Three New Iridoid Glucosides from the Roots of *Patrinia scabra*

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Received May 31, 2011, Accepted July 16, 2011

To probe the chemical constituents of *Patrinia scabra*, we undertook the phytochemical investigation on its roots, which led to the isolation and elucidation of three new iridoid glucosides, scabroside A-C (1-3), along with three known iridoids, jatamanin J (4), isopatriscabroside I (5), and loganic acid (6) from the aqueous fraction of the ethanolic extract of the roots. The structures and relative configurations of the three new compounds were elucidated by spectroscopic methods including IR, UV, MS, 1D and 2D NMR experiments. Compound 3 was an unusual iridoid with an oxygen bridge connecting C-3 and C-8.

**Key Words**: *Patrinia scabra*, Iridoid glucosides, Scabroside A, Scabroside B, Scabroside C

**Introduction**

*Patrinia scabra* Bunge is a perennial herb belongs to Valerianaceae family distributed in the northeastern part of China. The roots of *P. scabra* were used as a traditional medicine to treat malaria, dysentery, leukemia, gastric cancer and gynecological diseases. Previous phytochemical investigation on this plant revealed that the roots of *P. scabra* contain iridoids, flavonoids, terpenoids, and lignans. In this paper, we report the isolation of three new iridoid glucosides, scabroside A-C (1-3), along with three known iridoids, jatamanin J (4), isopatriscabroside I (5), and loganic acid (6) from the H2O-soluble fraction of the roots of this plant as shown in Figure 1. Their structures were established by mass-spectrometric and spectroscopic analyses, especially 2D-NMR techniques (1H-1H COSY, HMQC, HMBC, and NOESY), and comparison of their data with literature values. Herein, the isolation and structural elucidation of compounds 1-3 are described.

**Experimental Section**

**General Experimental Procedures.** Optical rotations were measured on a Horiba SEAP-300 polarimeter. A Hitachi UV 210A spectrophotometer was used to obtain the UV spectra in methanol (MeOH). IR spectra were taken on a Bruker Tensor 27 FT-IR spectrometer with KBr pellets. NMR spectra were measured on a Bruker AM-400 or a Bruker DRX-500 spectrometers with TMS as an internal standard. MS and HR-MS were performed on an API-QSTAR-Pulsar-I spectrometer. Column chromatography was carried out on C8 MB (100-400 μm, Fuji Silysia Chemical Co. Ltd.), MCI gel CHP-20P (75-150 μm, Mitsubishi Chemical Co.), Chromatorex ODS (30-50 μm, Fuji Silysia Chemical Co. Ltd.), and silica gel (200-300 mesh, Qingdao Haiyang Chemical Co. Ltd., P. R. China). Thin layer chromatography (TLC) was carried out on silica gel G precoated plates (Qingdao Haiyang Chemical Co. Ltd.), and spots were detected by spraying with 5% H2SO4 in EtOH followed by heating.

**Plant Material.** The dry roots of *Patrinia scabra* Bunge were purchased from Bozhou herbal market in March 2010 and identified by Prof. Dr. Kai-Jin Wang from the School of Life Sciences, Anhui University, where a voucher specimen (No. 20100303) was deposited.

