

# Indole Alkaloids and Other Constituents of *Rauwolfia serpentina*

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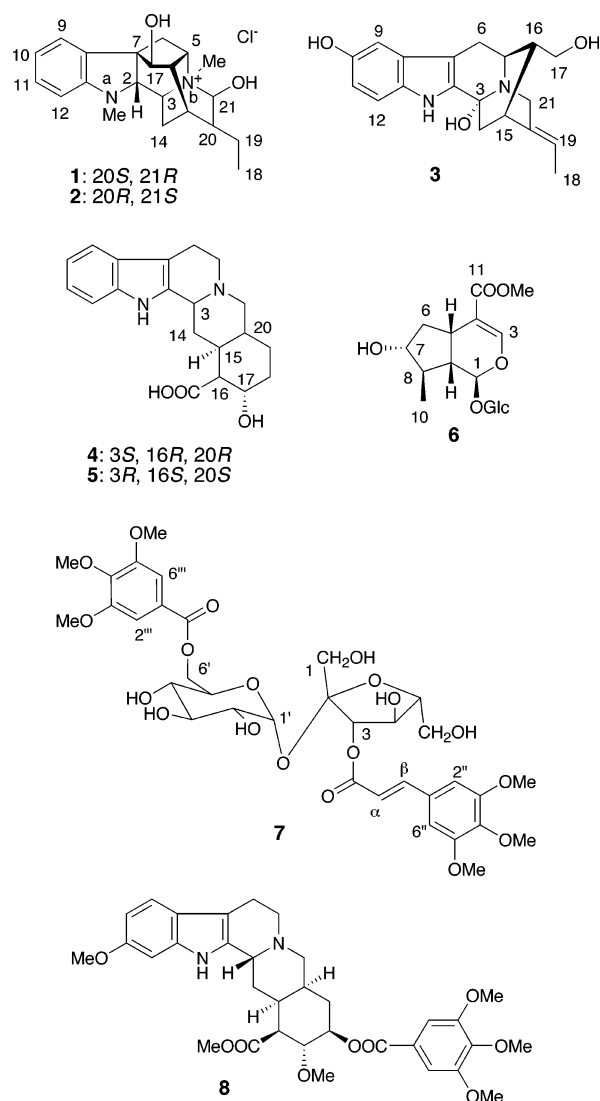
From the dried roots of *Rauwolfia serpentina* were isolated five new indole alkaloids, *N*<sub>1</sub>-methylajmaline (1), *N*<sub>1</sub>-methylisoajmaline (2), 3-hydroxysarpagine (3), yohimbic acid (4), isorauhimbinic acid (5), a new iridoid glucoside, 7-epiloganin (6), and a new sucrose derivative, 6'-*O*-(3,4,5-trimethoxybenzoyl)glomeratose A (7), together with 20 known compounds. The structures of the new compounds were determined by spectroscopic and chemical means. The inhibitory activities of the selected alkaloids on topoisomerase I and II and their cytotoxicity against the human promyelocytic leukemia (HL-60) cell lines were assessed.

*Rauwolfia serpentina* Benth., belonging to the Apocynaceae, grows in tropical and subtropical forests. In India, this plant has been used for the treatment of snake bites, feverish illnesses, and insanity for about 3000 years.<sup>1</sup> The biological-therapeutic significance of this plant has stimulated intensive studies, and many pharmacologically important alkaloids, such as reserpine with sedative and antihypertensive activities and ajmaline with antiarrhythmic activity, were isolated.<sup>2</sup> Because of an interest in indole alkaloids and related glycosides, alkaloidal and glycosidal fractions of *R. serpentina* were re-examined and the new indole alkaloids 1–5 and the new glycosides 6 and 7, together with 20 known compounds, were isolated. Herein, the isolation and structure elucidation of the new compounds and the inhibitory activities of the isolated alkaloids on topoisomerase I and II and their cytotoxicity against the HL-60 cancer cell lines are reported.

## Results and Discussion

The dried roots of *R. serpentina* were extracted with hot MeOH. The MeOH extract was successively partitioned between H<sub>2</sub>O and CHCl<sub>3</sub> and between H<sub>2</sub>O and *n*-BuOH. The *n*-BuOH layer was subjected to ODS column chromatography with H<sub>2</sub>O and MeOH and then purified by a combination of chromatographic procedures, affording the new alkaloids 1–5 and the new glycosides 6 and 7, along with 12 known alkaloids, ajmaline,<sup>3</sup> isoajmaline,<sup>3</sup> norajmaline,<sup>4</sup> (+)-tetraphyllicine,<sup>5</sup> raucaffricine,<sup>6</sup> normacusine B,<sup>7</sup> geissoschizol,<sup>8</sup> rhazimanine,<sup>9</sup> yohimbine,<sup>10</sup> isorauhimbine,<sup>10</sup> 18-hydroxyepialloyohimbine,<sup>10</sup> and methyl reserpate,<sup>10</sup> and five known glycosides, loganic acid,<sup>11</sup> 7-deoxyloganic acid,<sup>12</sup> secoxyloganin,<sup>13</sup> (+)-isolaricresinol 3a-*O*-β-D-glucopyranoside,<sup>14</sup> and glomeratose A.<sup>15</sup> The CHCl<sub>3</sub> layer was also purified to afford six known alkaloids, namely, reserpine (8),<sup>16</sup> reserpinine,<sup>16</sup> perakine,<sup>17</sup> yohimbine, isorauhimbine, and ajmaline, and a new glycoside, 7.

