Securinega Alkaloids from Flueggea leucopyra

Guo-cai WANG, a,b Ying WANG, a,b Xiao-qi ZHANG, a,b Yao-lan LI, a,b Xin-sheng YAO, a,b and Wen-cai YE a,b

a Institute of Traditional Chinese Medicine and Natural Products, Jinan University; and b Guangdong Province Key Laboratory of Pharmacodynamic Constituents of TCM and New Drugs Research, Jinan University; Guangzhou 510632, P. R. China. Received November 21, 2009; accepted December 21, 2009; published online December 24, 2009

Six new Securinega alkaloids (1, 3, 5, 7, 9, 10) together with four known ones were isolated from the twigs and leaves of Flueggea leucopyra. The structures of new compounds were established on the basis of the spectroscopic methods including UV, IR, HR-electrospray ionization (ESI)-MS, 1D and 2D NMR, and the absolute configurations of these new alkaloids were assigned by the modified Mosher’s method and the circular dichroism (CD) spectra.

Key words Flueggea leucopyra; Euphorbiaceae; Securinega alkaloid; absolute configuration

The Securinega alkaloids are a group of polycyclic compounds isolated from the plants of Securinega and Phyllanthus genera (Euphorbiaceae).1) Previous phytochemical investigations had led to the isolation of a number of Securinega alkaloids,1)–9) which exhibited antimalarial, antibacterial and antitumor activities.5)–11) Among the Securinega alkaloids, securinine was a major alkaloid obtained from the plant Securinega suffruticosais,12) and clinically applied to treat sequela of poliomyelitis and aplastic anemia.13) Pharmacology investigations indicated that securinine was a stereospecific GABA_ receptors antagonist with a significant central nervous system (CNS) activity.14,15) In searching for bioactive alkaloids from the Euphorbiaceae plants, we had isolated some chemical constituents from Securinega suffruticosais and Flueggea virosa.3) Flueggea leucopyra was a shrub which only distributed in Sichuan and Yunnan Provinces of China. Recently, our phytochemical study of F. leucopyra had resulted in the isolation of six new Securinega alkaloids together with four known ones (Fig. 1). This paper reports the isolation and structural elucidation of the new alkaloids (1, 3, 5, 7, 9, 10) from the twigs and leaves of F. leucopyra.

Results and Discussion

The air-dried twigs and leaves of F. leucopyra were extracted with 95% EtOH. The solution was evaporated in vacuo to get a residue. The residue was suspended in H_2O and then adjusted to pH 6 with 5% HCl. The acidic suspension was partitioned with CHCl_3 to remove the neutral component. The aqueous layer was basified with NH_3·H_2O and extracted with CHCl_3 to obtain a residue. Repeated column chromatography of the residue afforded six new compounds (1, 3, 5, 7, 9, 10) and four known compounds (2, 4, 6, 8). The known compounds were identified by comparison with the literature data as securinine (2),5) secu’amamine B (4),8) secu’amamine C (6),8) and fluggeain (8),6,7) respectively.

1 was obtained as yellow oil, and its molecular formula was determined as C_{13}H_{17}NO_4 on the basis of HR-electrospray ionization (ESI)-MS at m/z: 234.1127 (Calcd for C_{13}H_{17}NO_4, 234.1125). The IR spectrum of 1 implied the presence of hydroxyl group (3436 cm^{-1}) and α,β-unsaturated γ-lactone ring (1746, 1632 cm^{-1}). An α,β-unsaturated γ-lactone ring at δ_c 174.0, 168.8, 110.7 and 92.9, a double bond at δ_c 124.3 and 150.1, and an oxygenated methane at δ_c 64.9 were observed in the 1^1^C-NMR spectrum. Accordingly, the 1H-NMR spectrum showed three olefinic protons at δ_h 4.19 (1H, m) (Table 1) with J = 5.2 Hz), and an oxygenated proton at δ_h 9.2 Hz) and 6.77 (1H, dd, J = 9.2, 5.2 Hz), and an oxygenated proton at δ_h 4.19 (1H, m) (Table 1). All the data indicated that 1 was a securinine type alkaloid with a hydroxyl group.5)

