenoids (**1-4**) isolated from *Potentilla discolor* in this study were also urs-12-ene derivatives. Although the presence of ursolic acid (**1**) in *P. discolor* was reported,[11] to the best of our knowledge, this is the first report on the isolation of compounds **1-4** from this plant.

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