Alkaloid 1 was obtained as an amorphous powder and analyzed for C<sub>21</sub>H<sub>29</sub>N<sub>2</sub>O<sub>2</sub> from its HRSIMS. It showed UV maxima at 205, 244, and 288 nm, and IR bands at 3358, 1607, 1475, and 1466 cm<sup>-1</sup>. Its <sup>1</sup>H NMR spectrum exhibited signals for an ethyl group at δ 1.07 (3H, t, *J* = 7.5 Hz) and



1.56–1.72 (2H, m), a singlet for an *N*-methyl group at δ 2.79, and signals for four aromatic protons at δ 6.76 (brd, *J* = 7.5 Hz), 6.82 (td, *J* = 7.5 and 1.0 Hz), 7.16 (td, *J* = 7.5 and 1.0 Hz), and 7.54 (dd, *J* = 7.5 and 1.0 Hz). These spectroscopic features were similar to those of ajmaline,<sup>3</sup> a major alkaloid of *R. serpentina*, except for an additional

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singlet for an *N*-methyl group at  $\delta$  3.20. The *N*-methyl signal at  $\delta$  3.20 showed HMBC correlations with C-3 ( $\delta$  54.2), C-5 ( $\delta$  64.6), and C-21 ( $\delta$  98.4) and a NOESY interaction with H-5 ( $\delta$  3.73). Alkaloid **2** was isomeric with **1** from its HRSIMS. Its spectral features were closely similar to those of isoajmaline with an additional quaternary *N*-methyl signal at  $\delta$  3.09, which showed the same HMBC and NOESY correlation peaks as **1**. These results suggested that **1** and **2** are *N*<sub>b</sub>-methylated derivatives of ajmaline and isoajmaline, respectively.

The configurations of **1** and **2** were inferred by comparison of their NMR spectra with those of ajmaline and isoajmaline. The *R* configuration of C-17 in both alkaloids was suggested by the H-17 signal appearing around  $\delta$  4.40 as a broad singlet and the values of the chemical shifts of C-2, C-7, C-8, C-9, and C-17.<sup>3,18</sup> The chemical shifts of C-3, C-5, C-14, C-19, and C-20 in **1** and **2** were similar to those of ajmaline and isoajmaline,<sup>3</sup> respectively, except that the signals for C-3 and C-5 were shifted downfield by *N*<sub>b</sub>-methylation in both alkaloids, suggesting a 20*S*, 21*R* configuration in **1** and a 20*R*, 21*S* configuration in **2**. Furthermore, the cross-peaks between H-5 and H-19, between H-16 and H-19, and between H-3 and H-20 in the NOESY spectrum of **1**, and the cross-peaks between H-5 and H-20, between H-16 and H-20, and between H-15 and H-19 in the NOESY spectrum of **2**, supported **1** and **2** as *N*<sub>b</sub>-methylajmaline and *N*<sub>b</sub>-methylisoajmaline, respectively. This conclusion was finally confirmed by the treatment of ajmaline and isoajmaline with methyl iodide to afford **1** and **2**, respectively.

A third alkaloid, **3**, C<sub>19</sub>H<sub>22</sub>N<sub>2</sub>O<sub>3</sub>, was obtained as an amorphous powder. Its UV maxima at 205, 224.5, and 278 nm were characteristic of an indole alkaloid. Its <sup>1</sup>H NMR spectrum showed signals for oxymethylene protons (H<sub>2</sub>-17) at  $\delta$  3.46 (dd, *J* = 10.5 and 8.5 Hz) and 3.52 (dd, *J* = 10.5 and 6.5 Hz), signals for an ethylidene group at  $\delta$  1.65 (brd, *J* = 7.0 Hz) and 5.49 (q, *J* = 7.0 Hz), and an aromatic AMX spin system at  $\delta$  6.67 (dd, *J* = 8.5 and 2.0 Hz), 6.78 (d, *J* = 2.0 Hz), and 7.17 (d, *J* = 8.5 Hz). These <sup>1</sup>H NMR spectroscopic features were closely comparable to those of sarpagine,<sup>19</sup> except for the absence of a H-3 signal. The geometrical isomerism of the ethylidene group was inferred as *E* from the NOESY correlations between H-15 and H<sub>3</sub>-18 and between H-19 and H-21. The *R* configuration at C-16 was deduced from the chemical shifts of C-6, C-14, and C-17, which were in good accord with those of normacusine B, but not those of 16-epinormacusine B,<sup>7</sup> and from the NOESY correlations between H-6 and H-16 and between H-15 and H-17. The signal at  $\delta$  6.78 showed an HMBC correlation with C-7 and a NOESY correlation with H<sub>2</sub>-6 ( $\delta$  2.64 and 3.08), indicating a hydroxyl group at C-10. Furthermore, in its <sup>1</sup>H NMR spectrum, the signal for H-3 disappeared and in the <sup>13</sup>C NMR spectrum the signal for C-3 was observed at  $\delta$  84.7 as a quaternary carbon signal, suggesting an additional hydroxyl group at C-3. Accordingly, **3** was designated as 3-hydroxysarpagine.