Comparison of 1H- and 13C-NMR data of 1 (Table 1) with those of securitinine5) (2) revealed that the signals of the two compounds were very similar, except the absence of the methoxyl group in 1 as well as the upfield shift of C-4 from δ_c 72.8 in 2 to δ_c 64.9 in 1, suggesting that 1 had a hydroxyl group at C-4 position. This was further confirmed by the heteronuclear multiple bond connectivity (HMBC) correlations between H-4 (δ_h 4.19) and C-2 (δ_c 57.0) and C-6 (δ_c 43.7) (Fig. 2). The relative configuration of 1 was assigned as shown in Fig. 3 by the nuclear Overhauser effect spectroscopy (NOESY) correlations between H-2 and H-6a, between H-4 and H-6b, as well as between H-2 and H-8a. The circular dichroism (CD) spectrum of 1 showed Cotton effects at λ_{max} 216 nm (Δε = -3.34) and 312 nm (Δε = -17.37), which were similar to those of securitinine (2) [λ_{max} 227 nm (Δε = -1.13) and 302 nm (Δε = -19.36)]. Furthermore, the absolute configuration of 1 was confirmed by application of the modified Mosher’s method.16) Differences of proton chemical shift (Δδ values, δ_c−δ_h) between (S)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA) ester (1a) and (R)-MTPA ester (1b) (Fig. 4) indicated the presence of S configuration at C-4 in 1. Hence, the absolute configuration of 1 was assigned as 2S, 4S, 7S, and 9S. 1 was identified as 4α-hydroxyallosecurinine.

3 was isolated as yellow oil, and its HR-ESI-MS showed an [M+H]^+ ion peak at m/z: 266.1387 (Calcd for C_{14}H_{19}NO_4, 266.1387) for the molecular formula of C_{14}H_{19}NO_4. The presence of α,β-unsaturated γ-lactone ring

Fig. 1. Chemical Structures of Compounds 1—10

* To whom correspondence should be addressed. e-mail: chywc@yahoo.com.cn © 2010 Pharmaceutical Society of Japan
was suggested by its IR spectrum (1756, 1643 cm\(^{-1}\)). Fourteen carbon signals including one methyl, five methylenes, five methines, and three quaternary carbons were showed by the \(^{13}\)C and distortionless enhancement by polarization transfer (DEPT) NMR spectra. An \(\alpha,\beta\)-unsaturated \(\gamma\)-lactone ring (\(\delta_C 174.1, 172.2, 114.6, 91.0\)), two oxygenated methines (\(\delta_C 66.0, 81.0\)) and a methoxyl group (\(\delta_C 58.1\)) were displayed in the \(^{13}\)C-NMR spectrum. In the \(^1\)H-NMR spectrum, an olefinic proton (\(\delta_H 5.68, d, J=2.4\)) and two oxygenated protons (\(\delta_H 3.55 (m)\) and 4.14 (\(t, J=2.8\))) and a methoxy singlet (\(\delta_H 3.27, s\)) were observed. The above data indicated that 3 was a dihydrosecurinine type alkaloid with a methoxyl group and a hydroxyl group.

