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Iridoid Compounds from *Boschniakia rossica*

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Four iridoid compounds were isolated from methanol extract of *Boschniakia rossica* by repeated column chromatography. Their structures were determined as boschnaloside (**1**), boschnarol (**2**), bosnarol methylether (**3**), and 7-deoxy 8-epiloganic acid (**4**), respectively. Compound **2**, **3**, and **4** were isolated for the first time from this plant.

Key words : *Boschniakia rossica*, Orobanchaceae, Iridoid, Boschnaroside, Boschnarol, Boschnarol methylether, 7-Deoxy-8-epiloganic acid

INTRODUCTION

Bosniakia rossica (Cham. et Schldl.) Fedtsch. et Flerov (Orobanchaceae) is a paracytic plant growing on the root of *Alnus* species. The dried herb and stem of the plant have been used as a tonic or invigorating drug in Asia (Perry, 1980). In a district of northeast China, it has been used as antisenile agent in the form of alcoholic infusion (Tsuda *et al.*, 1994a, 1994b). The chemical constituents of the plant originated from Mt. Paekdu (Chang bai) have not been studied much except closely related species growing at Mt. Fuji in Japan. Most of chemical constituents such as boschnialactone, boschniakine (Sakan *et al.*, 1969), two iridoid glucosides such as boschnaloside and boschnaside (Murai *et al.*, 1982), (+)-pinoresinol-β-D-glucopyranoside, acylated oligosaccharide and phenylpropanoid glycosides named rossicaside A, B, C, and D (Konishi *et al.*, 1987) have been isolated from the latter one. On the other hand pharmacological activities have been studied with the methanol extract of the former one, which has been demonstrated that methanol extract of *B. rossica* has a reinforcement effect on decreased learning ability (Tsuda *et al.*, 1994a) and memory weakness as well as a free radical scavenging action (Tsuda *et al.*, 1994b). To elucidate the constituents and their phamacological activities of the plant collected from Mt. Paekdu (Chang bai), we have first undertaken the phytochemical studies. Four irodoid compounds were isolated from the MeOH extract of *B. rossica* by MCI-Gel CHP-20P and repeated silica gel column chromatography. Their structures were elucidated by physico-chemical properties and spectral data.

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MATERIALS AND METHODS

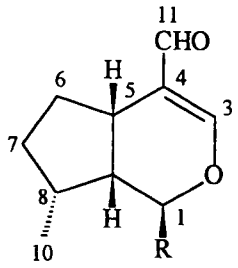
Melting points were determined on a Electrothermal 9100 melting point apparatus and were uncorrected. ^{1}H-NMR and ^{13}C-NMR spectra were obtained on a Varian Unity 300 MHz (^{1}H-NMR) and 75 MHz (^{13}C-NMR). Mass spectra were taken with a Hewlett-Packard 5989A spectrometer.

Plant materials

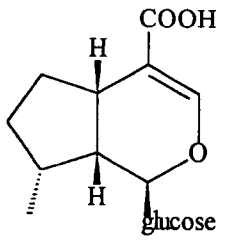
Whole plants were harvested from Mt. Paekdu (Chang bai) area. They were identified by Prof. Zong Zhu Yin in Yanbian University and the voucher specimen (CNU 96082) is deposited in the herbarium of College of Pharmacy, Chungnam National University.

Extration and isolation

The dried whole plants (1.5 kg) were sliced and extracted with MeOH at room temperature three times. The extract concentrated *in vacuo* was fractionated with dichloromethane and water. The aqueous layer was adsorbed in MCI-gel CHP20P and eluted with aqueous MeOH in a decreasing polarity (30%, 50%, 70% and 100% MeOH). The fraction of 50% MeOH was chromatographed by silica gel column chromatography using $CHCl_3$-MeOH-H_2O (10:2:0.1) to give compound **1** (653 mg) and compound **4** (450 mg). The dichloromethane fraction was partitioned again with hexane and 90% MeOH. The 90% MeOH extract was concentrated and the residue was fractionated on a silica gel column chromatography using a step gradient of n-hexane-ethylacetate (10:1, 5:1, 2:1, 1:1) and the



1 R = glucose
2 R = OH
3 R = OCH_3



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major fraction was further separated with a preparative TLC ($CHCl_3$-MeOH, 50:1). The crude compounds were finally purified by Sephadex LH20 to give compound **2** (12 mg) and compound **3** (9 mg).

Compound 1 (bosnaloside): Colorless needles, mp 90-91°C, UV λ_{max} (MeOH, log ε) nm: 248 (4.25), EIMS *m/z*: 344 (M^+), ^{1}H-NMR (pyridine-d_5) δ: 0.98 (3H, d, *J*= 7.2 Hz, H-10), 5.37 (1H, d, *J*=8.1 Hz, anomeric H), 5. 82 (1H, d, *J*=4.2 Hz, H-1), 7.39 (1H, s, H-3), 9.37 (1H, s, CHO) ^{13}C-NMR: Table I.

Compound 2 (bosnarol): Colorless oil, EIMS *m/z*: 182 (M^+) ^{1}H-NMR ($CDCl_3$) δ: 1.13 (3H, d, *J*=7.5 Hz, CH_3), 1.34 (1H, m), 1.40 (1H, m), 1.88 (1H, m), 2.09 (1H, q, *J*=7.2 Hz), 2.21 (1H, m), 2.31 (1H, m), 2.92 (1H, dd, *J*=7.2, 8.7 Hz), 5.27 (1H, d, *J*=7.5 Hz), 7.24 (1H, s), 9.18 (1H, s, CHO) ^{13}C-NMR: Table I.

