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Phytochemical Studies of Seeds of Medicinal Plants IV[1)]
Flavonoids and Triterpenoids from *Patrinia villosa* (THUNB.) JUSS.[2)]

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A flavonol glycoside, flavovilloside (**1**), was isolated from seeds (including a small amount of pericarps) of *Patrinia villosa* (THUNB.) JUSS. (Valerianaceae), and its structure was determined as 3-*O*-α-L-rhamnopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→6)-β-D-galactopyranosyl quercetin. Five known compounds, kaempferol-3-*O*-β-rhamninoside (**2**), 23-sulfates (as sodium salts) of 3β-hydroxyurs-12-en-28-oic acid (**3**) and 3β-hydroxyolean-12-en-28-oic acid (**4**), sulfapatrinosides I (**5**) and II (**6**), were also isolated. Of them, compounds **3** and **4** were isolated as naturally occurring products for the first time.

Keywords——*Patrinia villosa*; seed; flavonol glycoside; sulfated triterpenoid; sulfated triterpenoid glycoside

The Chinese crude drug, "Bai Jiang" (Haisho in Japanese), whole plants of *Patrinia scabiosaefolia* FISCHER (Ominaeshi in Japanese) and *P. villosa* (THUNB.) JUSS. (Otokoeshi in Japanese) (Valerianaceae), has been used in China as a diuretic, for treatment of fever and inflammation along with detoxication ("Qing Re Jie Du"), and for mobilization of blood circulation and treatment of statis ("Huo Xue Hua Yu").[3)]

In our recent studies,[1,4)] two glycosides of triterpenoid sulfates, sulfapatrinosides I (**5**) and II (**6**),[4)] and three isomeric pairs of ursolic acid and oleanolic acid glycosides[1)] were characterized as predominant constituents of seeds of *P. scabiosaefolia*. In our continuing phytochemical research of the genus Patrinia, we isolated a flavonol glycoside, flavovilloside (**1**) and five known compounds, *i.e.*, kaempferol-3-*O*-β-rhamninoside (**2**),[5)] 23-sulfates (in the forms of sodium salts) of 3β-hydroxyurs-12-en-28-oic acid (**3**)[4)] and 3β-hydroxyolean-12-en-28-oic acid (**4**),[4)] sulfapatrinosides I (**5**) and II (**6**),[4)] from seeds (including a small amount of pericarps) of *P. villosa*.

A *n*-BuOH soluble portion from a MeOH extract of the seeds was subjected to chromatographic and high-performance liquid chromatographic (HPLC) separations to give six compounds (**1**–**6**).

Flavovilloside (**1**), fine yellow plates of mp 210–213°C (decomp.), $[\alpha]_D$ −43.6° (MeOH), gave UV absorption maxima at 260 and 365 nm. The negative ion FAB-MS spectrum of **1** gave a quasimolecular ion $(M-H)^-$ at *m/z* 755 and three significant fragment ions at *m/z* 609 [(M-H)-146 (deoxyhexose unit)]$^-$, *m/z* 463 [609-146 (deoxyhexose unit)]$^-$ and *m/z* 301 [463-162 (hexose unit)]$^-$.

On methanolysis, **1** gave methyl galactoside and methyl rhamnoside in the sugar portion. The aglycone moiety of **1** was determined as quercetin (**7**), by the comparisons of the ^{1}H-NMR (TABLE I) and ^{13}C-NMR (TABLE II) data of **1** with the published data.[7)]

When the ^{13}C-NMR spectrum of **1** was compared with that of **7**, glycosylation shifts were observed at 2-C, 3-C, and 4-C of **1**. These results and UV absorption peak shifts produced on addition of some shift reagents[8)] indicated **1** to be a quercetin 3-*O*-dirhamnosyl-galactoside. Determination of the sugar portions: In the ^{1}H-NMR spectrum measured in MeOH-d_4, all protons of **1** were properly assigned with the aid of ^{1}H-^{1}H shift correlation spectroscopy (COSY), nuclear Overhauser effect spectroscopy (NOESY) and ^{1}H-^{13}C COSY, as shown in TABLE I. The doublet at δ 5.05 with a diaxial coupling (J=7.8 Hz) between 1″-H and 2″-H was assigned to the anomeric proton of β-galactopyranose (1″-H) linked to the 3-OH of **7**. The other two signals at δ 4.52 and 4.94 with a diequatorial coupling constant (each