**Extraction and Isolation.** The dry roots of *P. scabra* (18.0 kg) were powdered and extracted exhaustively with 90% ethanol at room temperature. The extracts were combined and concentrated under vacuum to give a residue (1.35...
kg). The residue was suspended in H₂O and extracted with petroleum ether, ethyl acetate and n-butanol successively to give petroleum ether (487 g), ethyl acetate (243 g), n-butanol (252 g) and aqueous fraction (478 g). The aqueous fraction was suspended in H₂O and then passed though a D101 resin column eluted with MeOH-H₂O (0:1-1.0) to afford fraction D (132 g). The fraction D was chromatographed on silica gel column eluted with CHCl₃-MeOH (from 7:1 to 1:1) to give two fractions D₁ and D₂. The fraction D₁ was subjected to RP-8 (MeOH-H₂O, 0:1-1:0) to yield fractions D₁₁-D₁₄. The fraction D₁₁ was purified by ODS (MeOH-H₂O, 0:1-1:0) to afford 4 (110 mg). The fraction D₁₂ was performed by MCI (MeOH-H₂O, 0:1-1:0) to obtain compound 2 (30 mg). Compound 3 (62 mg) was obtained from fraction D₁₃ on silica gel column eluted with CHCl₃-MeOH (8:1). Fraction D₁₄ was chromatographed on silica gel column eluted with CHCl₃-MeOH (10:1) to afford 1 (12 mg). Fraction D₂ was further subjected to MCI (MeOH-H₂O, 0:1-1:0) and then RP-8 (MeOH-H₂O, 0:1-1:0) to yield 5 (165 mg).

**Scabroside A (1)**. White powder; [α]D⁰²⁺ = +45.2 (c 0.47, MeOH); UV max MeOH (nm) (lg ε): 3416 (OH), 2973, 2928, 1728 (C=O), 1461, 1401, 1381, 1270, 1200, 1150, 1101, 1044, 922, 633, 582; ¹H- and ¹³C-NMR data: see Table 1; HR-ESI-MS (neg.): m/z 413.1215 [M+Cl]⁻ (calcd. 413.1214). Isolated from *Scabiosa japonica*.

**Scabroside B (2)**. White powder; [α]D⁰²⁺ = +78.2 (c 0.19, MeOH); UV max MeOH (nm) (lg ε): 3416 (OH), 2973, 2928, 1728 (C=O), 1461, 1401, 1381, 1270, 1200, 1150, 1101, 1044, 922, 633, 582; ¹H- and ¹³C-NMR data: see Table 1; HR-ESI-MS (neg.): m/z 377.1809 [M+H]⁺ (calcd. C₁₇H₂₆O₅ for 377.1811).

**Scabroside C (3)**. White powder; [α]D⁰²⁺ = −9.5 (c 0.30, H₂O); UV max MeOH (nm) (lg ε): 210 (2.72), 249 (2.20); IR (KBr): ν max cm⁻¹ 3424 (OH), 2935, 1638, 1449, 1381, 1324, 1294, 1165, 1130, 1077, 1037, 1013, 947, 634; ¹H- and ¹³C-NMR data: see Table 1; HR-ESI-MS (neg.) m/z: 413.1214 [M+Cl]⁻ (calcd. C₁₇H₂₆O₅Cl for 413.1214).

**Jataman J (4)**. White powder. ¹H-NMR (400 MHz, CD₃OD): δ 3.62 (1H, dd, J = 11.1, 7.5 Hz, H-1a), 3.71 (1H, dd, J = 11.1, 6.3 Hz, H-1b), 4.01 (2H, s, H-3), 3.11 (1H, td, J = 10.4, 7.8 Hz, H-5), 1.77 (1H, ddd, J = 13.3, 7.8, 1.9 Hz, H-6a), 2.23 (1H, m, H-6b), 3.85 (1H, dd, J = 5.3, 19.9 Hz, H-7), 2.23 (1H, m, H-9), 1.39 (3H, s, H-10), 5.08 (1H, s, H-11a), 5.27 (1H, s, H-11b); ¹³C-NMR (100 MHz, CD₃OD): δ 61.0 (t, C-1), 67.4 (t, C-3), 150.5 (s, C-4), 41.5 (d, C-5), 38.3 (t, C-6), 80.9 (d, C-7), 83.5 (s, C-8), 51.3 (d, C-9), 23.5 (q, C-10), 112.3 (t, C-11); FAB-MS (+): m/z: 225 [M+Na]⁺.

