Iridoid Esters from *Patrinia saniculaefolia*

Ren Bo AN,[a] Byung Sun MIN,[b] Min Kyun NA,[a] Hyeun Wook CHANG,[c] Kun Ho SON,[d] Hyun Pyo KIM,[e] Hyeong Kyu LEE,[b] KiHwan BAE,*[,a] and Sam Sik KANG[f]

[a] *College of Pharmacy, Chungnam National University; Daejeon 305–764, Korea:* [b] *Laboratory of Immunomodulator, Korea Research Institute of Bioscience & Biotechnology; P.O. Box 115, Yusung, Daejeon 305–333, Korea:* [c] *College of Pharmacy, Yeungnam University; Gyongsan 712–749, Korea:* [d] *Department of Food and Nutrition, Andong National University; Andong 760–749, Korea:* [e] *College of Pharmacy, Kangwon National University; Chunchon 200–701, Korea; and* [f] *Natural Products Research Institute, Seoul National University; Seoul 110–460, Korea.*

Received October 21, 2002; accepted January 4, 2003

Two new iridoids, named patridoid I (1) and patridoid II (2), were isolated from the whole plant of *Patrinia saniculaefolia* (Valerianaceae), together with the known one, nardostachin (3). The structures of compounds 1 and 2 were established on the basis of spectroscopic methods, including two dimensional (2D NMR) and high resolution fast atom bombardment mass spectrometry (HR-FAB-MS).

Key words patridoids I—II; iridoid; *Patrinia saniculaefolia*; nardostachin

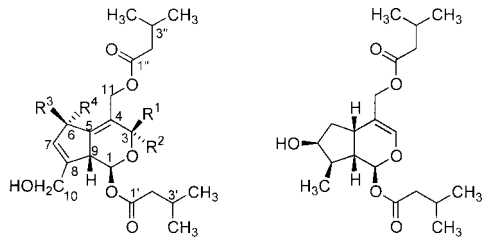
Patrinia saniculaefolia HEMSLEY (Valerianaceae) is a perennial herb and an endemic species in Korea. The genus Patrinia is taxonomically classified into four species, *P. saniculaefolia*, *P. scabiosaefolia*, *P. villos*a, and *P. rupestris*.[1] This genus is one of the valuable crude drugs which has been used in Korea and China as a traditional folk medicine for the treatment of initial stages of edema, appendicitis, endometriosis and inflammation.[2] Saponins, coumarins, iridoids, and flavonoids isolated from this genus have demonstrated sedative, antibacterial, and cytotoxic effects,[3—7] whereas there is no phytochemical report on *P. saniculaefolia*. In this paper, we describe the isolation and characterization of two iridoid diesters (**1**, **2**).

Results and Discussion

The dried whole plant of *P. saniculaefolia* was extracted with MeOH at room temperature. A hexane-soluble fraction of the MeOH extract was purified by silica gel and octadecyl silica (ODS) gel column chromatography to give three iridoids (**1**—**3**). A known was identified as nardostachin (**3**) isolated from *Nardostachys chinensis* (Valerianaceae), by comparing spectral data with those previously reported.[8]

Compound **1** was obtained as a yellow oil with a negative optical rotation ($[\alpha]_D$, −74°). The molecular formula of **1** was determined to be $C_{22}H_{34}O_8$ by high resolution fast atom bombardment mass spectrometry (HR-FAB-MS). The IR spectrum displayed strong absorptions at 3450 and 1740 cm^{-1}, which indicated the presence of hydroxyl as well as ester functionalities. The ^{1}H-NMR spectrum of **1** exhibited signals for a vinylic proton at δ 6.06 (1H, br s), two acetalic protons at δ 5.76 (1H, d, J=8.8 Hz) and 5.18 (1H, s), two sets of oxygen-bearing methylene protons at δ 4.13, 4.27 (each d, J=15.5 Hz) and 4.78 (2H, s), and two methines at δ 4.82 (1H, br s) and 3.59 (1H, d, J=8.8 Hz). The presence of these functional groups was further supported by ^{13}C-NMR and distortionless enhancement by polarization transfer (DEPT) spectral signals of **1**, observed at δ 126.7 (CH), 88.9 (CH), 98.1 (CH), 60.7 (CH_2), 79.2 (CH), and 46.8 (CH). Of these signals, the CH_2 signals at δ 4.13 and 4.27 in the ^{1}H-NMR, and δ 60.7 in the ^{13}C-NMR suggested the presence of a hydroxymethyl group in this iridoid skeleton. Furthermore, the ^{1}H-NMR spectrum of **1** showed the presence of two pairs of secondary dimethyls at δ 0.96 (6H, d, J=6.6 Hz) and 1.01 (6H, d, J=6.6 Hz), two methines at δ 2.16 and 2.10, and two methylenes at δ 2.29 (dd, J=18.2, 7.1 Hz) and 2.23 (dd, J=7.1, 1.8 Hz), which were assigned as two isovaleroyloxy groups. This result was further confirmed by the sequential loss of two m/z 102 ($C_5H_{10}O_2$) units in the electron impact mass (EI-MS) spectrum of **1**. The ^{1}H-NMR spectrum also exhibited two methoxy protons at δ 3.32 and 3.50. These data indicated an iridoid skeleton close to the structure of nardostachin (**3**).[8,9] In addition, the ^{13}C-NMR spectrum of **1** showed signals for olefinic carbons at δ 128.2, 139.2, 126.7, and 147.6, attributable to C-4, C-5, C-7, and C-8, respectively.