Alkaloids **4** and **5** were recognized as isomers from their HRSIMS. Their NMR spectroscopic features were closely comparable to those of yohimbine and isorauhimbine, respectively, except for the absence of a methoxyl signal. The alkaloids **4** and **5** were, therefore, assumed to be demethylated derivatives of yohimbine and isorauhimbine.<sup>10</sup> The stereochemistry of **4** and **5** was confirmed by detailed inspection of their NMR spectral data. The chemical shifts of C-3, C-5, C-6, C-14, C-15, C-16, C-20, and C-21 indicated C/*D* *trans* and D/*E* *trans* ring junctions (normal configuration) for **4** and C/*D* *cis* and D/*E* *cis* (epiallo

configuration) for **5**.<sup>10</sup> This was supported by the NOESY correlations (see Experimental Section). The coupling constants (**4**: *J*<sub>15,16</sub> = 11.0 Hz, *J*<sub>16,17</sub> = 2.0 Hz; **5**: *J*<sub>15,16</sub> = 4.0 Hz, *J*<sub>16,17</sub> = 11.0 Hz) indicated the configurations at C-16 and C-17 to be *R*, *S* in **4** and *S*, *S* in **5**. Accordingly, alkaloids **4** and **5** were presumed to be yohimbinic acid and isorauhimbinic acid, respectively. Finally, the structures of alkaloids **4** and **5** were confirmed by the methylation of **4** and **5** to afford yohimbine and isorauhimbine, respectively. Although the syntheses of **4** and **5** from yohimbine and isorauhimbine have been reported,<sup>20</sup> this is the first report of the isolation of these compounds as natural products.

Glucoside **6**, C<sub>17</sub>H<sub>26</sub>O<sub>10</sub>, was obtained as an amorphous powder. It showed a UV maximum at 235 nm, IR absorptions at 1695 and 1636 cm<sup>-1</sup>, and a <sup>1</sup>H NMR signal for an olefinic proton at  $\delta$  7.41 (d, *J* = 1.5 Hz), indicating an iridoid enol ether system conjugated with a carbonyl group.<sup>21</sup> Its <sup>1</sup>H NMR spectrum also exhibited a doublet for an acetal proton at  $\delta$  5.33 (*J* = 5.0 Hz), a doublet for an anomeric proton at  $\delta$  4.65 (*J* = 7.5 Hz), and a doublet for a secondary methyl group at  $\delta$  1.13 (*J* = 7.0 Hz). These spectroscopic data implied that **6** is loganin<sup>21</sup> or its stereoisomer. When its NMR spectra were compared with those of loganic acid isolated from this plant, there were differences in the coupling constants between H-6 and H-7 and between H-7 and H-8 (**6**: *J*<sub>6,7</sub> = 8.0, 8.0 Hz, *J*<sub>7,8</sub> = 0 Hz; loganic acid: *J*<sub>6,7</sub> = 4.5, 1.0 Hz, *J*<sub>7,8</sub> = 4.5 Hz), whereas *J*<sub>1,9</sub> (**6**: 5.0 Hz; loganic acid: 4.5 Hz), *J*<sub>5,9</sub> (**6**: 8.0 Hz; loganic acid: 9.0 Hz), and *J*<sub>8,9</sub> (**6**: 8.0 Hz; loganic acid: 9.0 Hz) were nearly identical. Compound **6** also differed from loganic acid in the chemical shifts of C-7, C-8, and C-10.<sup>11</sup> These spectroscopic features of **6** matched those of 7-epiloganin, which has been derived from swertiaside.<sup>21</sup> This is the first isolation of 7-epiloganin from a natural source.

Compound **7** was isolated as an amorphous powder and analyzed for C<sub>34</sub>H<sub>44</sub>O<sub>19</sub> from its HRSIMS. Its spectroscopic features were analogous to those of glomeratose A.<sup>15</sup> Acid hydrolysis of **7** liberated D-glucose and D-fructose, which were identified by GLC analysis of their thiazolidine derivatives.<sup>22</sup> Its <sup>1</sup>H NMR spectrum showed signals for a *trans*-3,4,5-trimethoxycinnamoyl group [ $\delta$  3.78 (3H, s, OMe), 3.86 (6H, s, 2 × OMe), 6.55 (1H, d, *J* = 16.0 Hz, H- $\alpha$ ), 6.95 (2H, s, 2 × ArH), 7.71 (1H, d, *J* = 16.0 Hz, H- $\beta$ )], signals for an  $\alpha$ -fructofuranosyl unit [ $\delta$  3.60 (1H, d, *J* = 12.0 Hz), 3.66 (1H, d, *J* = 12.0 Hz), 3.69 (1H, dd, *J* = 12.0, 3.0 Hz), 3.76 (1H, dd, *J* = 12.0, 6.0 Hz), 3.90 (1H, ddd, *J* = 8.0, 6.0, 3.0 Hz), 4.32 (1H, t, *J* = 8.0 Hz), 5.47 (1H, d, *J* = 8.0 Hz)], and signals for an  $\alpha$ -glucopyranosyl unit [ $\delta$  3.43 (1H, t, *J* = 10.0 Hz), 3.46 (1H, dd, *J* = 10.0, 3.5 Hz), 3.69 (1H, t, *J* = 10.0 Hz), 4.26 (1H, ddd, *J* = 10.0, 5.0, 2.0 Hz), 4.46 (1H, dd, *J* = 12.0, 5.0 Hz), 4.73 (1H, dd, *J* = 12.0, 2.0 Hz), 5.50 (1H, d, *J* = 3.5 Hz)]. The HMBC correlations between H-1 of the glucosyl unit and C-2 of the fructosyl unit and between H-3 of the fructosyl unit and carbonyl carbon ( $\delta$  167.7), which correlated with H- $\alpha$ , demonstrated a glomeratose A moiety as the basic skeleton. Furthermore, the <sup>1</sup>H NMR spectroscopic data of **7** showed a singlet for two aromatic protons at  $\delta$  7.32 and two singlets for three methoxyl groups at  $\delta$  3.81 (3H) and 3.85 (6H). The NOESY correlation between the aromatic protons ( $\delta$  7.32) and the methoxyl signal ( $\delta$  3.85) and the HMBC correlation between the aromatic proton ( $\delta$  7.32) and a carbonyl carbon signal ( $\delta$  167.6) indicated a 3,4,5-trimethoxybenzoyl group in **7**. Attachment of the 3,4,5-trimethoxybenzoyl group at the hydroxyl group at C-6 of the glucosyl unit was shown by the HMBC correlation between H<sub>2</sub>-6 of the glucosyl unit