Comparison of NMR data of 3 (Table 1) with those of secu’amamine B (8) (4) showed that the NMR signals of the two compounds were similar, except that 3 only had a methoxyl group as well as the \(^{13}\)C-NMR value at C-4 shifted from \(\delta_C 76.2\) in 4 to \(\delta_C 66.0\) in 3, suggesting that 3 had a methoxyl group at C-15 position and a hydroxyl group at C-4 position. It was further confirmed by the HMBC correlations between OCH\(_3\) (\(\delta_H 3.27\)) and C-15 (\(\delta_C 81.0\)) (Fig. 2). The NOESY correlations between H-2 and H-6\(a\), between H-6\(b\) and H-4, between H-2 and H-8\(a\), between H-8\(b\) and 15-OMe, as well as between 15-OMe and H-14\(b\) (Fig. 3) established the relative configuration of 3. The Cotton effects at \(\lambda_{\text{max}} 226\) nm (\(\Delta e+2.87\)) and 277 nm (\(\Delta e+0.76\)) in CD spectrum of 3 were similar to those of 4 (8). Therefore, the absolute configuration of 3 was assigned as \(2\text{S}, 4\text{S}, 7\text{S}, 9\text{S},\) and \(15\text{S}\). The structure of 3 was elucidated as 4\(\alpha\)-hydroxy-15\(\alpha\)-methoxy-14,15-dihydroallosecurinine.

\(5\) showed an [M+H]\(^+\) ion peak at \(m/z\) 280.1540 (Calcd for C\(_{15}\)H\(_{22}\)NO\(_4\), 280.1543) in the HR-ESI-MS spectrum, consistent with a molecular formula of C\(_{15}\)H\(_{22}\)NO\(_4\). The IR spectrum indicated the presence of \(\alpha,\beta\)-unsaturated \(\gamma\)-lactone ring (1758, 1644 cm\(^{-1}\)). The \(^1\)H- and \(^{13}\)C-NMR data of 5 (Table 1) were very similar to those of secu’amamine B (4) (8), suggesting that 5 was a dihydrosecurinine type alkaloid with
two methoxyl groups. The HMBC spectrum showed the correlations between OCH\(_3\) (δ\(_{II}\) 3.35) and C-4 (δ\(_C\) 77.4) as well as between OCH\(_3\) (δ\(_{II}\) 3.30) and C-15 (δ\(_C\) 81.0), indicating that two methoxyl groups were located at C-4 and C-15 positions, respectively. The NOE correlation between H-2 and H-14b (Fig. 3) indicated that the relative configuration at C-2 of 5 was opposite to those of 3 and 4. The absolute configuration of 5 was determined as 2R, 4S, 7S, 9S, and 15S by comparison of the CD spectrum of 5 with that of secu’amamine (6). Therefore, the structure of 5 was elucidated as 4α-methoxy-15β-methoxy-14,15-dihydrosecurinine.

7 was obtained as yellow oil. The molecular formula of 7 was determined as C\(_{13}\)H\(_{17}\)NO\(_3\) according to the HR-ESI-MS at \(m/z\) 222.1126 [M+H]\(^+\) (Calcd for C\(_{13}\)H\(_{17}\)NO\(_3\), 222.1125). The IR spectrum showed the presence of hydroxyl group (3442 cm\(^{-1}\)) and \(\alpha,\beta\)-unsaturated \(\gamma\)-lactone ring (1755, 1649 cm\(^{-1}\)). The NOESY correlations (Fig. 3) indicated that the methoxyl group was attached to C-14 position. The relative configuration of 7 was elucidated as 14\(\alpha\)-methoxy-13,14-dihydrosecurinine, suggesting the absolute configuration at C-14 of 7 was opposite to that of secu’amamine (6).

10 was isolated as yellow oil. Its molecular formula was determined as C\(_{13}\)H\(_{17}\)NO\(_3\) by the HR-ESI-MS at \(m/z\): 236.1286 [M+H]\(^+\) (Calcd for C\(_{13}\)H\(_{17}\)NO\(_3\), 236.1281). The IR spectrum of 9 implied the presence of \(\alpha,\beta\)-unsaturated \(\gamma\)-lactone ring (1755, 1649 cm\(^{-1}\)). Except for an additional methoxyl group (δ\(_{II}\) 3.41/δ\(_C\) 56.6), the 1\(H\)- and 1\(^3\)C-NMR data of 9 were similar to those of 7 (Table 2), suggesting 9 was a dihydrosecurinine type alkaloid with a methoxyl group. The HMBC correlations between OCH\(_3\) (δ\(_{II}\) 3.41) and C-14 (δ\(_C\) 79.8) indicated that the methoxyl group was attached to C-14 position. The relative configuration of 9 was assigned by NOESY correlations (Fig. 3). The CD spectrum (\(\lambda\)\(_{max}\) 208 nm (\(\Delta\varepsilon\) = 2.56), 234 nm (\(\Delta\varepsilon\) +2.25), and 284 nm (\(\Delta\varepsilon\) = 0.62)) of 9 was similar to those of 7 and 8, suggesting the absolute configuration of 9 was 2R, 6S, 8S, and 14R. Thus, the structure of 9 was elucidated as 14\(\beta\)-methoxy-13,14-dihydrosecurinine.

