# A Triterpenoid Saponin from *Patrinia scabiosaefolia*

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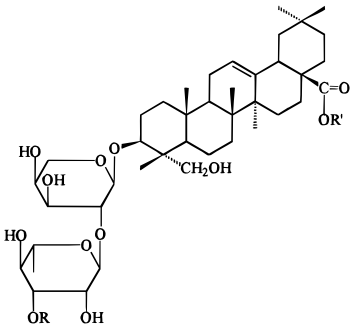
A new triterpenoid saponin, patrinia saponin H3 (**3**), was isolated from the aerial parts of *Patrinia scabiosaefolia* Fisch. and determined to be 3-*O*-*β*-D-glucopyranosyl(1→3)-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranosyl hederagenin 28-*O*-α-L-rhamnopyranosyl(1→4)-*β*-D-glucopyranosyl(1→6)-*β*-D-glucopyranosyl ester on the basis of NMR and FAB MS experiments and acid hydrolysis.

*Patrinia scabiosaefolia* Fisch. (Valerianaceae) is well-known in oriental traditional medicine. Its roots or whole plants have been used for treatment of various diseases including edema, appendicitis, endometritis, and inflammation.[1] Previous phytochemical investigation has revealed that the plants are rich in saponins. More than 20 saponins have been isolated[2] together with coumarins,[3] iridoids,[4] flavonoids,[5] and organic acids,[6] but no phytochemical study on the aerial parts of this plant has been reported in the literature so far. In our search for biologically active natural products, we investigated the saponin constituents of the aerial parts of *P. scabiosaefolia*, from which two saponins (**1** and **2**) together with rutin were isolated. The results were recently reported by one of us.[7] This paper describes the isolation and structure elucidation of a new saponin, patrinia saponin H3 (**3**), from this plant.




**1** R = R' = H

**2** R = H, R' = Rha(1→4)Glc(1→6)Glc

**3** R = Glc, R' = Rha(1→4)Glc(1→6)Glc

**3a** R = Glc, R' = H

Patrinia saponin H3 (**3**) was obtained as amorphous powder, mp 228−230 °C. An acid hydrolysis of **3** afforded hederagenin as an aglycon and arabinose, rhamnose, and glucose as the sugar components identified on TLC by comparison with authentic samples. Absolute configurations for sugars were determined to be L-form except for glucose (D-form), according to the procedure developed by Hara *et al*.[8] An alkaline hydrolysis of **3** yielded prosapogenin **3a**, mp 241−244 °C, which liberated arabinose, rhamnose, and glucose on acid hydrolysis. The negative ion mode FAB mass spectrum of **3** exhibited an $[M - H]^-$ ion at *m*/*z* 1381, which is consistent with a hexasaccharide glycoside carrying one arabinose, two rhamnose, three glucose, and an aglycon with molecular mass of 472. The remarkable intensity of the fragment ion at *m*/*z* 911 $[M - H - 146 - 162 - 162]^-$, which had an 80% intensity of the base peak of the spectrum strongly suggested that a trisaccharide chain corresponding to rhamnose−glucose−glucose was linked to hederagenin by an ester function.[9,10] The fragments at *m*/*z* 749 $[M - H - 146 - 162 - 162 - 162]^-$ and 603 $[M - H - 146 - 162 - 162 - 162 - 146]^-$ showed the presence of the linear sugar chain, and the sugar sequence would seem to be that of glucose−rhamnose−arabinose. The NMR spectral data revealed that the features of an oleanane type triterpene saponin whose hydroxyl group at C-3 and carboxyl group at C-28 are glycosylated.[11,12] It exhibited six anomeric proton signals at *δ* 4.95 (1H, d, *J* = 7.8 Hz), 5.47 (1H, d, *J* = 7.8 Hz), 6.26 (1H, br s), 4.97 (1H, d, *J* = 6.7 Hz), 5.83 (1H, br s) and 6.22 (1H, d, *J* = 8.0 Hz) in its $^1$H NMR spectrum. Prosapogenin **3a** showed an $[M - H]^-$ ion at *m*/*z* 911 in the negative ion mode FAB mass spectrum and three anomeric proton resonances at *δ* 4.95 (1H, d, *J* = 6.7 Hz), 5.39 (1H, d, *J* = 7.6 Hz), and 6.15 (1H, s) in its $^1$H NMR spectrum, suggesting that **3a** contains 1 mol each of arabinose, rhamnose, and glucose in the molecule. The analysis of these spectral data as well as the results of a sugar analysis indicated that both trisaccharide residues at C-3 and C-28 of hederagenin were determined to be *β*-D-glucopyranosyl-α-L-rhamnopyranosyl-α-L-arabinopyranose and α-L-rhamnopyranosyl-*β*-D-glucopyranosyl-*β*-D-glucopyranose, respectively. $^1$H−$^1$H COSY and HMQC experiments permitted assignments of the interglycosidic linkages by comparing the carbon chemical shifts observed with those of the corresponding methyl pyranosides, taking into account the known