**Table 3.** IC<sub>50</sub> Values of Purified Compounds for the Activities of Human DNA Topoisomerase I and II (topo I and II) and Human Cancer Cell Growth, Respectively<sup>a</sup>

compound	4	5	8	camptothecin	etoposide	DTA <sup>b</sup>
enzyme (IC <sub>50</sub> values, $\mu$ M)						
human topo I	30	40	160	20	> 1000	> 1000
human topo II	125	175	20	> 1000	40	38
cancer cells (IC <sub>50</sub> values, $\mu$ M)						
HL-60	84	125	67	130	150	64

<sup>a</sup> In the enzyme-inhibition assays, compounds were incubated with each enzyme (2 units). The enzymatic activity was measured as described in the Experimental Section. The cell survival rate was determined by a MTT assay. DNA topoisomerase activity and cell viability in the absence of the compound were taken as 100%. <sup>b</sup> DTA: dehydrotrametennoic acid.<sup>23</sup>

extracts were evaporated in vacuo. A portion (35 g) of the residue (105 g) was resuspended in H<sub>2</sub>O and extracted successively with CHCl<sub>3</sub> and *n*-BuOH. The residue from the CHCl<sub>3</sub> layers (4.9 g) was subjected to normal-phase MPLC. Elution with CHCl<sub>3</sub>–MeOH mixtures of indicated MeOH content gave six fractions, 1 (0%, 885 mg), 2 (0–5%, 239 mg), 3 (5%, 491 mg), 4 (5%, 593 mg), 5 (10%, 606 mg), and 6 (15%, 147 mg). Fraction 1 was submitted to normal-phase MPLC eluted with CHCl<sub>3</sub> to afford reserpine (70.2 mg). Fraction 2 was purified by preparative TLC (CHCl<sub>3</sub>–MeOH, 97:3) to yield **8** (34.4 mg). Fractions 3–6 were further purified by a combination of normal-phase MPLC (CHCl<sub>3</sub>–MeOH, 98:2–85:15) and preparative TLC (CHCl<sub>3</sub>–MeOH–NH<sub>4</sub>OH, 95:4.5:0.5, 90:9:1, 85:15:1.5, 80:20:2; CHCl<sub>3</sub>–acetone–Et<sub>2</sub>NH, 6:3:1), respectively. Fraction 3 yielded perakine (14.1 mg), ajmaline (13.8 mg), and yohimbine (11.4 mg); fraction 4, ajmaline (180.7 mg) and yohimbine (6.1 mg); fraction 5, **7** (47.0 mg) and ajmaline (227.8 mg); fraction 6, ajmaline (35.0 mg) and isorauhimbine (14.1 mg). The residue from the *n*-BuOH layers (7.5 g) was fractionated on reversed-phase MPLC. Elution with H<sub>2</sub>O–MeOH mixtures of indicated MeOH content gave five fractions, 1 (5%, 3.24 g), 2 (20%, 1.55 g), 3 (30–40%, 1.30 g), 4 (50%, 461 mg), and 5 (100%, 593 mg). Fraction 1 was submitted to normal-phase MPLC eluted with MeOH–CHCl<sub>3</sub> (2–10%) and was further purified by preparative TLC (CHCl<sub>3</sub>–MeOH–NH<sub>4</sub>OH, 70:30:3, CHCl<sub>3</sub>–MeOH–NH<sub>4</sub>OH, 90:9:1, CHCl<sub>3</sub>–MeOH, 8:2) to afford **1** (54.0 mg), **2** (50.6 mg), **3** (94.9 mg), **6** (3.9 mg), ajmaline (266 mg), isoajmaline (3.5 mg), normacusine B (8.1 mg), loganic acid (50.5 mg), norajmaline (3.9 mg), (+)-tetraphyllicine (9.4 mg), and rhazimanine (2.8 mg). Fraction 2 was submitted to column chromatography on DIAION HP-20 with MeOH–H<sub>2</sub>O and normal-phase MPLC with MeOH–CHCl<sub>3</sub> and was further purified by preparative TLC (CHCl<sub>3</sub>–MeOH–NH<sub>4</sub>OH, 70:30:3, CHCl<sub>3</sub>–MeOH–NH<sub>4</sub>OH, 90:9:1, CHCl<sub>3</sub>–MeOH, 8:2, C<sub>6</sub>H<sub>6</sub>–AcOEt–Et<sub>2</sub>NH, 7:2:1) to yield **1** (5.4 mg), **6** (5.9 mg), ajmaline (333 mg), isoajmaline (4.2 mg), yohimbine (25.4 mg), loganic acid (337 mg), geissoschizol (4.0 mg), and rhazimanine (7.4 mg). Fraction 3 was submitted to normal-phase MPLC eluted with MeOH–CHCl<sub>3</sub> and was further purified by preparative TLC (CHCl<sub>3</sub>–MeOH–NH<sub>4</sub>OH, 90:9:1, CHCl<sub>3</sub>–MeOH, 8:2, CHCl<sub>3</sub>–MeOH, 7:3, CHCl<sub>3</sub>–MeOH, 85:15, C<sub>6</sub>H<sub>6</sub>–AcOEt–Et<sub>2</sub>NH, 7:2:1) and preparative HPLC (MeOH–H<sub>2</sub>O, 65:35) to afford **4** (10.7 mg), **5** (48.4 mg), ajmaline (12.6 mg), yohimbine (32.2 mg), isorauhimbine (39.5 mg), 18-hydroxyepialloyohimbine (27.1 mg), glomeratose A (9.6 mg), geissoschizol (6.0 mg), 7-deoxyloganic acid (6.9 mg), secoxyloganin (13.5 mg), and (+)-isolariciresinol 3a-*O*- $\beta$ -D-glucopyranoside (12.8 mg). Fraction 4 was submitted to normal-phase MPLC eluted with MeOH–CHCl<sub>3</sub> and was further purified by preparative TLC (CHCl<sub>3</sub>–MeOH–NH<sub>4</sub>OH, 70:30:3, CHCl<sub>3</sub>–MeOH–NH<sub>4</sub>OH, 90:9:1, CHCl<sub>3</sub>–MeOH, 8:2, CHCl<sub>3</sub>–MeOH, 85:15, C<sub>6</sub>H<sub>6</sub>–AcOEt–Et<sub>2</sub>NH, 7:2:1) to afford **7** (48.9 mg), yohimbine (12.6 mg), isorauhimbine (42.0 mg), raucaffricine (6.4 mg), and methyl reserpate (7.2 mg). A part of the residue (107 mg) of fraction 5 was purified by preparative TLC (CHCl<sub>3</sub>–MeOH–NH<sub>4</sub>OH, 90:9:1) to afford isorauhimbine (32.4 mg).