**Isopatrisascabroside I (5)**. White powder. ¹H-NMR (400 MHz, CD₃OD): δ 4.58 (1H, d, J = 12.1 Hz, H-1a), 4.28 (1H, dd, J = 12.1, 5.4 Hz, H-1b), 2.87 (1H, m, H-4), 2.81 (1H, m, H-5), 1.62 (1H, dt, J = 13.5, 6.6 Hz, H-6a), 1.88 (1H, m, H-6b), 3.80 (1H, m, H-7), 2.26 (1H, dd, J = 10.3, 5.3 Hz, H-9), 1.31 (3H, s, H-10), 1.09 (3H, d, J = 6.5 Hz, H-11), 4.35 (1H, d, J = 7.7 Hz, H-7'), 3.14 (1H, t, J = 8.2 Hz, H-2'), 2.39-3.34 (3H, m, H-3', 4', 5'), 3.80 (1H, d, J = 11.7 Hz, H-6a), 3.65 (1H, dd, J = 11.8, 5.1 Hz, H-6b); ¹³C-NMR (100 MHz, CD₃OD): δ 67.3 (t, C-1), 182.0 (s, C-3), 38.8 (d, C-4), 39.3 (d, C-5), 34.2 (t, C-6), 88.2 (d, C-7), 82.2 (s, C-8), 47.9 (d, C-9), 23.9 (q, C-10), 14.3 (q, C-11), 105.7 (d, C-1'), 76.2 (d, C-2'), 78.8 (d, C-3'), 72.4 (d, C-4'), 78.4 (d, C-5'), 63.2 (t, C-6').

### Table 1.

<table>
<thead>
<tr>
<th>Position</th>
<th>¹H-NMR δ (ppm)</th>
<th>¹³C-NMR δ (ppm)</th>
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<tr>
<td>1</td>
<td>67.2 (t) 4.52 (dd, 11.2, 2.1)</td>
<td>60.3 (t) 3.65 (dd, 12.4)</td>
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<tr>
<td>2</td>
<td>176.1 (s) 4.90 (dd, 11.2, 6.2)</td>
<td>103.4 (d) 3.71 (dd, 12.4, 6.0)</td>
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<td>3</td>
<td>74.4 (s) 5.16 (m)</td>
<td>34.8 (d) 35.7 (d)</td>
</tr>
<tr>
<td>4</td>
<td>46.0 (d) 1.64 (m)</td>
<td>29.8 (t) 1.29 (dd, 13.6, 11.3, 6.2)</td>
</tr>
<tr>
<td>5</td>
<td>34.4 (t) 2.00 (dd, 12.6, 9.0, 3.4)</td>
<td>2.22 (m) 1.91 (m)</td>
</tr>
<tr>
<td>6</td>
<td>88.1 (d) 5.75 (t) 2.22 (m)</td>
<td>41.9 (t) 1.55 (m)</td>
</tr>
<tr>
<td>7</td>
<td>82.1 (s) 18.6 (s)</td>
<td>84.1 (s)</td>
</tr>
<tr>
<td>8</td>
<td>44.9 (d)</td>
<td>4.81 (d) 1.94 (m)</td>
</tr>
<tr>
<td>9</td>
<td>22.6 (q) 1.91 (g)</td>
<td>18.8 (g) 1.28 (s)</td>
</tr>
<tr>
<td>10</td>
<td>23.5 (q) 1.34 (s)</td>
<td>55.1 (q) 3.27 (s)</td>
</tr>
<tr>
<td>OCH₃</td>
<td>105.0 (d) 4.36 (d, 7.8)</td>
<td>97.7 (d) 4.41 (d, 7.7)</td>
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<td>Glucose</td>
<td>75.4 (d) 3.16 (d, 8.3)</td>
<td>73.8 (d) 2.93 (dd, 8.4, 3.1)</td>
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<tr>
<td>1</td>
<td>78.1 (d) 3.22 (m)</td>
<td>76.6 (d) 3.12 (m)</td>
</tr>
<tr>
<td>2</td>
<td>71.5 (d) 3.10 (m)</td>
<td>70.3 (d) 3.00 (m)</td>
</tr>
<tr>
<td>3</td>
<td>77.8 (d) 3.13 (m)</td>
<td>76.7 (d) 3.03 (m)</td>
</tr>
<tr>
<td>4</td>
<td>62.6 (t) 3.66 (dd, 11.9, 5.3)</td>
<td>61.3 (t) 3.37 (dd, 11.5, 5.7)</td>
</tr>
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</table>

