C_9-Iridoids from *Scrophularia buergeriana*

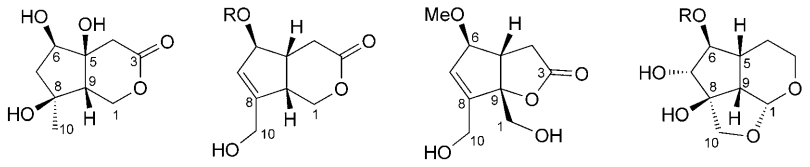
by **Shuang-Jun Lin**, **Chang-Heng Tan**, **Shan-Hao Jiang**, **Yi-Ming Li**, and **Da-Yuan Zhu***

State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, 201203, P. R. China
(phone: +86-21-50806728; fax: +86-21-50807088; e-mail: dyzhu@mail.shcnc.ac.cn)

Four new iridoids, buergerinins B–E (**1**–**4**), along with three known iridoids, were isolated from the roots of *Scrophularia buergeriana*. Their structures were identified on the basis of spectroscopic analysis.

Introduction. – The roots of the genus *Scrophularia* have been used in oriental medicine for the treatment of fever, swelling, constipation, neuritis, pharyngitis, and laryngitis [1]. Previous studies on this genus have resulted in some bioactive iridoids, phenylpropanoids, and their glycosides [2–8]. We have reported on the isolation, structure elucidation [9][10], as well as antioxidation [11], anti-inflammation, and anti-platelet-aggregation [12] effects of iridoid and phenylpropanoid glycosides from *S. ningpoensis*. Recently, the absolute configurations of buergerinins F and G [13], two iridoids with a novel skeleton, obtained from *S. buergeriana*, were derived through linear total syntheses [14].

In the present paper, we described the isolation and identification of four new iridoids from the titled plant, *i.e.*, buergerinins B–E (**1**–**4**), as well as of three known compounds: 7,8-didehydro-6β,10-dihydroxy-11-noriridomyrmecin (iridolactone; **5**) [15], ningpogenin [16], and pedicularis lactone [17].



1 **2** R = Me, **5** R = H, **3**, **4** R = Me, **6** R = H

Results and Discussion. – Buergerinin B (**1**), obtained as colorless needles, had the empirical molecular formula $C_9H_{14}O_5$ based on elemental analysis and EI-MS (*m*/*z* 203 ($[M+1]^+$). An IR band at 1737 cm^{-1}, in combination with a ^{13}C-NMR resonance at δ(C) 172.2, revealed the presence of a δ-lactone [18]. Detailed analyses of the ^1H-,

and ^{13}C-, and 2D-NMR data (see *Exper. Part*) established that buergerinin B corresponds to 5β-hydroxyjioglutolide[1]).

The ^{13}C-NMR (DEPT) spectrum displayed nine carbon signals: one Me, three CH_2, and two CH groups, and three C-atoms. In the ^{1}H-NMR spectrum, the signals at δ(H) 4.65 (*s*), 4.98 (*d*, J=6.4 Hz), and 4.93 (*s*) were disclosed to be due to protic functions, as inferred from deuterium-exchange experiments. The other signals were assigned to four isolated spin systems: O–CH_2–CH (δ(H) 2.17 (*dd*, J=9.4, 6.1), 4.02 (*dd*, J=11.5, 9.4), and 4.28 (*dd*, J=11.5, 6.1)), CH_2–CH(OH) (δ(H) 1.73 (*dd*, J=12.1, 8.9), 1.89 (*dd*, J=12.1, 5.5), 3.49 (*ddd*, J=8.9, 6.4, 5.5), and 4.98 (*d*, J=6.4)), an *AB*-type CH_2 (δ(H) 2.66, 2.43 (2*d*, J=14.7)), and a Me group (δ(H) 1.04 (*s*)). These data pointed to a structure related to jioglutolide, a C_9-iridoid with a cyclopenta[*c*]pyran moiety [18]. In contrast with jioglutolide, compound **1** lacked the ^{1}H- and ^{13}C-NMR signals for H–C(5), which were replaced by that of a quaternary C-atom, indicating a 5-hydroxylated jioglutolide. This assumption was further substantiated by HMQC and HMBC experiments (*Figure*).

The relative configuration of **1** was derived based on the NOESY correlations 5-OH/6-OH, 6-OH/8-OH, and 8-OH/H–C(9), which corroborated the *cis*-orientation of the three OH groups and H–C(9)[1]).