**N<sub>b</sub>-Methylajmaline (1):** white powder; [ $\alpha$ ]<sub>D</sub><sup>25</sup> +113° (c 1.1, MeOH); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 205 (4.40), 244 (3.86), 288 (3.40) nm; IR (KBr)  $\nu_{\max}$  3358, 1607, 1475, 1466 cm<sup>-1</sup>; <sup>1</sup>H NMR, Table 1; <sup>13</sup>C NMR, Table 2; NOESY, H-2/H-14, 17; H-3/N<sub>a</sub>Me, N<sub>b</sub>Me; H-5/H-21; H-6/N<sub>b</sub>Me; H-12/N<sub>a</sub>Me; H-14/H-17; H-15/H-

17, 19; H-21/N<sub>b</sub>Me; HMBC, H-2→C-6, 7, 8, 17, N<sub>a</sub>Me; H-9→C-7, 11, 13; H-10→C-8, 12; H-11→C-9, 13; H-12→C-8, 10; H-14→C-2, 20; H-15→C-17; H-16→C-6, 14; H-17→C-2, 5, 6, 15; H-18→C-20; N<sub>a</sub>Me→C-2, 13; N<sub>b</sub>Me→C-3, 5, 21; HRSIMS *m/z* 341.2225 (calcd for C<sub>21</sub>H<sub>29</sub>N<sub>2</sub>O<sub>2</sub>, 341.2230).

**N<sub>b</sub>-Methylisoajmaline (2):** white powder; [ $\alpha$ ]<sub>D</sub><sup>21</sup> +88° (c 1.2, MeOH); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 204 (4.41), 244 (3.88), 288 (3.42) nm; IR (KBr)  $\nu_{\max}$  3352, 1607, 1475, 1468 cm<sup>-1</sup>; <sup>1</sup>H NMR, Table 1; <sup>13</sup>C NMR, Table 2; NOESY, H-2/H-17; H-3, 5/N<sub>b</sub>Me; H-5/H-20; H-12/N<sub>a</sub>Me; H-15/H-19; H-16/H-20; H-21/N<sub>b</sub>Me; HMBC, H-2→C-6, 7, 8, 14, N<sub>a</sub>Me; H-6→C-2; H-9→C-7, 11, 13; H-10→C-8, 12; H-11→C-9, 13; H-12→C-8, 10; H-14→C-2, 16; H-15→C-3, 17, 21; H-16→C-6, 14; H-17→C-2, 5, 15; H-18→C-20; H-19→C-15, 21; N<sub>a</sub>Me→C-2, 13; N<sub>b</sub>Me→C-3, 5, 21; HRSIMS *m/z* 341.2237 (calcd for C<sub>21</sub>H<sub>29</sub>N<sub>2</sub>O<sub>2</sub>, 341.2230).