9 was isolated as yellow oil. Its molecular formula was determined as C\(_{13}\)H\(_{17}\)NO\(_3\) by the HR-ESI-MS at \(m/z\): 236.1286 [M+H]\(^+\) (Calcd for C\(_{13}\)H\(_{17}\)NO\(_3\), 236.1281). The IR spectrum of 9 implied the presence of \(\alpha,\beta\)-unsaturated \(\gamma\)-lactone ring (1755, 1649 cm\(^{-1}\)). Except for an additional methoxyl group (δ\(_{II}\) 3.41/δ\(_C\) 56.6), the 1\(H\)- and 1\(^3\)C-NMR data of 9 were similar to those of 7 (Table 2), suggesting 9 was a dihydrosecurinine type alkaloid with a methoxyl group. The HMBC correlations between OCH\(_3\) (δ\(_{II}\) 3.41) and C-14 (δ\(_C\) 79.8) indicated that the methoxyl group was attached to C-14 position. The relative configuration of 9 was assigned by NOESY correlations (Fig. 3). The CD spectrum (\(\lambda\)\(_{max}\) 208 nm (\(\Delta\varepsilon\) = 2.56), 234 nm (\(\Delta\varepsilon\) +2.25), and 284 nm (\(\Delta\varepsilon\) = 0.62)) of 9 was similar to those of 7 and 8, suggesting the absolute configuration of 9 was 2R, 6S, 8S, and 14R. Thus, the structure of 9 was elucidated as 14\(\beta\)-methoxy-13,14-dihydrosecurinine.