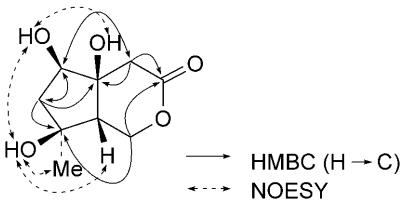


Figure. *Key 2D-NMR correlations for* **1**

Buergerinin C (**2**), a colorless oil, also incorporated a δ-lactone, as inferred from an IR absorption at 1736 cm^{-1} and the NMR data. The NMR data suggested that **2** was a 6-*O*-methylirdolactone, in agreement with the ESI-MS peaks at *m/z* 199 ($[M+1]^+$) and 211 ($[M+Na]^+$). The analysis of the ^{1}H- and ^{13}C-NMR data indicated a lactone, two CH_2, and three CH groups, a trisubstituted C=C bond, as well as a CH_2OH and a MeO function. All these signals were nearly identical with those of iridolactone (**5**), except for an additional MeO group (δ(H) 3.37 (*s*, 3 H); δ(C) 55.4) and minor differences at C(6) ($\Delta\delta$=+9.6), C(5) (−2.6), and C(7) (−2.8) for **2** relative to **5**. Thus, the structure of **2** was identified as 6-*O*-methyliridolactone.

The molecular formula $C_{10}H_{14}O_5$ was established for buergerinin D (**3**) by the ESI-MS signals at *m/z* 237 ($[M+Na]^+$) and 213 ($[M-H]^-$), revealing one more O-atom than in **2**. Besides a MeO group [δ(H) 3.39 (*s*), δ(C) 57.2], its ^{1}H- and ^{13}C-NMR spectra showed nine ^{13}C- and ten ^{1}H-NMR signals. The ^{1}H,^{1}H-COSY cross-peaks indicated a fragment of type −OC(O)CH_2CH(C)CH(O)CH=C, suggesting the same five-membered *B*-ring as in **2**. A strong IR band at 1755 cm^{-1}, and a ^2J value of 18.3 Hz for CH_2(4) in the ^{1}H-NMR spectrum indicated a pentacyclic lactone *A*-ring. The above evi-

[1]) Arbitrary atom numbering; for systematic names, see the *Exper. Part*.

dences suggested that **3** had a ring-contracted structure with a five-membered lactone ring *A* and an exocyclic hydroxymethyl group. With the exception of C(5) ($\Delta\delta = -4.2$), C(6) (+7.8), and C(7) (−2.9), all other resonances of **3** were basically isochronic with those of rehmaglutin C [19]. From these data, the structure of **3** was, thus, established as 6-*O*-methylrehmaglutin C.

Buergerinin E (**4**) had the empirical molecular formula $C_{10}H_{16}O_5$, as deduced by HR-ESI-MS (*m*/*z* 239.0891 ($[M+Na]^+$). The ^{13}C-NMR spectrum displayed one MeO, three CH_2, and five CH groups, as well as one quaternary C-atom. This suggested a tricycle C_9-iridoid, just like in the case of rehmaglutin A (**6**) [20]. The difference in their ^{13}C-NMR spectra was that **4** displayed a MeO signal, and a downfield resonance for C(6) ($\Delta\delta$ +7.7), and upfield signals for C(5) (−2.7) and C(7) (−1.8). Thus, the structure of **4** was identified as 6-*O*-methylrehmaglutin A. Considering the highly similar structures and identical signs of optical rotations ($[\alpha]_D^{19} = +43.6$ for **6** *vs.* $[\alpha]_D^{17} = 21.2$ for **4**), the absolute configuration of **4** was suggested to be the same as in **6**, *i.e.*, (1*R*,5*R*,6*S*,7*R*,8*S*,9*S*) [20].

Experimental Part

General. Column chromatography (CC): silica gel (200–300 or 400 mesh; *Qingdao Haiyang*, *Co*., China). M.p.: *Fisher-John* apparatus; uncorrected. Optical rotations: *Perkin-Elmer-341* polarimeter. IR Spectra: *Nicolet Magna-750* FT-IR spectrometer, with KBr pellets; in cm^{-1}. NMR Spectra: *Bruker DRX-400* instrument, at 400 (^{1}H) or 100 MHz (^{13}C); δ in ppm rel. to Me_4Si, *J* in Hz. ESI- and HR-ESI-MS: *LCQ-Deca* and *Q-Tof-Ultima* mass spectrometers, resp.; in *m*/*z* (rel. %).

Plant Material. The roots of *S. buergeriana* MIQ. (Scrophulariaceae) were collected in Chang Bai Mountains, Jilin Province, China, in 1996, and identified by Prof. *Zhong-Kai Yan*, *TCM Identification Committee of the China Association of Chinese Medicine*. A specimen (96–09) was deposited at the Herbarium of the Shanghai Institute of Materia Medica, Shanghai, P. R. China.