*Values may be interchangeable in the same column. *⁶ in 500 MHz.
Loganic Acid (6). White power. $^1$H-NMR (400 MHz, CD$_3$OD): $\delta$ 5.22 (1H, d, $J$ = 4.1 Hz, H-1), 7.29 (1H, s, H-3), 3.05 (1H, q, $J$ = 8.0 Hz, H-5), 1.62 (1H, dd, $J$ = 13.7, 5.9 Hz, H-6a), 2.18 (1H, dd, $J$ = 13.7, 7.8 Hz, H-6b), 3.99 (1H, t, $J$ = 5.1 Hz, H-7), 1.82 (1H, m, H-8), 1.97 (1H, td, $J$ = 9.0, 4.2 Hz, H-9), 1.04 (3H, d, $J$ = 6.8 Hz, H-10), 4.60 (1H, d, $J$ = 7.9 Hz, H-1), 3.15 (1H, d, $J$ = 8.5 Hz, H-2'), 3.32 (1H, m, H-3'), 3.21 (1H, m, H-4'), 3.33 (1H, m, H-5'), 3.61 (1H, dd, $J$ = 11.9, 5.4 Hz, H-6a), 3.84 (1H, d, $J$ = 11.9 Hz, H-6b); $^{13}$C-NMR (100 Hz, CD$_3$OD): $\delta$ 97.5 (d, C-1), 151.3 (d, C-3), 115.0 (s, C-4), 32.2 (d, C-5), 42.7 (t, C-6), 74.7 (d, C-7), 42.1 (d, C-8), 46.6 (d, C-9), 13.4 (q, C-10), 171.8 (s, C-11), 100.0 (d, C-1'), 75.1 (d, C-2'), 78.0 (d, C-3'), 71.6 (d, C-4'), 78.3 (d, C-5'), 62.7 (t, C-6'); ESI-MS (+) m/z 375 [M-H]$^+$.  

Results and Discussion

Scabroside A (1). Compound 1 obtained as white powder, has a molecular formula of C$_{17}$H$_{23}$O$_{11}$ based on HR-ESI-MS (neg.), showing a quasi-molecular ion peak at $m/z$ 413.1215 [M+Cl]$^-$ (C$_{16}$H$_{23}$O$_{10}$Cl, calcld. 413.1214). The IR spectrum indicated the presence of the hydroxy groups (3416 cm$^{-1}$) and a lactone carbonyl group (1728 cm$^{-1}$). The key HMBC and NOESY correlations of compounds 1-3.  