Compound 3 (bosnarol methylether): Colorless oil, EIMS *m/z*: 196 (M^+), ^{1}H-NMR ($CDCl_3$) δ: 1.08 (3H, d, *J*=7.2 Hz, CH_3), 3.45 (3H, s, OCH_3), 5.10 (1H, d, *J*= 3.6 Hz, H-1), 7.15 (1H, s, H-3), 9.30 (1H, s, CHO) ^{13}C-NMR: Table I.

Compound 4 (7-deoxy-8-epiloganic acid): Yellowish powder from MeOH, mp 209-210°C, EIMS *m/z*: 360 (M^+), ^{1}H-NMR (CD_3OD) δ: 1.08 (3H, d, *J*=6.9 Hz, H-10), 4.69 (1H, d, *J*=8.1 Hz, anomeric H), 5.45 (1H, d, *J*=4.8 Hz, H-1), 7.41 (1H, s, H-3) ^{13}C-NMR: Table I.

RESULTS AND DISCUSSION

The dried whole plants were extracted with MeOH. The extract was partitioned with CH_2Cl_2 and H_2O, and the CH_2Cl_2 soluble fraction was partitioned with hexane and 90% MeOH. The aqueous layer adsorbed in MCI-gel CHP20P was eluted with 50% MeOH and subjected to column chromatography on silica gel to afford compound **1** and compound **4**. The 90% MeOH extract was separated by repeated silica gel column chromatography, preparative TLC and Sephadex LH20 to afford compound **2** and **3**.

Compound **1**, colorless needles, mp 90-91°C, $C_{16}H_{24}O_8$, was isolated as major compound from this plant. The ^{1}H-NMR spectrum showed iridoidal characteristic peaks at δ 0.98 (d, *J*=7.2 Hz, CH_3), δ 5.37 (d, *J*=8.1 Hz, H-1'), δ 7.39 (s, H-3), and δ 9.37 (s, CHO). The ^{13}C-NMR spectrum indicated the presence of an α,β-unsaturated aldehyde group with olefinic carbon signals at δ 190.3, 162.19, and 125.0. The comparison of chemical shift revealed that compound **1** had a glucose in β-configuration and α-methyl group at C-10 (Bianco *et al.*, 1981) which was reported as boschnaloside (Boros *et al.*, 1990, El-Naggar *et al.*, 1980, Murai *et al.*, 1980).

Table I. ^{13}C-NMR data of iridoid compounds (75 MHz)

Carbon atom	**1** (pyridine-d_5)	**2** ($CDCl_3$)		**3** ($CDCl_3$)		**4** (CD_3OD)	
1	96.7	96.1	CH	102.6	CH	96.6	CH
3	162.1	162.7	CH	164.2	CH	153.1	CH
4	125.0	123.7	C	125.3	C	114.2	C
5	31.5	32.4	CH	32.3	CH	33.8	CH
6	30.6	30.3	CH_2	31.0	CH_2	32.8	CH_2
7	32.9	31.6	CH_2	32.5	CH_2	35.2	CH_2
8	35.9	36.4	CH	36.5	CH	38.1	CH
9	43.1	44.1	CH	43.0	CH	44.9	CH
1	16.3	16.4	CH_3	15.0	CH_3	17.2	CH_3
11	190.3	191.1	C	191.1	C	171.8	C
OCH_3	56.3			56.3	CH_3		
1'						100.2	CH
2'	100.5					75.3	CH
3'	74.8					78.5	CH
4'	78.5					72.3	CH
5'	71.6					78.9	CH
6'	79.0					63.5	CH_2

Compound **2**, $C_{10}H_{14}O_3$, colorless oil, showed molecular ion peak at *m/z* 182. The ^{1}H-NMR spectrum showed essentially identical with those of **1** except the signals arising from glucose moiety; a secondary methyl group at δ 1.13 (d, *J*=7.5 Hz, CH_3), an acetal proton at δ 5.27 (d, *J*=7.5 Hz, H-1), an olefinic proton at δ 7.24 (s, H-3), and an aldehydic proton at δ 9.18 (s, CHO). The relative configuration of C-10 was proved by its ^{13}C-NMR data, which showed α-methyl group at δ 16.4 (Justice *et al.*, 1992). The other ^{13}C- and ^{1}H-NMR data were in good agreement with those of **1**. This 8-epi-iridotrial compound was also observed in the biosynthesis of cornin in *Verbena* (Jensen *et al.*, 1989) and of antirrhinoside in *Antirrhinum* (Breinholt *et al.*, 1992). Probably, this compound was hydrolysed to the aglycone from **1** in this plant.

Compound **3**, $C_{11}H_{16}O_3$, colorless oil, showed molecular ion peak at *m/z* 196. The ^{13}C- and ^{1}H-NMR spectra were a close resemblance to those of **2**. However, compound **3** showed one methoxy group at δ 3.45 in ^{1}H- and δ 56.3 in ^{13}C-NMR spectra. From the spectral data, the structure of **3** was confirmed as boschnarol methylether, which was reported as hydrolysate of bosnaroside tetraacetate (Sakan *et al.*, 1969), but it was isolated for the first time from nature.

Compound **4**, $C_{16}H_{24}O_9$, mp 209-210°C, was slightly

yellowish powder. The ^{1}H-NMR spectrum showed signals for methyl group at δ 1.08 (d, H=6.9 Hz), an anomeric proton at δ 4.69 (d, *J*=8.1 Hz), a doublet at δ 5.45 (d, *J*=4.8Hz) for the acetalic H-1 (d, *J*=4.8 Hz) and an olefinic proton at δ 7.41 (s). By comparison with reported ^{13}C-NMR data (Bianco *et al.*, 1986), compound **4** was well accorded with 7-deoxy-8-epiloganic acid. This compound was isolated for the first time from this plant.

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