**3-Hydroxysarpagine (3):** white powder; [ $\alpha$ ]<sub>D</sub><sup>21</sup> +44° (c 1.0, MeOH); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 205 (4.36), 224.5 (4.23), 278 (3.75) nm; IR (KBr)  $\nu_{\max}$  3368, 1647, 1636, 1474 cm<sup>-1</sup>; <sup>1</sup>H NMR, Table 1; <sup>13</sup>C NMR, Table 2; NOESY, H-5/H-17; H-6/H-9, 16, 17; H-14/H-21; H-15/H-17, 18; H-19/H-21; H-17/H-19; HMBC, H-5→C-3, 7, 17; H-6→C-2, 7, 16; H-9→C-7, 11, 13; H-11→C-9, 13; H-12→C-8, 10; H-14→C-2, 3, 16, 20; H-15→C-3, 21; H-16→C-6, 20; H-17→C-5, 15; H-18→C-20; H-19→C-15, 21; H-21→C-19, 20; HRSIMS *m/z* 326.1641 (calcd for C<sub>19</sub>H<sub>22</sub>N<sub>2</sub>O<sub>3</sub>, 326.1631).

**Yohimbic acid (4):** white powder; [ $\alpha$ ]<sub>D</sub><sup>18</sup> -10° (c 0.63, MeOH); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 221 (4.28), 273sh (3.63), 279.5 (3.64), 288.5 (3.57) nm; IR (KBr)  $\nu_{\max}$  3405, 1632, 1576, 1571, 1406 cm<sup>-1</sup>; <sup>1</sup>H NMR, Table 1; <sup>13</sup>C NMR, Table 2; NOESY, H-3/H-5, 15, 21; H-5/H-21; H-6/H-9; H-14/H-16; H-15/H-21; H-16/H-20; H-19/H-21; HMBC, H-5→C-3, 7; H-6→C-2, 7; H-9→C-7, 11, 13; H-10→C-8, 12; H-11→C-9, 13; H-12→C-8, 10; H-16→C-14, CO; H-17→C-15, 19; H-18→C-16; H-21→C-3, 15; HRSIMS *m/z* 341.1867 (calcd for C<sub>20</sub>H<sub>25</sub>N<sub>2</sub>O<sub>3</sub>, 341.1866).

**Isorauhimbinic acid (5):** colorless crystalline solid, mp 234–236 °C (MeOH); [ $\alpha$ ]<sub>D</sub><sup>25</sup> -131° (c 0.25, pyridine); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 220 (4.47), 272 (3.82), 279 (3.82), 289 (3.74) nm; IR (KBr)  $\nu_{\max}$  3389, 1570, 1456 cm<sup>-1</sup>; <sup>1</sup>H NMR, Table 1; <sup>13</sup>C NMR, Table 2; NOESY, H-3/H-5; H-14/H-17; H-15/H-21; H-17/H-19, 21; H-19/H-21; HMBC, H-3→C-15; H-5→C-3, 7, 21; H-6→C-2; H-9→C-7, 11, 13; H-10→C-8, 12; H-11→C-9, 13; H-12→C-8, 10; H-14→C-20; H-18→C-17; H-21→C-3, 15, 19; HRSIMS *m/z* 341.1856 (calcd for C<sub>20</sub>H<sub>25</sub>N<sub>2</sub>O<sub>3</sub>, 341.1866).

**7-Epiloganin (6):** white powder; [ $\alpha$ ]<sub>D</sub><sup>25</sup> -18° (c 0.63, MeOH); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 235 (3.97) nm; IR (KBr)  $\nu_{\max}$  3400, 2957, 1695, 1636, 1439, 1097, 1080 cm<sup>-1</sup>; <sup>1</sup>H NMR (CD<sub>3</sub>OD, 500 MHz)  $\delta$  1.13 (3H, d, *J* = 7.0 Hz, H<sub>3</sub>-10), 1.35 (1H, dt, *J* = 13.0, 8.0 Hz, H-6), 1.74 (1H, dq, *J* = 8.0, 7.0 Hz, H-8), 1.80 (1H, td, *J* = 8.0, 5.0 Hz, H-9), 2.51 (1H, dt, *J* = 13.0, 8.0 Hz, H-6), 2.86 (1H, brq, *J* = 8.0 Hz, H-5), 3.18 (1H, t, *J* = 9.0, 7.5 Hz, H-2'), 3.26 (1H, dd, *J* = 9.0, 8.0 Hz, H-4'), 3.27–3.33 (1H, m, H-5'), 3.37 (1H, t, *J* = 9.0 Hz, H-3'), 3.65 (1H, dd, *J* = 12.0, 6.0 Hz, H-6'), 3.67 (1H, t, *J* = 8.0 Hz, H-7), 3.69 (3H, s, OMe), 3.89 (1H, dd, *J* = 12.0, 2.0 Hz, H-6'), 4.65 (1H, d, *J* = 7.5 Hz, H-1'), 5.33 (1H, d, *J* = 5.0 Hz, H-1), 7.41 (1H, d, *J* = 1.5 Hz, H-3); <sup>13</sup>C NMR (CD<sub>3</sub>OD, 125 MHz)  $\delta$  17.7 (C-10), 31.4 (C-5), 42.0 (C-6), 44.0 (C-8), 47.1 (C-9), 51.7 (OMe), 62.8 (C-6'), 71.7 (C-4'), 74.8 (C-2'), 78.1, 78.4 (C-5', 3'), 79.7 (C-7), 97.7 (C-1), 100.4 (C-1'), 113.3 (C-4), 152.5 (C-3), 169.5 (C-11); SIMS *m/z* 389 [M – H]<sup>-</sup>, 227; HRSIMS *m/z* 389.1466 (calcd for C<sub>17</sub>H<sub>25</sub>O<sub>10</sub>, 389.1449).