TABLE I. ^{1}H-NMR Spectral Data for **1** and **2** (600 MHz, in MeOH-d_4, δ)[a)]

	Aglycone			Inner galactosyl	
	1	**2**		**1**	2
6-H	6.20 (br. s)	6.21 (d, 2.0)	1″-H	5.05 (d, 7.8)	5.02 (d, 8.1)
8-H	6.38 (br. s)	6.39 (d, 2.0)	2″-H	3.85 (dd, 9.5, 7.8)	3.79 (dd, 8.1, 7.8)
2′-H	7.89 (d, 2.0)	8.09 (d, 8.9)	3″-H	3.61 (dd, 9.5, 3.5)	3.68 (dd, 7.8, 2.4)
3′-H	——	6.88 (d, 8.9)	4″-H	3.83 (d, 3.5)	3.77 (d, 2.4)
5′-H	6.87 (d, 8.5)	6.88 (d, 8.9)	5″-H	3.69 (br. t, 6.0)	3.66 (br. t, 6.0)
6′-H	7.61 (dd, 8.5, 2.0)	8.09 (d, 8.9)	6″-H$_2$	3.43 (dd, 10.0, 6.0)	3.41 (dd, 9.7, 6.0)
				3.76 (dd, 10.0, 6.0)	3.73 (dd, 9.7, 6.0)
	Middle rhamnosyl			Terminal rhamnosyl	
	1	**2**		**1**	2
1‴-H	4.52 (d, 1.5)	4.51 (d, 1.6)	1⁗-H	4.94 (d, 1.5)	4.93 (d, 1.6)
2‴-H	3.65–3.69[b)]	3.69 (dd, 3.2, 1.6)	2⁗-H	3.94 (dd, 3.5, 1.5)	3.93 (dd, 3.2, 1.6)
3‴-H	3.60 (dd, 9.5, 2.5)	3.55–3.58[c)]	3⁗-H	3.73 (dd, 9.5, 3.5)	3.73 (dd, 9.0, 3.2)
4‴-H	3.43 (t, 9.5)	3.42 (t, 9.0)	4⁗-H	3.35 (t, 9.5)	3.35 (t, 9.0)
5‴-H	3.57 (dq, 9.5, 6.0)	3.55–3.58[c)]	5⁗-H	3.65–3.69[b)]	3.60 (dq, 9.0, 6.0)
6‴-H$_3$	1.20 (d, 6.0)	1.18 (d, 6.0)	6⁗-H$_3$	1.14 (d, 6.0)	1.14 (d, 6.0)

[a)] Chemical shifts are given in δ-values with tetramethylsilane (TMS) as internal standard. Signs and figures given in parentheses refer to multiplicities and coupling constants (Hz).
[b,c)] Both multiplicities and coupling constants are not clear as they partly overlap.

TABLE II. ^{13}C-NMR Spectral Data for **1** and **2** (150 MHz, in MeOH-d_4, δ_C)