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effects of glycosidation.[13] A glycosidation shift was observed at the C-3 signal of the rhamnose residue by +10.5 ppm, i.e. the terminal glucose unit was linked at the C-3 position of a rhamnose unit. In the same manner, the chemical shift of the innermost arabinosyl C-2 at C-3 of hederagenin was displaced downfield to 75.7 ppm. On the basis of these results, the structure of the prosapogenin **3a** was determined to be hederagenin 3-*O*-*β*-D-glucopyranosyl(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside which has been isolated from the leaves of *Aralia elata*.[14] The sequence of the acyl sugar moieties at C-28 was α-L-rhamnopyranosyl-(1→4)-*β*-D-glucopyranosyl(1→6)-*β*-D-glucopyranose, because the chemical shifts of the carbon at C-6 on the glucose linked with the genin and the C-4 of the second glucose were both about 7 ppm downfield in comparison with those of the free sugar. In order to determine the definite squence and linkage positions among the sugar units and aglycon, a HMBC experiment was performed. In the HMBC experiment, the anomeric proton signals at $\delta$ 5.47 (d, $J$ = 7.8 Hz), 6.26 (br s), and 4.97 (d, $J$ = 6.7 Hz) assignable to glucose, rhamnose, and arabinose were correlated to the three-bond-coupled carbon signals at $\delta$ 83.0 (C-3 of rhamnose), 75.9 (C-2 of arabinose), and 81.2 (C-3 of hederagenin), respectively. Additional anomeric proton signals for acyl sugar moieties at $\delta$ 5.83 (br s), 4.95 (d, $J$ = 7.8 Hz), and 6.22 (d, $J$ = 8.0 Hz) assignable to rhamnose, glucose, and innermost glucose were correlated to the carbon signals at $\delta$ 78.7 (C-4 of glucose), 69.2 (C-6 of innermost glucose), and 176.5 (C-28 carbonyl carbon), respectively. In light of the above observations, the structure of the patrinia saponin H3 (**3**) was determined to be 3-*O*-*β*-D-glucopyranosyl(1→3)-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranosyl hederagenin 28-*O*-α-L-rhamnopyranosyl(1→4)-*β*-D-glucopyranosyl(1→6)-*β*-D-glucopyranosyl ester.

## Experimental Section

**General Experimental Procedures.** Melting points were measured on a Mitamura-Riken apparatus and are uncorrected. The optical rotations were determined on a Rudolph Autopol III automatic polarimeter. The IR spectra were obtained on a JASCO FT/IR-5300 spectrometer. The FAB mass spectrum was obtained in a 3-nitrobenzyl alcohol matrix in a negative ion mode on a VG-VSEQ spectrometer. The NMR spectra were measured in pyridine-$d_5$ on a Bruker AMX-500 instrument, and the chemical shifts were referenced to TMS. GC analysis was performed with a Hewlett-Packard 5890 Series II gas chromatograph equipped with an $H_2$ flame ionization detector. The column was HP-5 capillary column (30 m × 0.32 mm × 0.25 $\mu$m). Conditions: column temperature, 200 °C; injector and detector temperature, 290 °C; He flow rate, 30 mL/min. TLC was performed on silica gel 60$F_{254}$ (Merck) and cellulose plate (Art No. 5716, Merck).