**6'-O-(3,4,5-Trimethoxybenzoyl)glomeratose A (7):** white powder;  $[\alpha]_D^{25} -20^\circ$  (c 1.0, MeOH); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 214 (4.61), 272 (4.11), 303 (4.23) nm; IR (KBr)  $\nu_{\max}$  3385, 1719, 1506, 1128, 1057  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ , 500 MHz)  $\delta$  3.43 (1H, t,  $J = 10.0$  Hz, H-4'), 3.46 (1H, dd,  $J = 10.0$ , 3.5 Hz, H-2'), 3.60 (1H, d,  $J = 12.0$  Hz, H-1), 3.66 (1H, d,  $J = 12.0$  Hz, H-1), 3.69 (1H, dd,  $J = 12.0$ , 3.0 Hz, H-6), 3.69 (1H, t,  $J = 10.0$  Hz, H-3'), 3.76 (1H, dd,  $J = 12.0$ , 6.0 Hz, H-6), 3.78 (3H, s, OMe-4''), 3.81 (3H, s, OMe-4'''), 3.85 (6H, s, OMe-3''', 5'''), 3.86 (6H, s, OMe-3'', 5''), 3.90 (1H, ddd,  $J = 8.0$ , 6.0, 3.0 Hz, H-5), 4.26 (1H, ddd,  $J = 10.0$ , 5.0, 2.0 Hz, H-5'), 4.32 (1H, t,  $J = 8.0$  Hz, H-4), 4.46 (1H, dd,  $J = 12.0$ , 5.0 Hz, H-6'), 4.73 (1H, dd,  $J = 12.0$ , 2.0 Hz, H-6'), 5.47 (1H, d,  $J = 8.0$  Hz, H-3), 5.50 (1H, d,  $J = 3.5$  Hz, H-1'), 6.55 (1H, d,  $J = 16.0$  Hz, H- $\alpha$ ), 6.95 (2H, s, H-2'', 6''), 7.32 (2H, s, H-2''', 6'''), 7.71 (1H, d,  $J = 16.0$  Hz, H- $\beta$ );  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ , 125 MHz)  $\delta$  56.8 (OMe-3'', 5'', 3''', 5'''), 61.2 (OMe-4'', 4'''), 63.2 (C-6), 65.7 (C-1), 65.8 (C-6'), 71.9 (C-4'), 72.5 (C-5'), 73.2 (C-2'), 74.1 (C-4), 74.8 (C-3'), 79.7 (C-3), 84.2 (C-5), 93.3 (C-1'), 105.0 (C-2), 106.9 (C-2'', 6''), 108.2 (C-2''', 6'''), 117.8 (C- $\alpha$ ), 126.4 (C-1'''), 131.4 (C-1''), 141.4 (C-4'), 143.7 (C-4'''), 147.3 (C- $\beta$ ), 154.4 (C-3''', 5'''), 154.8 (C-3'', 5''), 167.6 (CO of benzoyl), 167.7 (CO of cinnamoyl); NOESY, H-1/H-3; H-2'', 6''/OMe-3'', 5''; HMBC, H-1 $\rightarrow$ C-2, 3; H-3 $\rightarrow$ C-4, CO; H-4 $\rightarrow$ C-6; H-1' $\rightarrow$ C-2, 3', 5'; H-6' $\rightarrow$ C-4', CO; H-2'', 6'' $\rightarrow$ C-4'',  $\beta$ ; OMe-3'', 5'' $\rightarrow$ C-3'', 5''; OMe-4'' $\rightarrow$ C-4'; H- $\beta$  $\rightarrow$ C-1'', 2'', 6'',  $\alpha$ ; H- $\alpha$  $\rightarrow$ C-1'', CO; H-2''', 6''' $\rightarrow$ C-1''', 4''', CO; OMe-3''', 5''' $\rightarrow$ C-3''', 5'''; OMe-4''' $\rightarrow$ C-4'''; SIMS  $m/z$  755 [M - H] $^-$ , 535, 237, 211; HRSIMS  $m/z$  755.2400 (calcd for  $\text{C}_{34}\text{H}_{43}\text{O}_{19}$ , 755.2400).

**Methylation of Ajmaline and Isoajmaline.** A solution of ajmaline (10.0 mg) and methyl iodide (3.82  $\mu\text{L}$ ) in MeCN (0.5 mL) was refluxed for 1 h. The concentrated reaction mixture was purified by preparative TLC ( $\text{CHCl}_3$ -MeOH-NH $_4\text{OH}$ , 70:30:3) and then treated with an anion-exchange resin (AG 1-X4) to afford **1** (9.8 mg):  $[\alpha]_D^{30} +109^\circ$  (c 0.23, MeOH); SIMS  $m/z$  341 [M] $^+$ . UV, IR,  $^1\text{H}$  NMR, and  $^{13}\text{C}$  NMR spectroscopic data were identical to those of **1** from *R. serpentina*.

