Rossicasins A, B and Rosicaside F, Three New Phenylpropanoid Glycosides from Boschniakia rossica

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Three phenylpropanoid glycosides have been isolated, together with the known phenylpropanoid glycosides rossicaside A (4), B (5), E (6), and trans-p-coumaryl alcohol 1-O-β-D-glucopyranosyl(1→4)-α-L-rhamnopyranosyl(1→3)-(4-O-trans-caffoyl)-O-β-D-glucopyranosyl(1→4)-α-L-rhamnopyranosyl(1→3)-(4-O-trans-caffoyl)-O-β-D-glucopyranosyl (8), from the aqueous extract of Boschniakia rossica (CHAM. et SCHLECH.) FEDTSCHEL. et FLEROV. Spectroscopic evidence led to the assignments of their structures as trans-p-coumaryl-6′-O-β-D-xylpyranosyl-1-O-β-D-glucopyranosyl (1), trans-p-coumaryl-(6′-O-α-L-arabinopyranosyl)-O-β-D-glucopyranoside (2) and 2-(3,4-dihydroxyphenyl)-R,S-2-ethoxy-ethyl-O-β-D-glucopyranosyl(1→4)-α-L-rhamnopyranosyl(1→3)(4-O-trans-caffoyl)-β-D-glucopyranoside (3), designated as rossicasin A, rossicasin B, and rossicaside F, respectively. Compound 7 was identified from the degradation reaction and this is the first isolation from a natural source.

Key words Boschniakia rossica; Orobancheaceae; phenylpropanoid glycoside; rossicasin A; rossicasin B; rossicaside F

Results and Discussion

The water-soluble fraction of the ethanolic extract of B. rossica was subjected to column chromatography by the procedure described in the Experimental section to yield seven phenylpropanoid glycosides (1—7) and an acylated oligosaccharide 8. Spectroscopic data obtained from compounds 4, 5, 6, 7, and 8 were in very good agreement with the literature data.

Rossicasin A (1) and rossicasin B (2) were found to be trans-p-coumaryl glycosides according to the 1H- and 13C-NMR data. Compounds 1 and 2 had identical quasi-molecular ions at m/z 443 [M–H]− in ESI-MS and HR-FAB-MS [M+H]+ ions at m/z 445.1715 and 445.1709, respectively, indicating the same molecular formula, C29H30O11. The ESI-MS spectra of 1 and 2 showed only the same fragment ion at m/z 311 [M–133]−, indicating a similar structure which loses a pentose [C5H7O5]− mass unit from the molecular structure. In the 1H-NMR spectrum of 1, signals at δ 4.29 (1H, dd, J = 12.5, 6.5 Hz, H-9), δ 4.48 (1H, dd, J = 12.5, 6.0 Hz, Hb-9), δ 6.17 (1H, dt, J = 16.0, 6.5 Hz, H-8), δ 6.59 (1H, d, J = 16.0 Hz, H-7), and δ 6.74/7.27 (each 2H, d, J = 8.5, H-3, -5/H-2, -6) suggested the presence of trans-p-coumaryl moieties that were identical with those of 2. In addition to the signals for the trans-p-coumaryl moiety, their

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was determined as trans-p-coumaryl 1-O-β-d-glucopyranosyl(1→4)-α-L-rhamnopyranosyl(1→3)-β-d-glucopyranoside. Compound 7 has been identified from the degradation reaction, and this is the first isolation from a natural source.

**Experimental**

**Apparatus** All melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. IR spectra were obtained as KBr pellets or film on a Nicolet Avatar 320 IR spectrometer. UV spectra were measured on a Hitachi U-3200 spectrophotometer in MeOH. 1H, 13C- and 2D-NMR spectra were measured with a Varian Inova-500 spectrometer with deuterated solvents as internal standard. ESI-MS and HR-FAB-MS were recorded on Finnigan LCQ and Finnigan/Thermo Quest MAT spectrometers, respectively. HPLC analysis was performed using a Shimadzu LC-8A or LC-10AT vp pump and SPD-10A vp UV–Vis detector or RIA-10A refractive index detector.

**Plant Material** The whole plant of *Boscinia kiosica* was purchased in Taipei, Taiwan, and verified by Mr. Hsi-Yu Chang, director of Feng Li Co., Inc., Taipei, Taiwan. A voucher specimen is deposited in the National Research Institute of Chinese Medicine, ROC.

**Isolation** The whole herb of *B. rossica* (9.6 kg) was extracted with 95% EtOH (601×4) under reflux. The ethanol extracts were combined and concentrated under vacuum to a volume of 1.51. The ethanolic extract was then partitioned successively between H₂O and EtOAc, followed by n-ButOH (each 11×3). A portion (200 g) of the H₂O extract (700 g) was subjected to column chromatography over Diaion HP-20 (10 cm×50 cm) with H₂O, 20% MeOH/H₂O, 50% MeOH/H₂O, and MeOH as the eluting solvents to give 4 fractions. Fr. 2 (40 g) was chromatographed over Sephadex LH-20 with aqueous MeOH (0—20%) and further purified by Cosmosil C₁₈ OPN 140 (20—40% MeOH in H₂O) to give Fr. 2-1 and 2-2. Fr. 2-1 was recrystallized with H₂O to give 8 (876 mg). Fr. 2-2 was further purified with semipreparative HPLC (column: Cosmosil NH₂, 5 μm, 25×250 mm; mobile phase: 80% CH₃CN/H₂O; flow rate: 16 ml/min, detector: UV 254 nm) to give compounds 1 (65 mg), 2 (13 mg) and 7 (190 mg). Fr. 3 (16 g) was chromatographed over a Sephadex LH-20 (0—60% MeOH in H₂O) to give Fr. 3-1—3-9. Fr. 3-5 was further purified with semipreparative HPLC (column: Inertsil 10 ODS, 22×250 mm; mobile phase: 18% CH₃CN/H₂O; flow rate: 16 ml/min, detector: UV 254 nm) to give compounds 3 (35 mg) and 4 (125 mg). Repeated chromatography of fraction Fr. 3-7 over Sephadex LH-20 (MeOH) and semipreparative HPLC (column: Inertsil 10 ODS, 22×250 mm; mobile phase: 50% MeOH/H₂O; flow rate: 15 ml/min, detector: UV 254 nm) yielded 5 (728 mg) and 6 (590 mg).

