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Studies on Components of *Patrinia scabiosaefolia*

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Abstract – Rutin, α-hederin and kalopanax saponin B and a mixture of hederagenin and 23-hydroxyursolic acid were isolated from the aerial parts of *Patrinia scabiosaefolia* Fisch.

Key words – *Patrinia scabiosaefolia*; Valerianaceae; rutin; α-hederin; kalopanax saponin B; hederagenin; 23-hydroxyursolic acid.

Patrinia scabiosaefolia Fisch. (Valerianaceae) is well known folk medicine for treatment of edema, appendicitis, endometritis and inflammation.[1] The previous phytochemical investigation has revealed that the plants are rich in saponins. More than 20 saponins have been isolated[2] together with coumarins,[3] iridoid,[4] flavonoids,[5] and organic acids.[6] Chinese medicines belonging to *Patrinia* species are given the generic name Herba cum Radice Patriniae(敗醬草).[7] Two species such as *P. scabiosaefolia* and *P. villosa* are used as this herb in Korea and have been used to clear heat, relieve toxicity, expel pus, dispel blood stasis and stop pain[7,8]. This paper describes the isolation and structure elucidation of four components from the aerial parts of *P. scabiosaefolia*.

MATERIALS AND METHODS

General experimental procedures – Mps were measured on a Mitamura-Riken apparatus, and are uncorrected. The IR spectra were obtained on a JASCO FT/IR-5300 spectrometer. The UV spectra were measured on a Gilford 2600 spectrophotometer. The NMR spectra were measured on either a Bruker AMX-500 (500 MHz) or a Gemini-2000 (300 MHz) instrument, and the chemical shifts were referenced to TMS. TLC was performed on silica gel $60F_{254}$ (Merck) and Cellulose plate (Art No. 5716, Merck).

Plant materials – The aerial parts of *P. scabiosaefolia* were collected at Wonju suburb, Kang Won Province in Sep. 1995. A voucher specimen(YHK95003) was deposited in the Dept. of Material Medica, Sanji Univ.

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 sucession between H_2O and $CHCl_3$, EtOAc, and then n-BuOH and gave 8.2 g, 2 g, and 11.1 g of the respective extracts. The EtOAc fraction was subjected to silica gel column chromatography eluting with increasing amounts of MeOH in EtOAc saturated with water to give compound 1 which was recrystallized from MeOH as pale yellowish powder (100 mg).

mp 189-190°

IR, υ_{max} (cm^{-1}) 3420(OH), 1650(α,β-unsaturated C=O), 1600, 1500, 1440(aromatic C=C), 1350, 1295, 1190, 1100-1000(glycosidic C-O), 870, 800.

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UV, υ_{max} MeOH 260 nm(log ε 4.35), 268 nm (sh, log ε 4.34), 300 nm(sh, log ε 4.00), 365(4.28):
λ_{max} MeONa 273 nm(log ε 4.45), 333 nm(log ε 4.03), 415 nm(log ε 4.44):
λ_{max} $AlCl_3$ 275 nm(log ε 4.46), 302 nm (sh, log ε 3.96), 437 nm(log ε 4.47):
λ_{max} $AlCl_3$+HCl 270 nm(log ε 4.39), 302 nm(sh, log ε 3.98), 365 nm(sh, log ε 4.21), 402 nm(log ε 4.27):
λ_{max} NaOAc 272 nm(log ε 4.41), 325 nm (log ε 4.04), 405 nm(log ε 4.32):
λ_{max} NaOAc+H_3BO_3 264 nm(log ε 4.47), 398 nm(sh, log ε 3.94), 387 nm(log ε 4.37)

^{1}H-NMR(300MHz, DMSO-d_6)δ: 0.97(3H, d, J=6Hz, Rha CH_3), 4.39(1H, brs, Rha H-1), 5.35(1H, d, J=8Hz, Glc H-1), 6.18(1H, d, J=1.8Hz, H-6), 6.36(1H, d, J=1.8Hz, H-8), 6.83 (1H, d, J=8Hz, H-5′), 7.51(1H, brs, H-2′), 7.52 (1H, brd, J=8Hz, H-6′), 12.59(1H, s, 5-OH).

^{13}C-NMR(75.5MHz, DMSO-d_6)δ: 17.9(C-6‴), 67.2(C-6″), 68.5(C-5‴), 70.2(C-4″), 71.6(C-2‴), 70.8(C-3‴), 72.1(C-4‴), 74.3(C-2″), 76.2(C-5″), 76.7(C-3″), 93.9(C-8), 99.0(C-6), 101.0(C-1‴), 101.5(C-1″), 104.3(C-10), 115.5(C-2′), 116.6(C-5′), 121.5(C-1′), 121.9(C-6′), 133.6(C-3), 145.1 (C-3′), 148.8(C-4′), 156.8(C-2), 157.0(C-9), 161.6 (C-5), 164.5(C-7), 177.8(C-4).

