# A Novel Iridoid from *Boschniakia rossica*

Soon-Ho YIM,[a] Hyun Jung KIM,[a] Yong Zhen LIU,[b] and Ik-Soo LEE*,[a]

[a] *College of Pharmacy and Research Institute of Drug Development, Chonnam National University; Gwangju 500–757, Korea: and* [b] *College of Pharmacy, Yanbian University; Yanji, Jilin 133000, P. R. China.*

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**A novel iridoid, (4*R*)-4-hydroxymethylboschnialactone (1), has been isolated from *Boschniakia rossica*, together with three previously known compounds, (24*S*)-3$\beta$-hydroxy-24-ethylcholest-5-en-7-one (2), (24*R*)-3$\beta$-hydroxy-24-ethylcholest-5-en-7-one (3) and methyl *p*-coumarate, using column chromatography. The structures of compounds were elucidated by spectroscopic methods.**

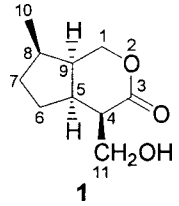
**Key words** *Boschniakia rossica*; Orobanchaceae; (4*R*)-4-hydroxymethylboschnialactone; 3$\beta$-hydroxy-24-ethylcholest-5-en-7-one

*Boschniakia rossica* FEDTSCH. *et* FLEROV (Orobanchaceae) is a parasitic plant grown on the roots of *Alnus maximowiczii* CALLIER (Betulaceae). Although its dried herb has been traditionally used as a tonic medicine in China, Korea and Japan, the tonic principle has not been yet described. It also attracts Felidae animals, and two compounds, boschniakine and boschnialactone, were isolated from this plant as being responsible for this activity.[1)] Some iridoids and phenylpropanoids were isolated from *B. rossica* in the previous studies.[2—8)]

Phytochemical investigation of this plant led to the isolation of a novel iridoid, (4*R*)-4-hydroxymethylboschnialactone (**1**), together with two previously known steroids, namely, (24*S*)-3$\beta$-hydroxy-24-ethylcholest-5-en-7-one (**2**) and (24*R*)-3$\beta$-hydroxy-24-ethylcholest-5-en-7-one (**3**), and a phenylpropanoid, methyl *p*-coumarate. Structures of the compounds were elucidated based on the spectroscopic methods.

Compound **1** was obtained as colorless needles with negative optical rotation $\{[\alpha]_D^{23} = -35.8°$ ($c$=0.1, MeOH)$\}$. Its $[M+1]^+$ peak was shown at $m/z$ 185.1173 (calcd for $C_{10}H_{17}O_3$: 185.1178) in high resolution (HR) EI-MS, which was compatible with the molecular formula $C_{10}H_{16}O_3$ with an index of hydrogen deficiency of three. The IR spectrum showed absorption bands for the hydroxyl and carbonyl groups at 3440 and 1731 $cm^{-1}$, respectively. The $^1$H- and $^{13}$C-NMR data, evident from the heteronuclear single quantum coherence (HSQC) experiment, exhibited a lactone moiety including an oxygenated $sp^3$ methylene [$\delta_H$ 4.03 (1H, dd, $J$=11.6, 14.8 Hz), 4.26 (1H, dd, $J$=5.4, 11.6 Hz); $\delta_C$ 67.1 (–$CH_2$O–), 175.9 (C=O)], a hydroxymethyl [$\delta_H$ 2.94 (OH, t, $J$=6.8 Hz), 3.75 (1H, m), 3.86 (1H, m); $\delta_C$ 61.3 (–$CH_2$OH)], a methyl attached to methine [$\delta_H$ 1.00 (3H, d, $J$=7.0 Hz); $\delta_C$ 14.3 (–$CH_3$)], two $sp^3$ methylenes [$\delta_H$ 1.61 (1H, dddd, $J$=2.9, 3.1, 6.9, 13.2 Hz), 1.92 (1H, dddd, $J$=7.0, 9.3, 11.2, 13.2 Hz) and 1.43 (1H, dddd, $J$=7.0, 11.2, 11.5, 12.5 Hz), 1.78 (1H, dddd, $J$=3.1, 5.5, 6.9, 12.5 Hz); $\delta_C$ 31.5, 32.7] and four $sp^3$ methines [$\delta_H$ 2.19 (1H, m), 2.27 (1H, ddt, $J$=2.9, 9.3, 11.6 Hz), 2.37 (1H, ddd, $J$=3.4, 6.8, 11.6 Hz), 2.47 (1H, dddd, $J$=5.4, 8.0, 11.6, 14.8 Hz); $\delta_C$ 36.9, 36.3, 45.8, 40.8] (Table 1). These spectral data suggested the compound **1** has a cyclopentane ring which is fused to a six-membered oxygen heterocycle.