The connectivities of **1** were established by the interpretation of significant HMBC correlations in the HMBC spectrum. Correlations between the signals at δ 3.59 (H-9)/6.06 (H-7) and δ 60.7 (C-10), and δ 5.18 (H-3) and δ 60.2 (C-11), confirmed that the oxygen-bearing methylenes were connected to C-4 and C-8, respectively. The latter oxymethylene was further linked with an isovaleryl group, which showed signals at δ 4.78 (H-11) and δ 172.8 (C-1″). Long-range correlation between at δ 5.76 (H-1) and δ 171.2 (C-1′) indicated the presence of the other isovaleryl group at C-1. The positions of methoxyl groups at C-3 and C-6 were confirmed by cross peaks between δ 3.50 (OCH_3) and δ 98.1 (C-3), and δ 3.32 (OCH_3) and δ 79.2 (C-6). Regarding the stereochem-



	R^1	R^2	R^3	R^4
1	H	OCH_3	OCH_3	H
2	OCH_3	H	OCH_3	H
4	OCH_3	H	H	OCH_3

3

Chart 1. Structures of Compounds from *Patrinia saniculaefolia*

* To whom correspondence should be addressed. e-mail: baekh@cnu.ac.kr

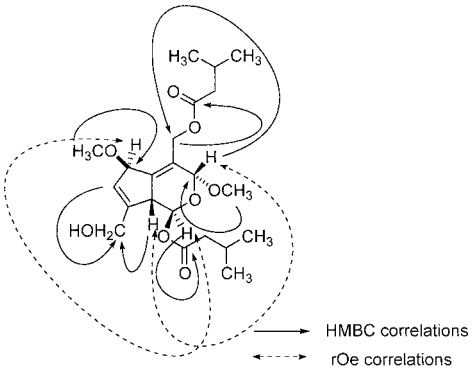


Fig. 1. Significant HMBC and rOe Correlations for **1**

Table 1. ^{1}H-NMR Spectral Data of Compounds **1**—**4** (600 MHz, in $CDCl_3$)

Position	1	2	3	4
1	5.76 d (8.8)	5.70 d (8.0)	5.96 d (3.8)	5.95 d (8.0)
3	5.18 s	5.27 s	6.31 br s	5.17 s
5			2.91 dt (7.9, 7.8)	
6	4.82 br s	5.04 br s	1.78 m	5.30 br s
			1.84 m	
7	6.06 br s	6.03 br s	4.15 m	6.01 t (1.6)
8			2.08 m	
9	3.59 d (8.8)	3.68 d (8.0)	2.13 m	3.56 d (7.7)
10	4.13 d (15.5)	4.15 d (15.4)	1.12 d (6.9)	4.20 s
	4.27 d (15.5)	4.25 d (15.4)		
11	4.78 s	4.60 d (12.6)	4.38 d (12.2)	4.82 d (12.5)
		4.99 d (12.6)	4.58 d (12.2)	4.94 d (12.5)
2′	2.29 dd (18.2, 7.1)	2.29 dd (10.7, 7.3)	2.22 t (7.4)	2.29 dd (7.1, 5.6)
3′	2.16 m		2.10 m	2.19 m
4′	1.01 d (6.6)		0.97 d (6.4)	1.01 d (6.6)
5′	1.01 d (6.6)		0.97 d (6.4)	1.01 d (6.6)
2″	2.23 dd (7.1, 1.8)	2.21 d (7.14)	2.19 d (7.0)	2.22 d (7.1)
3″	2.10 m	2.09 m	2.08 m	2.11 m
4″	0.96 d (6.6)	0.96 d (6.6)	0.97 d (6.4)	0.97 d (6.6)
5″	0.96 d (6.6)	0.96 d (6.6)	0.97 d (6.4)	0.97 d (6.6)
OCH_3(3)	3.50	3.46		3.50
OCH_3(6)	3.32	3.28		3.21