Extraction and Isolation: The air-dried roots (10 kg) of *S. buergeriana* were ground and extracted with 95% EtOH at r.t. The residue was taken up in H_2O and extracted with AcOEt and BuOH. After being defatted with petroleum ether (PE), the AcOEt extract was subjected to CC (SiO_2; gradient of 100, 50, 20, and 0% PE in AcOEt, then acetone): five fractions (*Fr. 1–5*). *Fr. 3* (20 g) was chromatographed (SiO_2; gradient of 0, 1, 2, 5, 10, and 20% MeOH in $CHCl_3$): six fractions (*Fr. 3.1–3.6*). *Fr. 3.1* was evaporated, and the residue was crystallized from acetone, which yielded colorless needles of **2** (85 mg). *Fr. 3.2* was repeatedly purified by CC (SiO_2; hexane/acetone 5:1) to furnish ningpogenin (3.4 g), iridolactone (**5**; 1.1 g), and pedicularis lactone (734 mg). Compounds **3** (12 mg) and **4** (9 mg) were isolated from *Fr. 3.3* by repeated CC (SiO_2; heaxane/acetone 5:1 → 2:1). Compound **1** (123 mg) was obtained from *Fr. 3.4* by repeated CC (SiO_2; heaxane/acetone 2:1, then AcOEt/acetone 2:1).

Buergerinin B (=*(4a*S*,5*R*,7*S*,7a*R*)-Hexahydro-4a,5,7-trihydroxy-7-methylcyclopenta[*c*]pyran-3(1*H*)-one*; **1**). Colorless needles (acetone). M.p. 159–160°. $[\alpha]_D^{23} = -23.1$ (*c* = 0.945, MeOH). IR (KBr): 3432, 1737, 1437, 1269, 1022. ^{1}H-NMR (400 MHz, (D_6)DMSO)[1]): 4.98 (*d*, *J* = 6.4, 6-OH); 4.93 (*s*, 8-OH); 4.65 (*s*, 5-OH); 4.28 (*dd*, *J*=11.5, 6.1, H_β–C(1)); 4.02 (*dd*, *J* = 11.5, 9.4, H_α–C(1)); 3.49 (*ddd*, *J* = 8.9, 6.4, 5.5, H–C(6)); 2.66 (*d*, *J* = 14.7, H_β–C(4)); 2.43 (*d*, *J* = 14.7, H_α–C(4)); 2.17 (*dd*, *J* = 9.4, 6.1, H–C(9)); 1.89 (*dd*, *J* = 12.1, 5.5, H_β–C(7)); 1.73 (*dd*, *J* = 12.1, 8.9, H_α–C(7)); 1.04 (*s*, Me(10)). ^{13}C-NMR (100 MHz, (D_6)DMSO)[1]): 172.2 (*s*, C(3)); 78.5 (*s*, C(5)); 75.3 (*d*, C(6)); 74.5 (*s*, C(8)); 66.0 (*t*, C(1)); 56.8 (*d*, C(9)); 48.2 (*t*, C(7)); 41.7 (*t*, C(4)); 24.9 (*q*, C(10)). EI-MS: 203 (5, $[M+1]^+$), 184 (12, $[M - H_2O]^+$), 166 (20), 140 (72), 126 (100). ESI-MS (pos./neg.): 225 ($[M+Na]^+$), 427 ($[2M+Na]^+$), 201 ($[M-H]^-$), 403 ($[2M-H]^-$). Anal. calc. for $C_9H_{14}O_5$: C 53.46, H 6.98, O 39.56; found: C 53.36, H 6.98, O 39.66.

Buergerinin C (=*(4a*R,5*S,7a*S)-4,4a,5,7a-Tetrahydro-7-(hydroxymethyl)-5-methoxycyclopenta[*c*]-pyran-3(1*H*)-one*; **2**). Colorless oil. $[\alpha]_D^{23} = -6.8$ (*c* = 1.894, MeOH). IR (KBr): 3412, 2900, 1736, 1388,

1243, 1080. ^{1}H-NMR (400 MHz, (D_6)DMSO)[1]): 5.75 (br. *s*, H–C(7)); 4.98 (*s*, 10-OH); 4.32 (*dd*, *J* = 11.6, 3.9, H_β–C(1)); 4.17 (*dd*, *J* = 11.6, 3.8, H_α–C(1)); 4.08, 4.07 (2 br. *d*, *J* = 14.2 each, CH_2(10)); 4.04 (br. *s*, H–C(6)); 3.39 (*m*, H–C(9)); 3.37 (*s*, MeO); 2.84 (*dd*, *J* = 14.9, 7.5, H_β–C(4)); 2.65 (*m*, H–C(5)); 2.42 (*dd*, *J* = 14.9, 4.3, H_α–C(4)). ^{13}C-NMR (100 MHz, (D_6)DMSO)[1]): 172.5 (*s*, C(3)); 148.4 (*s*, C(8)); 126.4 (*d*, C(7)); 91.0 (*d*, C(6)); 67.1 (*t*, C(1)); 58.5 (*t*, C(10)); 55.4 (*q*, MeO); 43.8 (*d*, C(9)); 40.8 (*d*, C(5)); 34.4 (*t*, C(4)). EI-MS: 199 (7, $[M+H]^+$), 180 (26), 167 (100, $[M-MeO]^+$), 137 (23), 109 (60). ESI-MS: 199 ($[M+H]^+$), 221 ($[M+Na]^+$). HR-ESI-MS: 221.0787 ($[M+Na]^+$, $C_{10}H_{14}NaO_4^+$; calc. 221.0790).