Aglycone moiety			Sugar moiety		
	1	**2**		**1**	**2**
			Inner galactosyl		
2-C	159.0[a)]	159.5[b)]	1″-C	106.2	105.8
3-C	136.0	135.9	2″-C	73.2	73.1
4-C	179.5	179.6	3″-C	75.2	75.1
5-C	162.9	163.0	4″-C	70.3	70.2
6-C	100.2	100.1	5″-C	75.3	75.4
7-C	166.3	166.2	6″-C	67.5	67.6
8-C	95.0	95.1	Middle rhamnosyl		
9-C	158.4[a)]	158.6[b)]	1‴-C	101.9	101.9
10-C	105.6	105.7	2‴-C	71.9	71.9
1′-C	122.8	122.7	3‴-C	79.6	79.6
2′-C	118.1	132.5	4‴-C	73.2	73.2
3′-C	145.8	116.2	5‴-C	70.0	70.1
4′-C	150.1	161.7	6‴-C	18.0	18.0
5′-C	116.2	116.2	Terminal rhamnosyl		
6′-C	123.1	132.5	1⁗-C	104.0	104.0
			2⁗-C	72.2	72.2
			3⁗-C	72.3	72.3
			4⁗-C	74.1	74.1
			5⁗-C	70.0	70.1
			6⁗-C	18.0	18.0

[a,b)] Assignments may be interchangeable in each column.

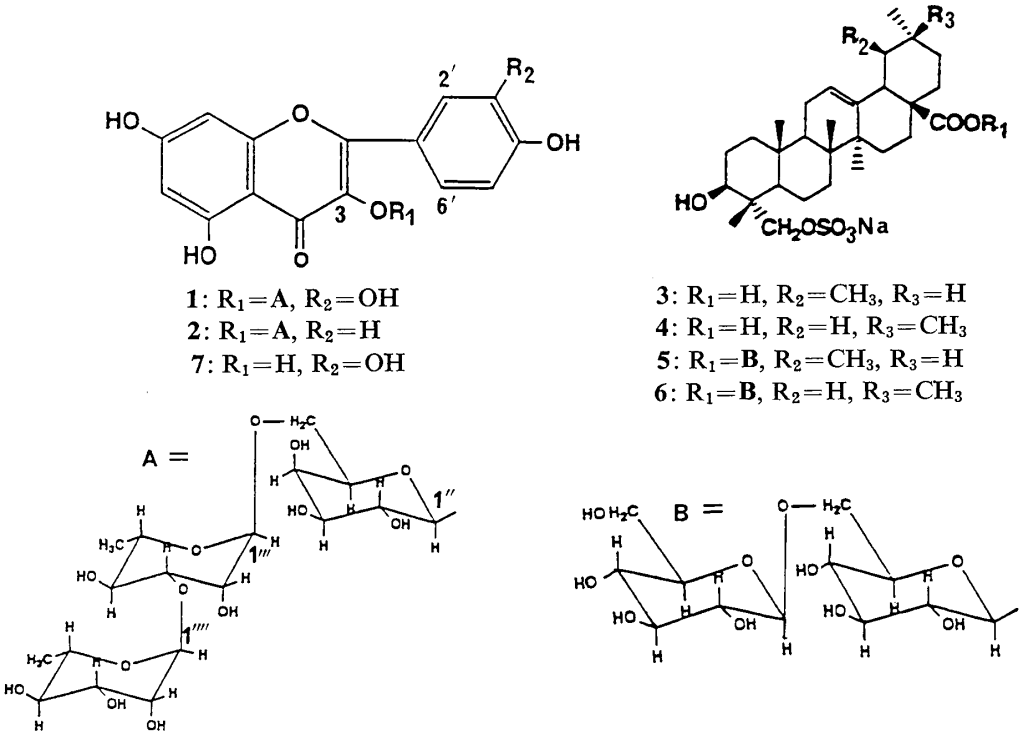

Chart 1.

1.5 Hz) were assigned to the anomeric protons of two α-rhamnopyranoses (1‴-H and 1⁗-H) linked to inner galactose and to middle rhamnose, respectively. The α-configurations of two rhamnopyranosyl moieties in **1** were also substantiated by the large ^{13}C-H coupling constants (J_{C1-H1}=each 169 Hz) of the anomeric carbons (1‴-C and 1⁗-C).[9]

