New Iridoid Esters from the Roots of *Patrinia scabiosaefolia*

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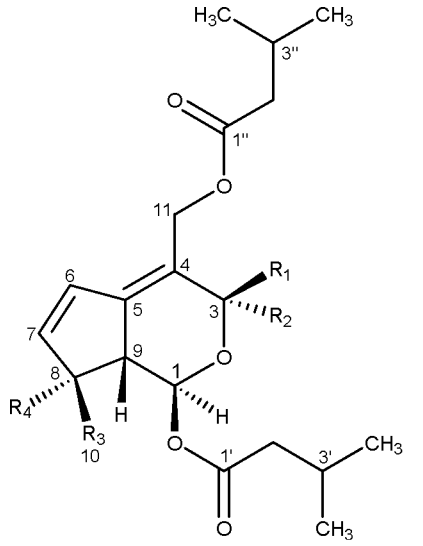
Patrinia scabiosaefolia Fischer (Valerianaceae) is a perennial plant distributed across mountainous regions and fields of Korea, Japan and China.[1] The roots of *P. scabiosaefolia* have been widely used in traditional medicine for the treatment of edema, appendicitis and inflammation.[2] Previous phytochemical work on the roots as well as aerial parts of *P. scabiosaefolia* has resulted in the isolation of saponins,[3-5] coumarins,[6] iridoids,[7] and triterpene lactone.[8] In addition, Bae *et al.* reported the isolation and characterization of two iridoid diesters, patridoid I and II, from the whole plant of *P. saniculaefolia*, a closely related species.[9] In a continuation of the phytochemical study on *P. scabiosaefolia*, we now report the isolation and structural elucidation of two new iridoids, 1-isovaleryloxy-3,8-dimethyloxy-4-(3-methyl-butyryloxymethyl)-8-hydroxymethyl-cyclopenta-4,6-diene[c]pyran, patriscadoid I (**1**), and 1-isovaleryloxy-3-methyloxy-4-(3-methyl-butyryloxymethyl)-8-hydroxymethyl-8-hydroxy-cyclopenta-4,6-diene[c]pyran, patriscadoid II (**2**).



	R_1	R_2	R_3	R_4
1	OCH_3	H	CH_2OH	OCH_3
2	OCH_3	H	CH_2OH	OH

Chart 1. Structures of Compounds **1** and **2** from *Patrinia scabiosaefolia*

The roots of *P. scabiosaefolia* were extracted with MeOH under reflux. The MeOH extract was suspended in water and then partitioned sequentially with equal volumes of dichloromethane, ethyl acetate, and *n*-butanol. A methylene chloride soluble fraction of the MeOH extract was purified by silica gel, MCI gel CHP 20P, and Lichroprep RP-18 column chromatography yielded two new iridoids, patriscadoid I, and II (Chart 1).

Compound **1** was obtained as a yellow oil and gave a molecular ion peak at *m/z* 449.2165 $[M+Na]^+$ in the HR-FAB-MS corresponding to the elemental formula $C_{22}H_{34}O_8$ and indicating five degrees of unsaturation. The IR spectrum indicated the presence of a hydroxyl group (3450 cm^{-1}) and an ester group (1730 cm^{-1}). The ^{1}H-NMR spectrum of **1** exhibited signals due to two olefinic protons at δ 6.88 (1H, d, J = 5.5 Hz) and 6.31 (1H, d, J = 5.5 Hz), two acetalic protons at δ 6.24 (1H, d, J = 7.0 Hz) and δ 5.22 (1H, s), one methine proton at δ 2.81 (1H, d, J = 6.5 Hz), and an isolated hydroxymethyl protons at δ 3.69 and 3.60 (each, d, J = 11.5 Hz). The presence of these functional groups was further supported by ^{13}C NMR signals for **1** observed at δ 132.6 (CH), 138.8 (CH), 90.4 (CH), 98.5 (CH), 45.4 (CH), and 67.2 (CH_2). Furthermore, the ^{1}H and ^{13}C-NMR spectra showed two doublet methyl signals at δ 0.94 (d, J = 6.5 Hz), 1.00 (d, J = 6.5 Hz), two methylene signals at δ 2.15, 2.07, two methine signals at δ 2.26, 2.19 (each, dd, J = 7.5, 4.5 Hz), and a carbonyl carbon signal at δ 172.0, 172.8, indicating two isovaleryloxy ester moieties which were confirmed by the 2D COSY of **1**. The ^{1}H-NMR spectrum also exhibited two methoxy protons at δ 3.44 and 3.09. These data suggested that the structure of **1** was an iridoid skeleton similar to that of patridoid II.[9] The connectivities of **1** were established by the HMBC spectrum. Correlations between the signals at δ 6.88 (H-6)/6.31 (H-7) and δ 45.4 (C-9), δ 2.81 (H-9) and δ 67.2 (C-10) confirmed that the isolated hydroxymethyl group was connected to C-8. In addition, correlations between signals δ 6.24 (H-1) and δ 172.0 (C-1'), δ 4.87 (H-11a)/4.65 (H-11b) and δ 172.8 (C-1") indicated that the isovaleryloxy group was connected to C-1 and C-4, respectively. The positions of the methoxy groups at C-3 and C-8 were confirmed by cross peaks between δ 3.44 (OCH_3) and