The BuOH fraction was passed through a porous polymer MCI gel (CHP 20P, 75-150 μ) column. After washing the column 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}-$_{507}$) and three subfractions (B_{101}-B_{103}), respectively. A portion of B_{101} (6.9 g) was chromatographed on silica gel with EtOAc saturated with water to give compound 2 which was recrystallized from MeOH as white needles (20 mg). mp 264-265°

IR, υ_{max} (cm^{-1}) 3410(OH), 1698(COOH), 1640, 1073, 1050, 1027(glycosidic C-O), 870, 806.

^{1}H-NMR(300MHz, pyridine-d_5)δ: 0.93(3H, s, CH_3), 0.94(3H, s, CH_3), 1.00(3H, s, CH_3), 1.03 (3H, s, CH_3), 1.06(3H, s, CH_3), 1.23(3H, s, CH_3), 1.64(3H, d, J=6.0Hz, Rha H-1), 3.29(1H, brdd, J=3.9, 13.2Hz, H-18), 3.74(1H, d, J=11.4Hz, H-23), 4.15(1H, d, J=11.4Hz, H-23), 4.29(1H, H-3), 5.12(1H, d, J=6.3Hz, Ara H-1), 5.45 (1H, brs, H-12), 6.25(1H, brs, Rha H-1).

^{13}C-NMR(75.5 MHz, DMSO-d_6)δ: Table I.

A portion of B_{103} (1.19 g) was subjected to silica gel column chromatography eluting with increasing amounts of MeOH in EtOAc saturated with water to give compound 3 which was crystallized from MeOH as white amorphous powder (430 mg).

mp 225-228°

IR, υ_{max} (cm^{-1}) 3426(OH), 1730(C=O), 1638, 1458, 1387, 1350, 1295, 1190, 1059(glycosidic C-O), 814 (trisubstituted C=C).

^{1}H-NMR(500 MHz, pyridine-d_5)δ: 0.84(3H, s, 29-CH_3), 0.85(3H, s, 30-CH_3), 0.95(3H, s, 25-CH_3), 1.05(3H, s, 24-CH_3), 1.10(3H, s, 26-CH_3), 1.14(3H, s, 27-CH_3), 1.61(3H, d, J=6.1 Hz, Rha H-1), 1.68(3H, d, J=6.2 Hz, Rha H-1), 3.72(1H, d, J=10.5Hz, H-23), 4.08(1H, d, J=10.5Hz, H-23), 4.24(1H, t, J=9.6Hz, H-3), 4.97 (1H, d, J=7.8Hz, Glc H-1), 5.09(1H, d, J=6.1Hz, Ara H-1), 5.37(1H, brs, H-12), 5.83(1H, brs, Rha H-1), 6.21(1H, brs, Rha H-1), 6.21 (1H, d, J=8Hz, Glc H-1).

^{13}C-NMR(125MHz, DMSO-d_6)δ: Table I.

The fraction B_{204} (220 mg) was applied over silica gel column eluting with hexane-EtOAc (8:5, 4:3, 1:1), EtOAc and then EtOAc saturated with water to yield subfractions. The subfraction eluted with EtOAc was concentrated and recrystallized from MeOH to afford compound 4 as amorphous white powder (15 mg). This compound was identified as a mixture of two compounds (4a and 4b) by NMR data.

Table I. ^{13}C-NMR data for compounds 2-4

Carbon No.	2	3	4a	4b	Carbon No.	2	3
C-1	38.8	39.0	38.9		Ara C-1	104.4	104.3
C-2	26.0	26.2	27.8	26.2	C-2	75.7	75.8
C-3	81.0	81.0	73.5		C-3	74.7	74.7
C-4	43.4	43.5	43.0		C-4	69.3	69.5
C-5	47.6	47.7	48.7	48.2	C-5	65.7	65.7
C-6	18.0	18.1	18.6		Rha C-1	101.7	101.6
C-7	32.7	32.5	33.1	33.3	C-2	72.3	72.3
C-8	39.6	39.9	39.9	40.1	C-3	72.5	72.5
C-9	48.0	48.2	48.2		C-4	74.1	74.2
C-10	36.7	36.9	37.3	37.2	C-5	69.6	69.7
C-11	23.3	23.3	23.8	24.0	C-6	18.4	18.4
C-12	122.6	122.9	122.8	125.9	Glc C-1		95.6
C-13	144.8	144.1	145.1	139.5	C-2		74.1
C-14	42.0	42.1	42.3	42.6	C-3		78.2
C-15	28.2	28.3	28.4	28.8	C-4		70.8
C-16	23.8	23.8	23.8	25.0	C-5		78.0
C-17	46.5	47.0	46.8	48.2	C-6		69.1
C-18	42.1	41.6	42.1	53.7	Glc C-1		104.8
C-19	46.3	46.1	46.5	39.6	C-2		75.2
C-20	30.8	30.7	31.0	39.5	C-3		76.5
C-21	34.1	34.0	34.3	31.1	C-4		78.7
C-22	33.1	32.8	33.3	37.5	C-5		77.1
C-23	64.0	63.9	68.0		C-6		61.3
C-24	13.8	13.9	13.2		Rha C-1		102.7
C-25	15.9	16.2	16.0		C-2		72.5
C-26	17.3	17.5	17.5		C-3		72.7
C-27	26.0	26.0	26.2	23.8	C-4		73.9
C-28	180.3	176.5	180.6	179.8	C-5		70.3
C-29	33.1	33.1	33.3	17.5	C-6		18.5
C-30	23.6	23.7	23.8	21.5			