Detailed interpretation of heteronuclear multiple bond correlation (HMBC) spectral data resulted in the elucidation of the structure of **1** (Table 1). H-1, H-4 and H-11 protons




**1**

Table 1. NMR Spectral Data of **1** in $CDCl_3$

| No. | $\delta_C$ [a)] | | $\delta_H$ [a,b)] | HMBC | NOESY |
|---|---|---|---|---|---|
| 1 | 67.1 t | $\alpha$ | 4.03 (1H, dd, 11.6, 14.8) | C-5, C-9 | H-9 |
| | | $\beta$ | 4.26 (1H, dd, 5.4, 11.6) | C-3, C-5, C-9 | H-9 |
| 3 | 175.9 s | | — | — | — |
| 4 | 45.8 d | | 2.37 (1H, ddd, 3.4, 6.8, 11.6) | C-3, C-5, C-6, C-11 | H-5, H-11a, H-11b |
| 5 | 36.3 d | | 2.27 (1H, ddt, 2.9, 9.3, 11.6) | C-4, C-11 | H-4, H-6$\alpha$, H-9 |
| 6 | 31.5 t | $\alpha$ | 1.92 (1H, dddd, 7.0, 9.3, 11.2, 13.2) | C-4, C-5, C-7 | H-5, H-7$\alpha$ |
| | | $\beta$ | 1.61 (1H, dddd, 2.9, 3.1, 6.9, 13.2) | C-4, C-7, C-8, C-9 | H-7$\beta$, OH |
| 7 | 32.7 t | $\alpha$ | 1.78 (1H, dddd, 3.1, 5.5, 6.9, 12.5) | C-5, C-6, C-9 | H-6$\alpha$ |
| | | $\beta$ | 1.43 (1H, dddd, 7.0, 11.2, 11.5, 12.5) | C-6, C-8, C-9, C-10 | H-6$\beta$ |
| 8 | 36.9 d | | 2.19 (1H, m) | C-1, C-6, C-7, C-9, C-10 | H-9, H-10 |
| 9 | 40.8 d | | 2.47 (1H, dddd, 5.4, 8.0, 11.6, 14.8) | C-1, C-6, C-7, C-8 | H-1$\alpha$, H-1$\beta$, H-5, H-8 |
| 10 | 14.3 q | | 1.00 (3H, d, 7.0) | C-7, C-8, C-9 | H-8 |
| 11 | 61.3 t | a | 3.75 (1H, m) | C-3, C-4, C-5 | H-4 |
| | | b | 3.86 (1H, m) | C-3 | H-4 |
| | | | 2.94 (OH, t, 6.8) | | H-6$\beta$ |

*a*) Assignments are based on HSQC and HMBC experiments; TMS was used as the internal standard. *b*) Integrals, multiplicities and coupling constants ($J$ in hertz) in parentheses.

* To whom correspondence should be addressed. e-mail: islee@chonnam.chonnam.ac.kr

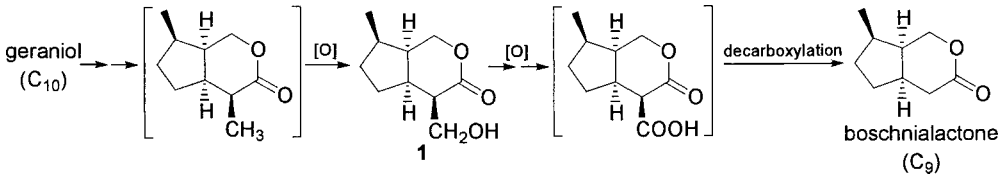



Fig. 1. Hypothetical Biosynthetic Pathway of Boschnialactone

showed correlations with carbonyl carbon at $\delta_C$ 175.9 (C-3) of lactone moiety. The $sp^3$ methine protons at C-5 and C-9 were correlated with C-4, C-6, C-7 and C-1, C-7, C-6, C-8, respectively. Also the methyl group at $\delta_C$ 14.3 was assigned to C-10 based on the HMBC correlation of its protons to C-7, C-8 and C-9. The hydroxymethyl group at $\delta_C$ 61.3 was assigned to C-11 by correlations between its protons and C-3, C-4, C-5.