δ values in ppm and coupling constants (in parentheses) in Hz.

istry of **1**, the β-orientation of the isovaleryloxy group at C-1 was deduced from the coupling constant of H-1 (d, J=8.8 Hz) between H-1 and H-9, which indicated an axial–axial relationship. Two methoxy groups at δ 3.50 (OCH_3-3) and δ 3.32 (OCH_3-6) were assigned as α- and β-orientations, respectively, as evidenced by rOe correlations observed from δ 5.18 (H-3) to δ 3.59 (H-9) and δ 4.82 (H-6) to δ 5.76 (H-1) in the rotating-frame nOe spectroscopy (ROESY) spectrum (Fig. 1). From these results, the structure of compound **1** was elucidated as shown, and named patridoid I.

Compound **2** was obtained as a yellow oil. HR-FAB-MS showed a molecular formula, $C_{22}H_{34}O_8$, corresponding to a molecular ion peak at *m/z* 449.2148 $[M+Na]^+$. The ^{1}H-, ^{13}C-, and 2D-NMR spectral data of **2** were very similar to those of **1**, except for a significant difference in the chemical shift of C-1. Comparison of the chemical shift of **2** at C-1 with that of **1** revealed a downfield shift to δ 92.8 (+3.9 ppm) in the ^{13}C-NMR spectrum. This suggested that the methoxy group at C-3 has a β-orientation, compared to that of 3-epilomurin with phlomurin isolated from *Phlomis aurea* (Lamiaceae).[10]

Table 2. ^{13}C-NMR Spectral Data of Compounds **1**—**4** (150 MHz, in $CDCl_3$)

Carbon	1	2	3	4
1	88.9	92.8	91.6	93.3
3	98.1	98.2	139.5	98.6
4	128.2	130.7	114.3	131.4
5	139.2	139.9	32.0	139.7
6	79.2	79.7	39.8	82.0
7	126.7	126.5	74.9	127.1
8	147.6	147.8	40.5	145.7
9	46.8	46.9	45.0	46.3
10	60.7	60.6	12.8	60.6
11	60.2	59.3	63.8	59.9
1′	171.2	171.8	171.0	172.1
2′	43.3	43.2	43.5	43.3
3′	25.4	25.5	25.8	25.5
4′	22.3	22.3	22.4	22.4
5′	22.3	22.3	22.4	22.4
1″	172.8	172.7	172.8	172.8
2″	43.3	43.3	43.5	43.5
3″	25.7	25.7	25.7	25.7
4″	22.3	22.3	22.4	22.4
5″	22.3	22.3	22.4	22.4
3-OCH_3	56.1	55.7		55.9
6-OCH_3	55.4	54.5		53.3

This was further confirmed by rOe correlations from δ 5.70 (H-1)/5.04 (H-6) to δ 5.27 (H-3) in the ROESY spectrum. Consequently, **2** was assigned as a diastereomer of **1** and named patridoid II. Patridoid II was gradually changed to **4** as a diastereomer at C-6 in the presence of oxygen. This was identified by the downfield shift of C-6 by 2.1 ppm, compared with that of **2** in the ^{13}C-NMR spectrum, as well as rOe correlation between δ 5.30 (H-6) and δ 3.56 (H-9) in the ROESY spectrum.

Experimental

Optical rotations were measured on a JASCO DIP-370 digital polarimeter. UV spectra were obtained on a Milton Roy 3000 spectrometer. IR spectra were determined on a JASCO Report-100 spectrometer (KBr plate). ^{1}H-, ^{13}C-NMR, DEPT, ^{1}H–^{1}H COSY, HMQC, HMBC, and ROSEY spectra were recorded on Bruker DMX 600 NMR spectrometer with $CDCl_3$ as a solvent, the chemical shifts being represented as ppm with tetramethylsilane as an internal standard. EI-MS was measured with a JMX-AX 505 HAD mass spectrometer. HR-FAB-MS was obtained on JEOL HX 110 Mass spectrometer.

Plant Material *P. saniculaefolia* was collected during August 1999 at Mt. Chiri, Jeonnam Province, Korea, and identified by Professor KiHwan Bae of the College of Pharmacy, Chungnam National University, Korea. A voucher specimen (CNU 2017) has been deposited in the herbarium of the College of Pharmacy, Chungnam National University, Korea.