Buergerinin D (= *(3a*S*,4*R*,6a*S*)-3,3a,4,6a-Tetrahydro-6,6a-bis(hydroxymethyl)-4-methoxy-2*H*-cyclopenta[*b*]furan-2-one*; **3**). Colorless oil. $[\alpha]_D^{17} = -28.1$ (*c* = 0.615, acetone). IR (KBr): 3441, 1755, 1737, 1664. ^{1}H-NMR (400 MHz, $CDCl_3$)[1]): 6.10 (br. *s*, H–C(7)); 4.29 (br. *s*, CH_2(10)); 4.12 (br. *s*, H–C(6)); 3.90 (*d*, *J* = 11.9, H_β–C(1)); 3.80 (*d*, *J* = 11.9 Hz, H_α–C(1)); 3.39 (*s*, MeO); 2.98 (*dd*, *J* = 18.4, 11.1, H_β–C(4)); 2.81 (*m*, H–C(5)); 2.45 (*dd*, *J* = 18.3, 5.2, H_α–C(4)). ^{13}C-NMR ($CHCl_3$)[1]): 176.3 (*s*, C(3)); 147.2 (*s*, C(8)); 131.8 (*t*, C(7)); 99.6 (*s*, C(9)); 88.8 (*d*, C(6)); 65.5 (*t*, C(10)); 58.5 (*t*, C(1)); 57.2 (*q*, MeO); 45.8 (*d*, C(5)); 34.5 (*t*, C(4)). EI-MS: 213 (4, $[M-H]^+$), 196 (16, $[M-H_2O]^+$), 183 (96, $[M-MeO]^+$). ESI-MS (pos./neg.): 237 ($[M+Na]^+$), 213 ($[M-H]^-$). HR-ESI-MS: 237.0734 ($[M+Na]^+$, $C_{10}H_{14}NaO_5^+$; calc. 237.0739).

Buergerinin E (= *(2a*S*,3*R*,4*S*,4a*R*,7a*R*,7b*S*)-Hexahydro-4-methoxy-2*H*-1,7-dioxacyclopent[*cd*]indene-2a,3(3*H*)-diol*; **4**). Colorless oil. $[\alpha]_D^{17} = 21.2$ (*c* = 0.617, acetone). IR (KBr): 3360 (sh), 2920, 1647, 1148, 1039. ^{1}H-NMR (400 MHz, $CDCl_3$)[1]): 5.32 (*d*, *J* = 4.7, H–C(1)); 4.43 (*d*, *J* = 10.1, H_β–C(10)); 4.03 (*d*, *J* = 9.0, H–C(7)); 3.92 (*td*, *J* = 12.2, 2.4, H_β–C(3)); 3.59 (*ddd*, *J* = 11.8, 5.0, 2.2, H_α–C(3)); 3.54 (*s*, MeO); 3.51 (br. *d*, *J* = 9.2, H–C(6)); 3.37 (*d*, *J* = 10.0, H_α–C(10)); 2.21 (br. *s*, H–C(5)); 2.16 (br. *d*, *J* = 5.6, H–C(9)); 1.77 (*tt*, *J* = 14.0, 2.5, H_α–C(4)); 1.59 (br. *d*, *J* = 14.1, H_β–C(4)). ^{13}C-NMR (100 MHz, $CDCl_3$): 99.8 (*d*, C(1)); 84.4 (*s*, C(8)); 83.2 (*d*, C(7)); 83.1 (*d*, C(6)); 70.1 (*t*, C(10)); 59.2(*t*, MeO); 55.9 (*t*, C(3)); 43.1 (*d*, C(9)); 32.2 (*d*, C(5)); 21.7 (*t*, C(4)). EI-MS: 217 (5), 216 (4, M^+), 215 (13), 199 (18), 184 (28), 168 (47), 167 (50), 166 (58), 137 (56), 102 (100). ESI-MS: 239 ($[M+Na]^+$). HR-ESI-MS: 239.0891 ($[M+Na]^+$, $C_{10}H_{16}NaO_5^+$; calc. 239.0895).

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