**Plant Material.** The aerial parts of *P. scabiosaefolia* were collected in September 1995 near Wonju City in Kangwon Province, Korea, and authenticated by one of us (Y.H.K.). A voucher specimen (YHK95003) was deposited in the herbarium of the Natural Products Research Institute, Seoul National University.

**Extraction and Isolation.** Fresh aerial parts of *P. scabiosaefolia* (710 g) were extracted three times with MeOH at room temperature. The MeOH extract was evaporated under reduced pressure to dryness, which was partitioned in succession between $H_2O$ and $CHCl_3$, EtOAc, and then *n*-BuOH and afforded 8.2, 2, and 11.1 g of the respective extracts. The BuOH fraction was passed through a porous polymer MCI gel (CHP 20P, 75−150 $\mu$m) column. After the column was washed with $H_2O$, the adsorbed materials were eluted successively with 20% and 50% aqueous MeOH, and MeOH to yield four ($B_{201}$−$B_{204}$), seven ($B_{501}$−$B_{507}$), and three subfractions ($B_{101}$−$B_{103}$), respectively. The subfraction $B_{103}$ (1.19 g) was repeatedly chromatographed on silica gel 60 using EtOAc saturated with $H_2O$ with increasing amounts of MeOH as eluent to yield saponin **2** (430 mg) and then **3** (53 mg). The structure of **2** was confirmed by direct comparison with an authentic sample obtained from *Pulsatilla koreana* Nakai.[15]

Patrinia saponin H3 (**3**) was obtained as amorphous powder (MeOH): mp 228−230 °C; $[\alpha]^{20}_D$ −13.4° (*c* 0.6, pyridine); IR (KBr) $\nu_{max}$ 3437, 2934, 1734, 1637, 1453, 1385, 1070, 912, 814 cm$^{-1}$; $^1$H NMR (pyridine-$d_5$, 500 MHz) 0.84, 0.86, 0.95, 1.09, 1.12, 1.15 (18H, all s, 6 × Me), 1.52 (3H, d, $J$ = 6.0 Hz, Rha Me), 1.68 (3H, d, $J$ = 6.2 Hz, Rha Me), 3.14 (1H, dd, $J$ = 13.4, 4.0 Hz, H-18), 3.91 (1H, d, $J$ = 10.5 Hz, H-23), 4.31 (1H, d, $J$ =10.5 Hz, H-23'), 4.24 (1H, dd, $J$ = 11.4, 3.8 Hz, H-3), 4.95 (1H, d, $J$ = 7.8 Hz, Glc H-1 at C-28), 4.97 (1H, d, $J$ = 6.7 Hz, Ara H-1), 5.37 (1H, br s, H-12), 5.47 (1H, d, $J$ = 7.8 Hz, Glc H-1), 5.83 (1H, br s, Rha H-1 at C-28), 6.22 (1H, d, $J$ = 8.0 Hz, Glc H-1 at C-28), 6.26 (1H, br s, Rha H-1); $^{13}$C NMR (pyridine-$d_5$, 125.8 MHz) 81.2 (C-3), 122.9 (C-12), 144.1 (C-13), 64.0 (C-23), 176.5 (C-28), 104.9 (Ara C-1), 75.9 (Ara C-2), 74.0 (Ara C-3), 69.7 (Ara C-4), 66.5 (Ara C-5), 101.4 (Rha C-1), 71.7 (Rha C-2)[a], 83.0 (Rha C-3), 73.0 (Rha C-4), 69.8 (Rha C-5), 18.5 (Rha C-6), 106.8 (Glc C-1), 75.3 (Glc C-2), 78.5 (Glc C-3)[b], 71.6 (Glc C-4)[a], 78.6 (Glc C-5)[b], 62.5 (Glc C-6), 95.6 (Glc C-1), 74.0 (Glc C-2), 78.3 (Glc C-3), 70.9 (Glc C-4), 78.0 (Glc C-5), 69.2 (Glc C-6), 104.8 (Glc C-1), 75.1 (Glc C-2), 76.5 (Glc C-3), 78.7 (Glc C-4), 77.1 (Glc C-5), 61.3 (Glc C-6), 102.7 (Rha C-1), 72.5 (Rha C-2), 72.7 (Rha C-3), 73.9 (Rha C-4), 70.3 (Rha C-5), 18.5 (Rha C-6) ([a,b]assignments may be interchangeable); negative ion FAB MS, see text.