### Table 2. NMR Data of 7, 9 and 10 (CDCl\(_3\), \(J\) in Hz)

<table>
<thead>
<tr>
<th>Position</th>
<th>(\delta_C)</th>
<th>(\delta_{II})</th>
<th>(\delta_C)</th>
<th>(\delta_{II})</th>
<th>(\delta_C)</th>
<th>(\delta_{II})</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>65.6</td>
<td>3.14(^a)</td>
<td>66.7</td>
<td>3.22(^a)</td>
<td>66.9</td>
<td>3.15 (dd, 8.8, 6.4)</td>
</tr>
<tr>
<td>3</td>
<td>28.9</td>
<td>1.89(^a)</td>
<td>29.1</td>
<td>1.91(^a)</td>
<td>30.3</td>
<td>1.90(^a)</td>
</tr>
<tr>
<td>4</td>
<td>26.6</td>
<td>1.81(^a)</td>
<td>26.5</td>
<td>1.96(^a)</td>
<td>27.9</td>
<td>1.95(^a)</td>
</tr>
<tr>
<td>5</td>
<td>57.2</td>
<td>3.36 (m)</td>
<td>57.6</td>
<td>3.47 (m)</td>
<td>58.6</td>
<td>3.36 (m)</td>
</tr>
<tr>
<td>6</td>
<td>66.0</td>
<td>3.10(^a)</td>
<td>63.3</td>
<td>3.24(^a)</td>
<td>64.7</td>
<td>3.22 (m)</td>
</tr>
<tr>
<td>7</td>
<td>29.5</td>
<td>2.33 (dd, 11.6, 6.4)</td>
<td>31.5</td>
<td>2.51 (dd, 11.6, 6.4)</td>
<td>31.2</td>
<td>2.27 (dd, 11.6, 6.4)</td>
</tr>
<tr>
<td>8</td>
<td>91.8</td>
<td>—</td>
<td>91.4</td>
<td>—</td>
<td>92.5</td>
<td>—</td>
</tr>
<tr>
<td>10</td>
<td>172.9</td>
<td>—</td>
<td>172.8</td>
<td>—</td>
<td>173.9</td>
<td>—</td>
</tr>
<tr>
<td>11</td>
<td>111.1</td>
<td>5.66 (d, 2.0)</td>
<td>110.6</td>
<td>5.64 (d, 2.4)</td>
<td>112.3</td>
<td>5.59 (d, 2.0)</td>
</tr>
<tr>
<td>12</td>
<td>172.9</td>
<td>—</td>
<td>171.5</td>
<td>—</td>
<td>173.9</td>
<td>—</td>
</tr>
<tr>
<td>13</td>
<td>32.2</td>
<td>2.93(^a)</td>
<td>29.4</td>
<td>3.22(^a)</td>
<td>30.1</td>
<td>2.88 (d, 12.8)</td>
</tr>
<tr>
<td>14</td>
<td>69.1</td>
<td>4.24 (t, 4.4)</td>
<td>79.8</td>
<td>3.34 (dd, 6.4, 2.0)</td>
<td>79.1</td>
<td>3.67 (dd, 4.8, 4.4)</td>
</tr>
<tr>
<td>14-OCH(_3)</td>
<td>56.6</td>
<td>3.41 (s)</td>
<td>58.1</td>
<td>3.26 (s)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(\lambda\)\(_{max}\) 261 nm (\(\Delta\varepsilon\) = 2.67), 238 nm (\(\Delta\varepsilon\) = 2.67), and 265 nm (\(\Delta\varepsilon\) = 0.27).

Hence, the absolute configuration of 7 was assigned as 2R, 6S, 8S, and 14R. 7 was identified as 14\(\beta\)-hydroxy-13,14-dihydrosecurinine.

### General Experimental Procedures

**Optical rotations** were determined on a JASCO P-1020 polarimeter. **UV spectra** were obtained on a JASCO V-550 UV/VIS spectrophotometer. **CD spectra** were measured on a JASCO J-720 spectrometer. **IR spectra** were obtained on a JASCO FTIR-480 plus spectrometer. **\(\gamma\)H-, \(\gamma\)C- and 2D-NMR spectra** were determined on a Bruker-
AV-400 spectrometer in CDCl3. ESI-MS spectra were run on a HP-1100 HPLC/ESI spectrometer. For column chromatography, silica gel (200—300 mesh, Qingdao Marine Chemical Factory, P. R. China), Sephadex LH-20 (Pharmacia) and ODS (YMC) were used. TLC analyses were carried out using precoated silica gel GF254 plates (Qingdao Marine Chemical Factory, P. R. China). HPLC separations were performed on a COSMOSIL C18 preparative column (5 μm, 20×250 mm).

Plant Material. The twigs and leaves of *E. leucopeyrara* were collected in Nujiang county, Yunnan province of China, in September of 2006. The plant was authenticated by Prof. Guang-xiong Zhou of Jian University. A voucher specimen (No. 0609614) was deposited in the Institute of Traditional Chinese Medicine and Natural Products, Jinan University, Guangzhou, P. R. China.

Extraction and Isolation. The air-dried twigs and leaves of *E. leucopeyrara* (8.5 kg) were extracted with 95% EtOH and the solution was evaporated to get a residue (1045 g). The residue was dissolved in H2O to form a suspension and then adjusted to pH 6 with 5% HCl. The acidic suspension was partitioned with CHCl3 to remove the neutral components. The aqueous phase was basified with 2% NH3·H2O to pH 8 and then extracted with CHCl3 to obtain a total alkaloid part (42 g), which was subjected to silica gel chromatography (CH3OH–H2O, 60:40) to yield 3-4 (346 mg) was purified by HPLC (CH3OH–H2O–(CH3CH2)3N, 10:1) to afford 8 (12 mg), 9 (11 mg) and 10 (9 mg). Fraction 4 (2.76 g) was purified by Sephadex LH-20 column (CH3OH and silica gel) to yield 5 (12 mg) and 6 (19 mg), respectively.