IR, υ_{max} (cm^{-1}) 3434(OH), 2926(CH), 1701 (COOH), 1638(C=C), 1460, 1044, 870, 800, 604.

^{1}H-NMR(300 MHz, pyridine-d_5)δ: 0.94(3H, s, CH_3), 0.99(3H, s, CH_3), 1.02(3H, s, CH_3), 1.06(3H, s, CH_3), 1.07(3H, s, CH_3), 1.25(3H, s, CH_3), 3.32(1H, dd, J=3.4, 10.2Hz, H-18), 3.75(1H, d, J=10.2Hz, H-23), 4.21(1H, d, J=10.2Hz, H-23), 5.52(1H, brs, H-12) for **4a**: δ:0.95 (3H, d, J=5Hz, CH_3), 0.99(3H, s, CH_3), 1.00 (3H, d, J=6Hz, CH_3), 1.07(3H, s, CH_3), 1.09 (3H, s, CH_3), 1.19(3H, s, CH_3), 2.65(1H, d, J =11.1Hz, H-18), 3.75(1H, d, J=10.2Hz, H-23), 4.21(1H, d, J=10.2Hz, H-23), 5.52(1H, brs, H -12) for **4b**.

^{13}C-NMR(75.5 MHz, pyridine-d_5)δ: Table I.

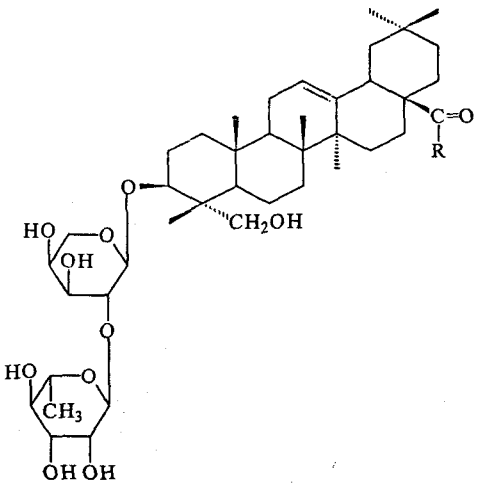
Acid hydrolysis - Compound 1, 2 and 3 (5 mg each) was refluxed with 5% H_2SO_4 for 1 h. The reaction solution was diluted with H_2O and extracted with ether. The H_2O layer was neutralized with $BaCO_3$, filtered and concentrated to give the sugar moiety. Glucose and rhamnose from compound 1, rhamnose and arabinose from compound 2 and glucose, rhamnose and arabinose from compound 3 were identified by cellulose TLC with authentic samples (pyridine-EtOAc-HOAc-H_2O=36:36:7:21). The ether layer was evaporated to yield the aglycones, quercetin from compound 1 and hederagenin

from 2 and 3, which were identified by direct comparison with authentic samples.

Alkaline hydrolysis of compound 3 – Compound 3 (20 mg) was refluxed with 5% alcoholic NaOH solution for 30 min, neutralized with dil-HCl and filtered. The residue was recrystallized from MeOH to afford prosapogenin. The physical properties and NMR spectra of the prosapogenin were in good agreement with compound 2 by direct comparison.