**Acid Hydrolysis of 3.** Saponin **3** (10 mg) was refluxed with 5% HCl in 60% aqueous dioxane (10 mL) for 2 h. The reaction solution was evaporated under reduced pressure, and the hydrolysate was extracted with ether. The ether extract was evaporated to yield aglycon hederagenin, which was identified by direct comparison with an authentic sample. The $H_2O$ layer was neutralized with $Ag_2CO_3$ and filtered, and the filtrate was concentrated under reduced pressure. The residue was compared with standard sugars by cellulose TLC [pyridine−EtOAc−HOAc−$H_2O$ (36:36:7:21)], which showed the sugars to be arabinose, rhamnose, and glucose.

**Determination of the Absolute Configuration of Sugars of 3.** A sample of **3** (10 mg) was treated as above, the dried sugar mixture was dissolved in pyridine (0.1 mL), and then the solution was added to a pyridine solution (0.1 mL) of L-cysteine methyl ester hydrochloride (2 mg) and warmed at 60 °C for 1 h. The solvent was evaporated under a $N_2$ stream and dried in vacuo. The residue was trimethylsilylated with TMS−HT (0.1 mL) at 60 °C for 30 min. After the addition of *n*-hexane

and water, the *n*-hexane layer was removed and checked by GC. The retention times ($t_R$) of the peaks were 11.0 (L-arabinose), 14.1 (L-rhamnose), and 22.5 min (D-glucose).

**Alkaline Hydrolysis of Saponin 3.** Saponin **3** (25 mg) was refluxed with 3% KOH (10 mL) for 30 min, and on usual workup the prosapogenin **3a** (15 mg) was obtained. Prosapogenin **3a** was obtained as amorphous powder (MeOH): mp 241−244 °C; $[\alpha]^{20}_D$ +8.0° (*c* 0.4, pyridine); $^1$H NMR (pyridine-$d_5$, 500 MHz) 0.85, 0.86, 0.93, 0.94, 1.03, 1.17 (18H, all s, 6 × Me), 1.47 (3H, d, $J$ = 5.9 Hz, Rha Me), 4.95 (1H, d, $J$ = 6.7 Hz, Ara H-1), 5.39 (1H, br s, H-12), 5.40 (1H, d, $J$ = 7.6 Hz, Glc H-1), 6.16 (1H, br s, Rha H-1); $^{13}$C NMR (pyridine-$d_5$, 125.8 MHz) 81.1 (C-3), 123.0 (C-12), 144.3 (C-13), 63.9 (C-23), 180.2 (C-28), 104.8 (Ara C-1), 75.7 (Ara C-2), 74.8 (Ara C-3), 69.6 (Ara C-4), 66.2 (Ara C-5), 101.4 (Rha C-1), 71.5 (Rha C-2)[a], 82.9 (Rha C-3), 72.9 (Rha C-4), 69.6 (Rha C-5), 18.3 (Rha C-6), 106.7 (Glc C-1), 75.4 (Glc C-2), 78.3 (Glc C-3)[b], 71.6 (Glc C-4)[a], 78.5 (Glc C-5)[b], 62.4 (Glc C-6) ([a,b]assignments may be interchangeable); negative ion FAB MS, see text.

**Acid Hydrolysis of Prosapogenin 3a.** Prosapogenin **3a** was hydrolyzed on a TLC plate in concentrated HCl vapor at room temperature for 2 h, and on usual workup arabinose, glucose, and rhamnose were identified.

**Determination of the Absolute Configuration of Sugars of 3a.** A sample of **3a** (5 mg) was treated in the same manner as **3**, and the absolute configurations of sugars were found to be also in the same form as those of **3**.

## References and Notes

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