**Rosassin A** (1) Colorless needles (MeOH), mp 168—170°C ([α]D < 0° (MeOH), UV λmax (MeOH) nm (ε): 234 (4.02), 3455, 3322 (OH), 1605, 1522 (C=O), 1451, 1398, 1351, 1035. 1055. 1H-NMR (CD₃OD) δ: 3.22 (IH, t, J=11.0 Hz, Ha-5), 3.52 (IH, t=9.0 Hz, H-3*), 3.35 (IH, t=9.0 Hz, H-3*), 3.79 (IH, dd, J=12.5, 6.5 Hz, Ha-9), 4.36 (1H, d, J=12.5, 3.5 Hz, Hb-5), 7.27 (2H, d, J=8.5 Hz, H-2, H-6). 13C-NMR (CD₃OD) δ: 66.9 (C-5*), 69.7 (C-6*), 71.1 (C-14*), 71.2 (C-9*), 71.4 (C-4*), 74.8 (C-2*), 75.0 (C-2*), 76.9 (C-5*), 77.7 (C-3*), 77.9 (C-9*), 103.1 (C-1*), 105.5 (C-1*), 116.3 (C-3*), 123.3 (C-8*), 128.9 (C-2*), 129.7 (C-13*), 134.3 (C-7*), 158.4 (C=O). ESI-MS/m/z: 443 [M+H]-, 311 [M+133]-. HR-FAB-MS/m/z 445.1715 [M+1]+. (Calcd 445.1710 for C₂₅H₂₂O₈).

**Acid Hydrolysis of 1** A solution of 1 (5 mg) in 2 N H₂SO₄ (3 ml) was refluxed in a water bath for 2 h. H₂O was added to the solution, the mixture washed with CHCl₃, the aqueous phase neutralized with BaCO₃, and then the precipitate was filtered off. The filtrate was concentrated and examined by HPLC (Phenomenex Luna 5 μ NH₂, 250×4.6 mm, 65% acetonitrile/H₂O, 1.2 ml/min, RI detector). D-glucuronic acid (tₘ=4.50 min) and D-xylene (tₘ=4.03 min) were detected by comparing them with the retention times (τₘ) of authentic samples.

**Rosassin B** (2) Brown syrup. [α]D < 51.7° (c=0.29, H₂O). UV λmax (MeOH) nm (ε): 263 (4.02). IR νcm⁻¹: 3395 (OH), 1609, 1514, 1435, 1372, 1062, 1009 (C=O). 1H-NMR (CD₃OD) δ: 3.24 (1H, t, J=8.0 Hz, H-2*), 3.36 (2H, m, H-3*), 3.45 (1H, m, H-5*), 3.54 (2H, m, H-7*), 3.62 (1H, dd, J=9.0, 6.5 Hz, H-2*), 3.75 (1H, dd, J=11.5, 5.5 Hz, H-6*), 3.81 (1H, brs, H-4*), 3.88 (1H, dd, J=12.5, 3.5 Hz, H-5*), 4.12 (1H, dd, J=7.5, 1.5 Hz, H-6*), 4.32 (1H, dd, J=11.0, 8.0 Hz, H-7*), 6.78 (2H, d, J=8.5 Hz, H-2, H-6), 6.89 (1H, dd, J=16.0, 6.5 Hz, H-8), 6.95 (1H, d, J=16.0 Hz, H-7), 7.64 (2H, d, J=8.5 Hz, H-3, H-5), 7.27 (2H, d, J=8.5 Hz, H-2, H-6). 13C-NMR (CD₃OD) δ: 66.9 (C-5*), 69.7 (C-6*), 71.1 (C-14*), 71.2 (C-9*), 71.4 (C-4*), 74.8 (C-2*), 75.0 (C-2*), 76.9 (C-5*), 77.7 (C-3*), 77.9 (C-9*), 103.1 (C-1*), 105.5 (C-1*), 116.3 (C-3*), 123.3 (C-8*), 128.9 (C-2*), 129.7 (C-13*), 134.3 (C-7*), 158.4 (C=O). ESI-MS/m/z: 443 [M+H]-, 311 [M+133]-. HR-FAB-MS/m/z 445.1715 [M+1]+. (Calcd 445.1710 for C₂₅H₂₂O₈).
Acid Hydrolysis of 2 A mixture of 2 (3 mg) and 2 x H2SO4 (3 ml) was heated in a water bath for 2h. The products α-glucose (tα = 4.8 min) and L-arabinose (tβ = 3.68 min) were isolated in the HPLC analysis, as described for I.

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References