Extraction and Isolation The dried whole plant (1.5 kg) of *P. saniculaefolia* was extracted with MeOH (2 l) three times at room temperature for 7 d. The MeOH extract (150 g) was suspended in water and then partitioned with hexane. The hexane soluble fraction (19.0 g) was subjected to column chromatography on silica gel and eluted using a stepwise gradient of hexane and acetone to afford three fractions. Fraction **2** (8.62 g) was further subjected to column chromatography on silica gel eluted with CH_2Cl_2–EtOAc (20 : 1) and then chromatographed on an reversed-phase ODS gel column eluted with MeOH–H_2O (70 : 30) to give **1** (40.7 mg), **2** (70.7 mg), and **3** (70.0 mg).

Patridoid I (**1**): Yellow oil; $[\alpha]_D^{23}$: −74.0° (c=0.5, MeOH); UV (MeOH) λ_{max} (log ε) 203 (4.05) nm; IR ν_{max} cm^{-1}: 3450, 1740, 1290, 1100 cm^{-1}; ^{1}H- and ^{13}C-NMR, see Tables 1 and 2; EI-MS (rel. int.) *m/z*: 324 ($M^+-C_5H_{10}O_2$) (25), 262 (22), 231 (34), 222 (64), 202 (38), 190 (100), 183 (74), 165 (62); HR-FAB-MS (+NaI) *m/z*: 449.2151 $[M+Na]^+$ (Calcd for $C_{22}H_{34}O_8Na$, 449.2151).

Patridoid II (**2**): Yellow oil; $[\alpha]_D^{23}$: −36.0° (c=0.5, MeOH); UV (MeOH) λ_{max} (log ε) 206 (3.88) nm; IR ν_{max} 3450, 1740, 1290, 1100 cm^{-1}; ^{1}H- and ^{13}C-NMR, see Tables 1 and 2; HR-FAB-MS (+NaI) *m/z*: 449.2148

$[M+Na]^+$ (Calcd for $C_{22}H_{34}O_8Na$, 449.2151).

Nardostachin (**3**): Colorless oil; $[\alpha]_D^{23}$: −80.9° (c=0.4, MeOH); UV (MeOH) λ_{max} (log ε) 212 (3.63) nm; IR ν_{max} 3450, 1722, 1668, 1320, 1250, 1180 cm^{-1}; ^1H- and ^{13}C-NMR, see Tables 1 and 2; EI-MS m/z: 266 ($M^+-C_5H_{10}O_2$) (11), 220 (12), 195 (21), 182 (10), 164 (100), 146 (10), 136 (19), 85 (46); FAB-MS m/z: 391.1 $[M+Na]^+$.

Compound **4**: Yellow oil; $[\alpha]_D^{23}$: +54.0° (c=0.5, MeOH); UV (MeOH) λ_{max} (log ε) 205 (3.68) nm; IR ν_{max} 3450, 1740, 1290, 1100 cm^{-1}; ^1H- and ^{13}C-NMR, see Tables 1 and 2; HR-FAB-MS m/z: 449.2154 $[M+Na]^+$ (Calcd for $C_{22}H_{34}O_8Na$, 449.2151).

Acknowledgments This research was supported by a grant (PF002104-00) from the Plant Diversity Research Center of the 21st Century Frontier Research Program funded by the Ministry of Science and Technology of the Korean government. We are grateful to KBSI for ^1H- and ^{13}C-NMR spectral measurements.

References

1) Lee Y. N., "Flora of Korea," Kyo-Hak Publishing Co., Ltd., Seoul, 1996, p. 756.
2) Lee T. B., "Illustrated Flora of Korea," Hyangmoon Publ. Co., Seoul, 1980, p. 714.
3) Kang S. S., "Triterpenoid Saponins," Seoul National University Press, Seoul, 1996, pp. 216—218.
4) Choi J. S., Woo W. S., *Arch. Pharmacal Res.*, **7**, 121—126 (1984).
5) Kouno I., Koyama I., Jiang Z.-H., Tanaka T., Yang D.-M., *Phytochemistry*, **40**, 1567—1568 (1995).
6) Kouno I., Yasuda I., Mizoshiri H., Tanaka T., Marubayashi N., Yang D.-M., *Phytochemistry*, **37**, 467—472 (1994).
7) Larchenko V. A., Fursa N. S., Drozd G. A., *Khim. Prir. Soedin.*,**1979**, 852—853 (1979).
8) Bagchi A., Oshima Y., Hikino H., *Planta Med.*, **54**, 87—88 (1988).
9) Boros C. A., Stermitz F. R., Harris G. H., *J. Nat. Prod.*, **53**, 72—80 (1990).
10) Kamel M. S., Mohamed K. M., Hassanean H. A., Ohtani K., Kasai R., Yamasaki K., *Phytochemistry*, **55**, 353—357 (2000).