RESULTS AND DISCUSSION

Compound 1 showed positive reaction at Shinoda test. The IR spectra of compound 1 showed the presence of OH groups (3420 cm^{-1}), α,β-unsaturated C=O (1650 cm^{-1}), aromatic C=C groups (1600, 1500 and 1440 cm^{-1}) and glycosidic C-O bands (1100-1000 cm^{-1}). From these data, compound 1 was supposed to be a flavonoid glycoside. Its UV spectra showed a peak due to band I at 365 nm, indicating that compound 1 was supposed to be a flavonol glycoside.[9] On addition of CH_3ONa, the band I was bathochromic shifted to 415nm with increasing intensity indicating that OH group should be located at C-4′ position. On addition of NaOAc band II exhibited a bathochromic shift (12 nm). The bathochromic shift (22 nm) of band I upon addition of $NaOAc+H_3BO_3$ suggested to be *ortho* dihydroxyl groups in its structure. On addition of $AlCl_3$, band I was also bathochromic shifted. Consequently this compound was supposed to be a flavonol which had free hydroxyl groups on C-5,7,3′,4′ positions.[9] This suggestion was verified by acid hydrolysis of this compound. Acid hydrolysis of compound(1) gave quercetin and glucose and rhamnose, indicating that the sugar moieties were linked to C-3 position of quercetin. The ^1H-NMR spectra of compound(1) showed the presence of two anomeric protons at δ5.35 ppm (d, J=8Hz) for glucose and δ4.39(brs) ppm for rhamnose in addition to five aromatic protons for quercetin moiety. The chemical shift values and coupling constants suggested that the disaccharide moiety was rhamnosylglucose and was linked at C-3 of quercetin. The signal of the C-6 of glucose appeared at lower field (δ67.2) by 5.3 ppm due to the glycosylation shift in the ^{13}C-NMR spectrum demonstrating that rhamnosylglucose unit was rutinose.[9] From the above results the structure of compound 1 established as 3-O-α-L-rhamnopyranosyl(1 → 6)-β-D-glucopyranosyl quercetin, rutin. This compound has not been isolated from this plant.



2 R=-OH 3 R=Rha(1 → 4)glc(1 → 6)glc-O-

Compound 2 and 3 were assumed to be saponins on the basis of their positive colouration in Liebermann-Burchard and Molisch reagents. Acid hydrolysis of both compounds yielded hederagenin as the common aglycone and rhamnose and arabinose from 2 and rhamnose, arabinose and glucose from 3 as sugar components, respec-

tively. Alkaline hydrolysis of 3 gave compound 2 suggesting to be bisdesmoside. It showed signals for the typical terminal rhamnosyl unit as well as a downfield shifted carbon signal for arabinose C-2 in the ^{13}C-NMR spectra suggesting that compound 2 was α-hederin. Therefore the structure of 2 was determined as hederagenin 3-O-α-L-rhamnopyranosyl(1e → 2)-α-L-arabinopyranoside which was isolated from the roots of this plant[3] and several other plants.[2] Compound 3 exhibited five anomeric proton signals at δ4.97 (1H, d, J=7.8 Hz), 5.09 (1H, d, J=6.1 Hz), 5.83 (1H, brs), 6.21 (1H, brs) and 6.21 (1H, d, J=8.0 Hz) in its ^1H-NMR spectrum. Taking into account of two anomeric protons in compound 2 at δ5.12 (1H, d, J=6.3 Hz) and 6.25 (1H, brs), the acylated sugar moieties in compound 3 have three anomeric protons at δ4.97 (1H, d, J=7.8 Hz), 5.83 (1H, brs) and 6.21 (1H, d, J=8Hz) suggesting that 3 contains one mole each of α-L-arabinopyranose, α-L-rhamnopyranose and β-D-glucopyranose in the molecule. 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. Accordingly, compound 3 was determined to be kalopanax saponin B which was confirmed by direct comparison with an authentic sample (pulsatila saponin F).[10] This compound has also found in other plants such as *Acanthopanax, Akebia, Anemone, Aralia, Astrantia, Caltha, Hedera, Pulsatilla* and *Kalopanax* plants[2] but this is the first report from the genus *Patrinia*.

Compound 4 gave positive colouration in Liebermann-Burchard reaction. It showed an IR absorption band for an acid at 1701 cm^{-1}, suggesting compound 4 was an acidic triterpene. It showed signals for 2 pairs of trisubstituted double bonds (δ_C 122.8, 145.1; δ_H 3.32, dd, J=3.4, 10.2 Hz for 4a; δ_C 125.9, 139.5; δ_H 2.65, d, J=11.1 Hz for 4b) in its NMR spectra.[11] Therefore, compound 4 was established as a mixture of olean and ursan. Moreover, the presence of 3β,23-dihydroxy groups (δ_C 73.5; 68.0) indicated that this compound was a mixture of hederagenin(4a) and 23-hydroxyursolic acid(4b).[11] The 23-sulfates of these compounds have already been isolated from *Patrinia villosa*.[12] But this is the first time that 23-hydroxyursolic acid has been obtained from *Patrinia* plants. The structures of the other saponins isolated from this plant are now under investigation.

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