In a similar manner, isoajmaline (3.2 mg) gave **2** (2.2 mg):  $[\alpha]_D^{25} +74^\circ$  (c 0.22, MeOH); SIMS  $m/z$  341 [M] $^+$ . UV, IR, and  $^1\text{H}$  NMR spectroscopic data were identical to those of **2** from *R. serpentina*.

**Methylation of 4 and 5.** A methanolic solution (0.25 mL) of **4** (5.1 mg) was methylated with (trimethylsilyl)diazomethane and purified by preparative TLC ( $\text{CHCl}_3$ -MeOH-NH $_4\text{OH}$ , 90:9:1) to afford yohimbine (1.8 mg):  $[\alpha]_D^{25} +77^\circ$  (c 0.18, pyridine); EIMS  $m/z$  354 [M] $^+$ . UV, IR,  $^1\text{H}$  NMR, and  $^{13}\text{C}$  NMR spectroscopic data were identical to those of authentic yohimbine.

In a similar manner, **5** (10.0 mg) gave isorauhimbine (3.6 mg):  $[\alpha]_D^{25} -78^\circ$  (c 0.36, pyridine); EIMS  $m/z$  354 [M] $^+$ . UV, IR,  $^1\text{H}$  NMR, and  $^{13}\text{C}$  NMR spectroscopic data were identical to those of authentic isorauhimbine.

**Acid Hydrolysis of Compound 7.** Compound **7** (1 mg) was heated at  $95^\circ\text{C}$  with dioxane (0.5 mL) and 5%  $\text{H}_2\text{SO}_4$  (0.5 mL) for 1 h. After neutralization with Amberlite IRA-400 (OH $^-$  form), the reaction mixture was concentrated and the residue was passed through a Sep-Pak C $_{18}$  cartridge with  $\text{H}_2\text{O}$ . The eluate was concentrated and the residue was treated with L-cysteine methyl ester hydrochloride (1 mg) in pyridine (0.125 mL) at  $60^\circ\text{C}$  for 1 h. The solution was then treated with *N,O*-bis(trimethylsilyl)trifluoroacetamide (0.05 mL) at  $60^\circ\text{C}$  for 1 h. The supernatant was applied to GLC; GLC conditions: column, Supelco SPB-1, 30 m  $\times$  0.25 mm; column temperature,  $230^\circ\text{C}$ ;  $\text{N}_2$  flow rate, 0.8 mL/min;  $t_R$  of derivatives, D-glucose 12.9 min, L-glucose 13.4 min, D-fructose 11.1 min, L-fructose 10.8 min. D-Glucose and D-fructose were detected from **7**.

**DNA Topoisomerase Assays.** Recombinant human DNA topoisomerase I and II (topo I and II) (2 units/ $\mu\text{L}$ , each) were purchased from TopoGen, Inc. (Columbus, OH). Relaxation activity of topo II was determined by detecting the conversion of supercoiled plasmid DNA to its relaxed form.<sup>24</sup> The topo II reaction was performed in 20  $\mu\text{L}$  of reaction mixture containing 50 mM Tris-HCl buffer (pH 8.0), 120 mM KCl, 10 mM  $\text{MgCl}_2$ , 0.5 mM ATP, 0.5 mM dithiothreitol, supercoiled pUC19 DNA (0.25  $\mu\text{g}$ ), 2  $\mu\text{L}$  of inhibitor solution [10% dimethyl sulfoxide

(DMSO)], and 2 units of topo II. The reaction mixtures were incubated at  $37^\circ\text{C}$  for 30 min and terminated by adding 2  $\mu\text{L}$  of loading buffer consisting of 5% sarkosyl, 0.0025% bromophenol blue, and 25% glycerol. The mixtures were subjected to 1% agarose gel electrophoresis in TAE (Tris-acetate-EDTA) running buffer. The agarose gel was stained with ethidium bromide (EtBr), and DNA was visualized on a UV transilluminator. Relaxation activity of topo I was analyzed in the same manner described above except that the reaction mixtures contained 10 mM Tris-HCl (pH 7.9), pBR322 DNA (0.25  $\mu\text{g}$ ), 1 mM EDTA, 150 mM NaCl, 0.1% bovine serum albumin (BSA), 0.1 mM spermidine, 5% glycerol, and 2 units of topo I. One unit was defined as the amount of enzyme capable of relaxing 0.25  $\mu\text{g}$  of DNA in 15 min at  $37^\circ\text{C}$ .

**Cell Culture and Measurement of Cell Viability.** A human promyelocytic leukemia cell line, HL-60, was obtained from the Health Science Research Bank (Osaka, Japan). The cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum, penicillin (100 units/mL), and streptomycin (100  $\mu\text{g}/\text{mL}$ ) at  $37^\circ\text{C}$  in a humid atmosphere of 5%  $\text{CO}_2/95\%$  air. For the cell growth assay, HL-60 cells were plated at  $3 \times 10^5$  cells into each well of 96-well microplates with various concentrations of the alkaloids. These compounds were dissolved in phosphate-buffered saline (PBS) at a concentration of 10 mM as a stock solution. The stock solutions were diluted to the appropriate final concentrations with growth medium just before use. The cell viability was determined by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) assay.<sup>25</sup>

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