Scabroside B (2). Compound 2 obtained as white powder, has a molecular formula of C$_{17}$H$_{23}$O$_{11}$ based on HR-ESI-MS (neg.), showing a quasi-molecular ion peak at $m/z$ 377.1809 [M-H]$^+$ (C$_{17}$H$_{23}$O$_{11}$, calculated. 377.1811). The IR spectrum showed the hydroxyl absorption at 3420 cm$^{-1}$. The $^1$H-NMR spectrum (Table 1) exhibited signals for two tertiary methyl groups at $\delta$ 1.35 (s) and 1.34 (s), two methylene groups at $\delta$ 4.52 (dd, $J$ = 11.2, 2.1 Hz, H-1a), 4.90 (dd, $J$ = 11.2, 6.2 Hz, H-1b), 1.64 (m, H-6a), and 2.00 (ddd, $J$ = 12.6, 9.0, 3.4 Hz, H-6b), as well as three methine groups at $\delta$ 2.29 (dd, $J$ = 10.7, 5.9, 1.2 Hz), 2.78 (m), and 3.81 (dd, $J$ = 5.0, 4.1 Hz). The $^{13}$C-NMR (DEPT) spectrum (Table 1) showed 17 carbon signals including one methoxyl at $\delta$ 44.9 (C-6), 34.8 (C-9), 86.1 (C-7), two quaternary carbons at $\delta$ 74.4 (C-4) and 82.1 (C-8), and a lactone carbonyl carbon signal at $\delta$ 176.1 (C-3), as well as six carbon signals of one glucosyl moiety. The $^1$H-$^1$H COSY spectrum showed the connectivities of the proton coupling sequence for the C(1)-C(9)-C(5)-C(6)-C(7) fragment. The HMBC correlations (Figure 2) of H-1, H-5 with C-3, H-5 with C-4, -11, H-1, -6' with C-8, and H-7, -9 with C-10 were observed. Detailed analysis of the $^1$H-$^1$H and $^1$H-$^1$C correlations, exhibited in the $^1$H-$^1$H COSY, HMOC and HMBC spectra, allowed the establishment of an iridomyrmecin-type iridolactone structure for 1. The long range $^1$H-$^1$C correlations between the proton of GlcH-1 and C-7 confirmed that the glucosyl moiety was located at C-7 of the iridolactone. The anomeric proton of the glucosyl moiety signal appearing as a doublet at $\delta$ 4.36 (d, $J$ = 7.8 Hz) suggested a $\beta$-configured glucose unit. All the carbons of the glucosyl moiety were assigned through direct $^1$H-$^1$C correlations in the HMOC spectrum and were situated between $\delta$ 62.6 and 78.1 except for that at the anomeric position, which was assigned to the signal at $\delta$ 105.0.

The relative configuration of 1 was determined by the 2D-NOESY spectrum and NMR spectra. Comparison of the

Figure 2. The key HMBC and NOESY correlations of compounds 1-3.
study of the correlations of H-7, H-9 with C-8 in HMBC (Figure 2) led to the establishment of a cyclopenta [c] pyran-type iridoid structure for 2. The methoxyl proton signal was correlated to the C-3 signal in the HMBC spectrum, indicating that the methoxyl group was linked to C-3 of the iridoid. The HMBC correlations of Me-10 with C-8, and GlcH-1 with C-8, suggested that the 10-methyl and the glucosyl moiety were located at C-8 of the iridoid. The anomeric proton of the glucosyl moiety signal appearing as a doublet at δ 4.41 (d, J = 7.7 Hz) suggested a β-configured glucose unit. All the carbons of the glucosyl moiety were assigned through direct 1H-13C correlations in the HMBC spectrum and were situated between δ 61.3 and 76.7 except for that at the anomeric position, which was assigned to the signal at δ 97.7.

The relative configuration of 2 was determined by the 2D-NOESY spectrum, and based upon comparison of NMR data of 2 with those reported in the literature. According to the data in the literature, the chemical shift of C-9 at a relatively high field (δ 48.1) indicated that the glucosyl moiety at C-8 was α-oriented and the methyl group at C-8 was β-oriented.3-11 The NOESY correlations (Figure 2) between H-10 and H-9, H-10 and H-7, H-9 and H-5, H-5 and H-11, H-10 with H-6α [δ 1.29 (dd, J = 13.6, 11.3, 6.2 Hz)], H-OCH3 with H-6β [δ 1.91 (m), but no NOE SYG correlations between H-9 and H-4, H-9 and H-OCH3, indicated that H-3, H-5, H-7, H-9 and two methyl groups were in β-orientation, the methoxyl group and the glucosyl moiety were α-oriented. Therefore, the structure of 2 was deduced as an iridoid glucoside derivative, named scabroside B (Figure 1).