4α-Hydroxyallosecurinine (1): Yellow oil; [α]D 20 = −102 (c=0.07, MeOH); UV (MeOH) λmax (log ε) 218 (1.65), 257 (1.97) nm; CD (MeOH) [α]D 20 = 32 (1.85) nm; CD (MeOH) [α]D 20 = 32 (1.85) nm; Me2SO [α]D 20 = 400.1529 [M–H]+; HR-ESI-MS m/z: 450 [M–H]+ [HR-ESI-MS m/z: 450 [M–H]+ [HR-ESI-MS m/z: 450 [M–H]+ [HR-ESI-MS m/z: 450 [M–H]+ [HR-ESI-MS m/z: 450 [M–H]+ [HR-ESI-MS m/z: 450 [M–H]+ [HR-ESI-MS m/z: 450 [M–H]+. (C13H17NO3Na, 258.1101).

Preparation of MTPA Esters of 1. 1 (4.5 mg) was dissolved in 0.5 ml of dried pyridine and treated with (R)-(+)-α-methoxy-α-(trifluoromethyl) phenylacetyl chloride (10 μl). The reaction product was stirred at room temperature overnight and then dried in vacuum. The reaction mixture was poured into water (5 ml) and extracted with EtOAc (5 ml). The EtOAc extract was purified by silica gel column chromatography (n-hexane–EtOAc (70:30) to yield (S)-MTPA ester (1a, 3.2 mg). (R)-MTPA ester (1b, 2.7 mg) was obtained using the same method of treatment by 1 (4.3 mg) with (S)-(+)-α-methoxy-α-(trifluoromethyl) phenylacetyl chloride.

(S)-MTPA Ester (1a): 1H-NMR (CDCl3, 400 MHz) δ: 1.63 (1H, m, H-3b), 1.96 (1H, d, J=14.8 Hz, H-3b), 2.10 (1H, m, H-5b), 2.20 (1H, d, J=11.6 Hz, H-8b), 2.57 (1H, dd, J=16.0, 9.6 Hz, H-5a), 2.81 (1H, dd, J=14.0, 12.8 Hz, H-6b), 3.12 (1H, br d, J=11.6 Hz, H-8a), 3.55 (1H, overlapped, H-6a), 3.60 (3H, s, OMe of MTPA), 4.12 (1H, dd, J=14.0, 3.2 Hz, H-7), 4.81 (1H, br s, H-2), 5.39 (1H, m, H-4), 6.06 (1H, brs, H-12), 6.70 (1H, dd, J=8.6, 6.4 Hz, H-15), 7.01 (1H, d, J=8.8 Hz, H-14), 7.27 and 7.45 (phenyl protons of MTPA); ESI-MS m/z: 450 [M+H]+; HR-ESI-MS m/z: 450.1519 [M+H]+; HR-ESI-MS m/z: 450.1519 [M+H]+; HR-ESI-MS m/z: 450.1519 [M+H]+; HR-ESI-MS m/z: 450.1519 [M+H]+; HR-ESI-MS m/z: 450.1519 [M+H]+; HR-ESI-MS m/z: 450.1519 [M+H]+; HR-ESI-MS m/z: 450.1519 [M+H]+.

Acknowledgments. This work was supported by grants from the Cheung Kong Scholars Program (to W. C. Y.), the National Science Fund for Distinguished Young Scholars (No. 30625039), the Joint Fund of NSFC-Guangdong Province (U0932004), the National Natural Science Foundation of China (No. 20902038) and the China Postdoctoral Science Foundation (No. 20070410841).

References