The stereochemistry of **1** was established based on the nuclear Overhauser effect spectroscopy (NOESY) experiment (Table 1). The NOESY spectrum showed correlations of H-5/H-4, H-5/H-6$\alpha$, H-5/H-9 and H-8/H-9, in addition to those of H-6$\alpha$/H-7$\alpha$ and H-6$\beta$/H-7$\beta$. These data indicated that compound **1** has the same stereochemistry at C-5, C-8 and C-9 as that of boschnialactone[1] and the hydroxymethyl group bound to C-4 is in the $\beta$ position. Thus the structure of compound **1** was established to be (4*R*)-4-hydroxymethylboschnialactone.

Compound **1** is postulated to be a possible intermediate precursor of boschnialactone isolated from *B. rossica* by Sakan *et al.*[1] (Fig. 1).

Compounds **2** and **3** were obtained as a mixture, which were identified as (24*S*)-3$\beta$-hydroxy-24-ethylcholest-5-en-7-one and (24*R*)-3$\beta$-hydroxy-24-ethylcholest-5-en-7-one by direct comparison of their spectral data with those reported in the literature.[9—11] Presence of these compounds in this plant is reported for the first time herein.

## Experimental

**General** Optical rotation was measured on a JASCO DIP 1000 digital polarimeter. UV spectra were recorded on a JASCO V-530 UV/Vis spectrophotometer. IR spectra were recorded using a JASCO FT-IR 300E spectrophotometer. $^1$H (500 MHz) and $^{13}$C (125 MHz) NMR spectra were run on a Varian Unity INOVA 500 spectrometer in $CDCl_3$, using TMS as the internal standard. Mass and HR EI-MS spectra were determined on a Micromass Platform II GC/LC and a JEOL JMS-700 Mstation mass spectrometer, respectively. HPLC was performed on a Waters 600E multisolvent delivery system using Waters $\mu$Porasil$^{TM}$ and Supelco silica HPLC columns.

**Plant Material** The whole plant of *Boschniakia rossica* was obtained from Mt. Baekdu (Chang bai), China in August, 2000. The voucher specimen was deposited in the College of Pharmacy, Chonnam National University.

**Extraction and Isolation** The air-dried *B. rossica* (769 g) was extracted with 80% MeOH and evaporated *in vacuo* to give a crude extract (157 g), which was successively extracted with *n*-hexane, $CHCl_3$, EtOAc and *n*-BuOH. The $CHCl_3$ extract (1.1 g) was chromatographed on silica gel using a $CHCl_3$–MeOH gradient system (99 : 1→1 : 1) to give six fractions, and fraction 1 (270 mg) was chromatographed on a silica gel HPLC column (Waters, $\mu$Porasil$^{TM}$, 19×150 mm) by *n*-hexane–$CHCl_3$ gradient system (95 : 5→80 : 20) to give two fractions. Subfraction 2 (147 mg) was purified by HPLC (Supelco, 4.6×150 mm) using $CHCl_3$–MeOH (98 : 2) to afford **1** (6.3 mg). The *n*-hexane extract (5.5 g) was subjected to silica gel column chromatography with an *n*-hexane–EtOAc solvent system (20 : 1→1 : 1) to provide seven fractions. Fraction 4 (564 mg) was rechromatographed on a silica gel MPLC using $CHCl_3$–MeOH (98 : 2) to give seven fractions. Subfraction 3 (141 mg) was purified by HPLC (Waters, $\mu$Porasil$^{TM}$, 19×150 mm) eluting with $CH_2Cl_2$–EtOAc (20 : 1) to give a mixture of **2** and **3** (6.0 mg).

(4*R*)-4-Hydroxymethylboschnialactone (**1**): Colorless needles; mp 46—47 °C ($CHCl_3$); $^1$H- and $^{13}$C-NMR (Table 1); IR (KBr) cm$^{-1}$: 3440, 2950, 1731, 1631, 1164, 1033; UV $\lambda_{max}$ ($CHCl_3$) 248 nm; HR EI-MS *m/z* 185.1173 (calcd for $C_{10}H_{16}O_3$: 185.1178); EI-MS *m/z* 185 $[M+1]^+$, 154, 139; $[\alpha]_D^{23} = -35.8°$ (*c*=0.1, MeOH).

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