Interglycosidic linkages in the dirhamnosyl-galactosyl part of **1**: In the ^{13}C-NMR spectrum (TABLE II), the galactosyl 6″-C and rhamnosyl 3‴-C, resonating at δ 67.5 and 79.6 ppm, were observed in more downfield (by *ca.* 7 and *ca.* 9 ppm, respectively) than the corresponding signals of usual galactopyranosyl and rhamnopyranosyl residues.[7] In the NOESY experiments, two significant NOE cross peaks were observed between the anomeric H (1⁗-H) of terminal rhamnose and 3‴-H of middle rhamnose, and between the anomeric H (1‴-H) of middle rhamnose and 6″-H_2 of inner galactose. Three significant cross peaks (between 1⁗-H and 3‴-C, 1‴-H and 6″-C, and 1″-H and 3-C) were shown in the heteronuclear multiple bond connectivity (HMBC) spectrum. On the basis of these evidences, **1** was assigned as 3-*O*-α-L-rhamnopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→6)-β-D-galactopyranosyl quercetin.[10] Compound **1** per-*O*-acetate was characterized as a compound from rhamnaceous plants,[6a] *Rhamnus catharticus* and *R. saxatilis* subsp. *saxatilis*. But no data on **1** itself have been given in literature. So, this is the first report of isolation of the original glycoside (**1**) and its ^{1}H- and ^{13}C-NMR data (TABLES I and II, respectively).

Compound **2**, fine yellow crystals of mp 198–200°C, $[\alpha]_D$ −37.6° (MeOH), has been characterized as 3-*O*-α-L-rhamnopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→6)-β-D-galactopyranosyl kaempferol (=kaempferol-3-*O*-β-rhamninoside).[5,6] Compounds **3** and **4** showed a carboxyl band at 1690 cm^{-1} and a typical sulfate S=O stretching vibration at 1240 cm^{-1} in the IR spectra. These compounds were determined to be 23-sulfates (in the forms of sodium salts) of 3β-hydroxyurs-12-en-28-oic acid and 3β-hydroxyolean-12-en-28-oic acid, respectively, by direct comparisons with the respective authentic samples.[4] In this work, both compounds were isolated for the first time as naturally occurring compounds. Isolated triterpenoid glycosyl sulfates, **5** and **6**, were identified as sulfapatrinosides I and II by direct comparison with the respective authentic specimens.[4]

Experimental

All melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. IR and UV spectra were run with a JASCO A-302 instrument and a Shimadzu UV-3000 spectrometer, respectively. ^{1}H- (600 MHz) and ^{13}C-NMR (150 MHz) spectra were measured with a GE-OMEGA 600 spectrometer in MeOH-d_4 (as a solvent) with tetramethylsilane as an internal standard. Negative ion FAB-MS spectra were obtained

with a JEOL JNM-DX300 spectrometer under the following conditions: accelerating voltage, 2–3 kV; emission current, 30 mA; matrix, triethanolamine; collision gas, Xe. Optical rotations were determined on a JASCO DIP-140 digital polarimeter. GLC was carried out on a Shimadzu GC-7AG gas chromatograph under the following conditions: column, 1.5% SE-52 on Chromosorb WAW DMCS (2 m×3 mm i.d.); detector, flame ionization detector (FID); column temperature, 180°C; carrier N_2 gas, 30 ml/min. Preparative HPLC was carried out on a Waters 600E instrument with a U6K septum-less injector, a Lambda-Max Model 480 photospectrometer or on a Waters instrument with an M 6000A pump and a series R-401 differential refractometer. In each case, a reversed-phase TSK-GEL ODS-120T column was used.

Plant material——Seeds of *P. villosa* were harvested at the Kitakuwata-gun, Kyoto Pref. in 1987. The plants used in the present study was identified by one of us (H.M.). A voucher specimen is deposited in the herbarium of the Faculty of Pharmaceutical Science, Setsunan University.