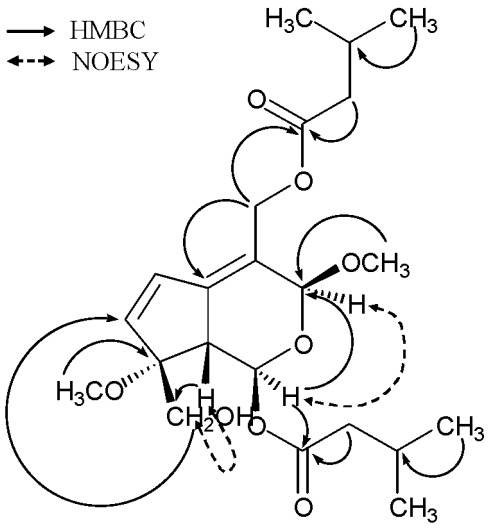


Figure 1. Key HMBC and NOESY correlations for **1**.

δ 98.5 (C-3) and between δ 3.09 (OCH_3) and δ 87.7 (C-8). Concerning the stereochemistry of **1**, the β-orientation of the isovaleryloxy group at C-1 was determined by comparison of the NMR spectral data with those reported in the literature and NOESY experiments.[10] The coupling constant of H-1 of **1** compared with that of patridoid II, isolated from *P. saniculaefolia*, was almost the same.[9,11-12] In addition, the two methoxy groups at δ 3.44 (OCH_3-3) and δ 3.09 (OCH_3-8) were assigned as β- and α-orientations, respectively, by NOESY experiments. In the NOESY spectrum, correlation peaks between H-1(δ 6.24) and H-3 (δ 5.22), and between H-9 (δ 2.81) and H-10 (δ 3.69) indicating that H-1/H-3 and H-9/H-10 possess the same plane. Based on the above spectral evidence, the structure of **1** was elucidated as 1-isovaleryloxy-3,8-dimethyloxy-4-(3-methyl-butyryloxymethyl)-8-hydroxymethyl-cyclopenta-4,6-diene-[c]pyran, and named as patriscadoid I.

Compound **2** was obtained as a yellow oil and gave a molecular ion peak at *m/z* 435.2009 $[M+Na]^+$ in the HR-FAB-MS corresponding to the elemental formula $C_{21}H_{32}O_8$ and indicating five degrees of unsaturation. The ^{1}H- and ^{13}C-NMR spectra of **2** (see Table 1) were quite similar with those of **1**, except for one additional hydroxyl group (δ 83.2) and one less methoxyl group (δ 52.5) than **1**. Comparison of the chemical shift of **2** at C-8 and C-7 with that of **1** revealed an upfield shift to δ 83.2 (−4.5 ppm) and a downfield shift to δ 141.5 (+2.7 ppm), respectively, in the ^{13}C-NMR spectrum.[11] Moreover, the methoxyl proton peak at δ 3.09 disappeared in the ^{1}H-NMR spectrum. The configurations of the isovaleryloxy group at C-1 and the hydroxyl group of C-8 of **2** were equivalent with those of **1**. In the NOESY spectrum, correlation peaks were observed between H-1(δ 6.30) and H-3(δ 5.23), and between H-9 (δ 2.86) and H-10 (δ 3.74).[13-14] Based on the above spectral evidence, the structure of **2** was elucidated as 1-isovaleryloxy-3-methyloxy-4-(3-methyl-butyryloxymethyl)-8-hydroxymethyl-8-hydroxy-cyclopenta-4,6-diene[c]pyran, and named as patriscadoid II.

Patriscadoid I, and II were examined for the inhibitory effect on the IL-6 production in TNF-α stimulated MG-63 cells.[15] and the LPS-induced nitric oxide production using murine macrophage RAW 264.7 cells.[16] Both compounds showed no inhibitory activity.