Scabroside C (3). Compound 3 obtained as white powder, has a molecular formula of C16H20O16 based on HR-ESI-MS (neg.), showing a quasi-molecular ion peak at m/z 413.1214 [M+Cl]−. The IR spectrum showed the hydroxyl absorption at 3424 cm−1. The 1H-13C NMR spectrum (Table 1) exhibited signals for one tertiary methyl signal at δ1 1.28 (s), two methylene signals at δ1 1.55 (m, H-6a), 2.18 (m, H-6b), and 3.90 (m, H-11) as well as six methine signals at δ1 1.91 (t, J = 3.8 Hz), 2.05 (dd, J = 4.8, 2.8 Hz), 2.19 (m), 3.91 (m), 5.05 (d, J = 3.1 Hz), and 5.48 (d, J = 2.8 Hz), respectively. The 13C-NMR (DEPT) spectrum (Table 1) showed 16 carbon signals, including one methyl signal at δc 18.8 (C-10), two methylene signals at δc 41.9 (C-6) and 71.6 (C-11), six methine signals at δc 30.6 (C-5), 43.1 (C-9), 48.0 (C-4), 79.8 (C-7), 90.3 (C-1), and 92.7 (C-3), and one quaternary signal at δc 84.1 (C-8), as well as six carbon signals of one glucosyl moiety. The 1H-13C COSY spectrum showed the connectivities of the proton coupling sequences for the C(1)-C(9)-C(5)-C(6)-C(7), C(5)-C(4)-C(3) and C(4)-C(11) fragments. In HMBC spectrum, correlations were observed between H-1 and C-3, H-5 and C-4, -11, H-6, -7, -10 and C-8, H-7, -10 and C-9. Further analysis of NMR data and the 1H-13C COSY and 13C-1H HMBC connectivities (Figure 2) revealed the presence of a cyclopenta [c] pyran-type iridoid skeleton. The long range 1H-13C correlation of GlcH-1 with C-11 suggested that the glucosyl moiety was located at C-11. The C(3)-atom was linked to C-8 forming an oxo bridge, based on the downfield-shift value of C-3, and the existence of HMBC between the 3-proton and the 8-carbon. The anomeric proton of the glucosyl moiety signal appearing as a doublet at δ 4.36 (d, J = 7.9 Hz) suggested a β-configured glucose unit. All the carbons of the glucosyl moiety in 3 were assigned through direct 1H-13C correlations in the HMBC spectrum and were situated between δ 61.7 and 76.8 except for that at the anomeric position, which was assigned to the signal at δ 103.5.

The relative configuration of 3 was determined by the 2D-NOESY spectrum, and by comparison of NMR data with those reported in the literature.3 The H-10 at C-8 was determined to be β-oriented on the basis of the C-9 chemical shift (δ 43.1), which is at relatively high field.3-11 Hence, the formation of intramolecular acetal of C-3 with C-8 confirmed the H-3 to be in β-orientation. The NOESY spectrum (Figure 2) exhibited correlations of H-10 with H-9, H-5 with H-9, H-9 with H-6a, and H-4 with H-6b, but there was no correlations of H-5 with H-1 and H-7, indicating the α-, α-, β-, α- and β-orientations of H-1, H-4, H-5, H-7 and H-9, respectively. Therefore, the structure of compound 3 was determined as shown in Figure 1, and named scabroside C.

Three known iridoids, jatamamin J (4),7 isopatriscabroside I (5),7 and loganic acid (6),8 were also isolated from the H2O-soluble fraction of the roots of this plant. Their structures were determined by spectral data and their comparison with literature values.

Acknowledgments. This work was financially supported by the Postgraduate Academic Innovation Foundation of Anhui University. The authors are grateful to the staffs of the analytical group at the Modern Experiment Technology Center, Anhui University and the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences for their measuring spectral data.

References