Extraction and isolation of compounds 1–6——Crushed seeds (including a small amount of pericarps) (233 g) were extracted three times with MeOH (1.5 l×3). The combined extracts (39.1 g) were dissolved in MeOH (100 ml) and poured into Et_2O (1.2 l). The resultant insoluble precipitate (23.3 g), collected by filtration, was suspended in H_2O and extracted with *n*-BuOH. The residue (13.3 g) obtained from the *n*-BuOH layer was subjected to silica gel column chromatography and the fractions containing **1–6** were further purified by Amberlite XAD-2 chromatography and/or reversed phase HPLC to give **1** (56 mg), **2** (22 mg), **3** (33 mg), **4** (66 mg), **5** (18 mg), and **6** (30 mg). The physical and spectral properties of **1–6** were as follows.

Flavovilloside (**1**), yellow fine plates of mp 210–213°C (decomp.) (MeOH), $[\alpha]_D$ −43.6° (c=0.40, MeOH). UV λ_{max} (MeOH) nm (log ε): 260 (4.27), 365 (4.19). +NaOMe: 275, 330, 410; +AcONa: 275, 325, 380; +$AlCl_3$: 275, 435. IR ν(KBr) cm^{-1}: 3400, 1645, 1595, 1060. Negative ion FAB-MS m/z (%): 755 {$[M(C_{33}H_{40}O_{20})-H]^-$, 100}, 609 [(M-H-146)$^-$, 21], 463 [(609-146)$^-$, 21], 301 [(463-162)$^-$, 77]. ^{1}H- and ^{13}C-NMR data are given in Tables I and II, respectively. Kaempferol-3-*O*-β-rhamninoside (**2**),[5,6] yellow fine crystals of mp 198–200°C (decomp.) (MeOH-Et_2O) (ref. 6a, mp 205–210°C), $[\alpha]_D$ −37.6° (c=0.25, MeOH) [ref. 6a, −38.6° (MeOH); 6b, −42.7° (MeOH)]. UV λ_{max} (MeOH) nm (log ε): 268 (4.25), 350 (4.18). +NaOMe: 276, 327, 402; +AcONa; 274, 306, 372; +$AlCl_3$: 276, 306, 352, 400. IR ν(KBr) cm^-: 3400, 1640, 1595, 1060. Negative ion FAB-MS m/z (%): 739 {$[M(C_{33}H_{40}O_{19})-H]^-$, 100}, 593 [(M-H-146)$^-$, 10], 447 [(593-146)$^-$, 7], 285 [(447-162)$^-$, 70]. ^{1}H- and ^{13}C-NMR data are given in Tables I and II, respectively. Compounds **3** (mp 228–230°C), **4** (mp 223–225°C), **5** (mp 239–242°C), and **6** (mp 242–244°C) were identified as 23-sulfate (as sodium salt) of 3β-hydroxy-urs-12-en-28-oic acid, 23-sulfate (as sodium salts) of 3β-hydroxy-olean-12-en-28-oic acid, sulfapatrinoside I, and sulfapatrinoside II, respectively, by direct comparisons with the respective authentic samples.[4]

Methanolysis of 1——A solution of **1** (*ca.* 3 mg) in 5% anhydrous HCl-MeOH (2 ml) was refluxed for 5 h. The reaction mixture was neutralized with Ag_2CO_3. The inorganic precipitate was filtered off and the filtrate was concentrated under reduced pressure to give a residue. The residue was trimethylsilylated with *N*,*O*-bis(trimethylsilyl)-trifluoroacetamide-py., and subjected to GLC analysis to demonstrate the presence of methyl galactoside and methyl rhamnoside.

References and Notes

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2) A part of this work was presented at the 42nd Meeting of Kinki Branch, Pharmaceutical Society of Japan, Kyoto, 1992, Abstract of Papers, p. 67.
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5) This compound has been isolated from *Rhamnus catharticus*,[6a] *R. alaternus*,[6a] and *R. leptophylla*[6b] (Rhamnaceae). This is the first report of isolation of **2** from another family. Further, this compound was characterized as its per-*O*-acetate in the literature. The ^{1}H- and ^{13}C-NMR data (Tables I and II, respectively) of the original glycoside are reported here for the first time.
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10) Whether the sugars are D or L was not determined. But, galactose and rhamnose in **1** were probably in D and L forms, respectively, as these sugars occur in these forms in natural products.