Table1. ^{1}H- and ^{13}C-NMR Spectral Data of Compounds 1 and **2**

No	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
1	6.24 d (7.0)	90.4	6.30 d (7.5)	91.7
3	5.22 s	98.5	5.23 s	98.8
4		124.3		125.5
5		143.1		143.0
6	6.88 d (5.5)	132.6	6.69 d (5.5)	130.3
7	6.31 d (5.5)	138.8	6.33 d (5.5)	141.5
8		87.7		83.2
9	2.81 d (6.5)	45.4	2.86 d (7.5)	46.1
10	3.69 d (11.5)	67.2	3.74 d (11.0)	68.0
	3.60 d (11.5)		3.60 d (11.0)	
11	4.87 d (12.5)	60.1	4.81 d (12.5)	60.1
	4.65 d (12.5)		4.67 d (12.5)	
1'		172.0		172.3
2'	2.26 dd (7.5, 4.5)	43.3	2.28 d (7.5)	43.5
3'	2.15 m	25.5	2.11 m	25.6
4'	1.00 d (6.5)	22.3	1.00 d (6.5)	22.4
5'	1.00 d (6.5)	22.3	1.00 d (6.5)	22.4
1"		172.8		172.9
2"	2.19 dd (7.5, 4.5)	43.5	2.20 d (7.5)	43.3
3"	2.07 m	25.7	2.08 m	25.7
4"	0.94 d (6.5)	22.4	0.95 d (6.5)	22.4
5"	0.94 d (6.5)	22.4	0.95 d (6.5)	22.4
OCH_3 (1)				
OCH_3 (3)	3.44 s	55.9	3.46 s	55.9
OCH_3 (8)	3.09 s	52.5		

NMR data were obtained in 500 MHz for ^{1}H and 125 MHz for ^{13}C in $CDCl_3$

Experimental

General Procedures. Optical rotations were measured using an Autopol-IV polarimeter. UV spectra were obtained on a Shimadzu UV/Visible Spectrophotometer. The IR spectra were measured in KBr pellets using an IMS 85 (Bruker). The NMR spectra were recorded on a Varian Unity Inova 500 spectrometer. HR-FAB-MS was recorded on a JEOL JMS 700 mass spectrometer. TLC and the preparative TLC were carried out on precoated Silica gel 60 F_{254} (Merck, art. 5715) and RP-18 F_{254s} (Merck, art. 15389) plates. Column chromatography was performed on Silica gel 60 (Merck, 40-63 and 63-200 μm), MCI gel CHP 20P (Mitsubishi Chemical Co., 70-150 μm), and Sephadex LH-20 (Sigma, 25-100 μm).

Plant Material. The roots of *Patrinia scabiosaefolia* were collected from the Herbarium of the College of Pharmacy, Chosun University, Korea in May 2006 and identified by Professor Emeritus Young Hee Moon of College of Pharmacy, Chosun University, Korea. Voucher specimens were

deposited in the Herbarium of the College of Pharmacy, Chosun University, Korea (CSU-975-17).

Extraction and Isolation. The air-dried roots of *Patrinia scabiosaefolia* (0.6 Kg) were cut and extracted with MeOH (3 L × 3) at 60 °C for 3 hrs (×3). The MeOH extract (89.86 g) was suspended in water (1.0 L) and then partitioned sequentially with equal volumes of dichloromethane, ethyl acetate and *n*-butanol. Each fraction was evaporated *in vaccuo* to yield the following residues: CH_2Cl_2 (26.27 g), EtOAc (2.06 g), *n*-BuOH (21.9 g), and water (64.10 g) extract. A portion of the CH_2Cl_2 soluble fraction (10.0 g) was subjected to column chromatography over silica gel (400 g) eluted with an *n*-hexane-acetone = 10:1 → 1:1 gradient system. The fractions were combined based on their TLC pattern to form subfractions designated as D1-D16. Subfraction D5 (64.9 mg) was further purified by column chromatography over silica gel eluted with a $CHCl_3$-MeOH = 100:1 → 1:1 gradient system to afford three subfractions (D51-D53). Subfraction D53 (10.9 mg) was purified by Lichropep RP-18 column chromatography (MeOH-H_2O = 2:1) to give compound **1** (2.4 mg). Subfraction D52 (9.6 mg) was purified by MCI gel CHP 20P column chromatography (MeOH- H_2O = 2:1) to give compound **2** (2.0 mg).

Patriscadoid I (**1**): Yellow oil. $[\alpha]_D^{25}$: -6.0° (c 1.0, MeOH); UV λ_{max} (MeOH) nm (log ε) 277 (4.20); IR (KBr) cm^{-1}: 3450, 1730, 1290, 1100; ^1H- and ^{13}C-NMR, see Table 1; HR-FAB-MS *m/z* 449.2165 $[M+Na]^+$ (calcd for $C_{22}H_{34}O_8Na$: 449.2151).

Patriscadoid II (**2**): Yellow oil. $[\alpha]_D^{25}$: +51.97° (c 0.08, MeOH); UV λ_{max} (MeOH) nm (log ε) 277 (4.10); IR (KBr) cm^{-1}: 3450, 1730, 1290, 1100; ^1H- and ^{13}C-NMR, see Table 1 ; HR-FAB-MS *m/z* 435.2009 $[M+Na]^+$ (calcd for $C_{21}H_{32}O_8Na$: 435.1995).

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