Securinega Alkaloids from *Flueggea leucopyra*

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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—8)] which exhibited antimalarial, antibacterial and antitumor activities.[8—11)] Among the Securinega alkaloids, securinine was a major alkaloid obtained from the plant *Securinega suffruticosa*,[12)] and clinically applied to treat sequela of poliomyelitis and aplastic anemia.[13)] Pharmacology investigations indicated that securinine was a stereospecific $GABA_A$ receptor 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 suffruticosa* and *Flueggea virosa*.[3,4)] *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 vacuum* 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 $NH_3 \cdot H_2O$ 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 securitinine (**2**),[5)] secu'amamine B (**4**),[8)] secu'amamine C (**6**),[8)] and fluggeainol (**8**),[6,7)] respectively.

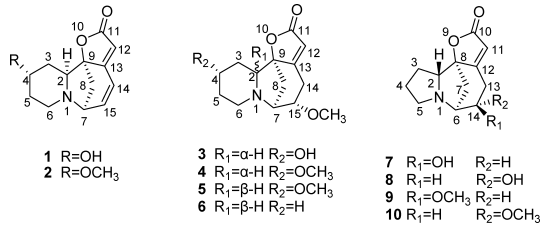

Fig. 1. Chemical Structures of Compounds **1**—**10**

1 was obtained as yellow oil, and its molecular formula was determined as $C_{13}H_{15}NO_3$ on the basis of HR-electrospray ionization (ESI)-MS at *m/z*: 234.1127 $[M+H]^+$ (Calcd for $C_{13}H_{16}NO_3$, 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 methine at δ_C 64.9 were observed in the ^{13}C-NMR spectrum. Accordingly, the ^{1}H-NMR spectrum showed three olefinic protons at δ_H 5.71 (1H, br s), 6.64 (1H, d, J=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 ^{1}H- and ^{13}C-NMR data of **1** (Table 1) with those of securitinine[5)] (**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 ($\Delta\varepsilon$ −3.34) and 312 nm ($\Delta\varepsilon$ −17.37), which were similar to those of securitinine (**2**) [λ_{max} 227 nm ($\Delta\varepsilon$ −1.13) and 302 nm ($\Delta\varepsilon$ −19.36)]. Furthermore, the absolute configuration of **1** was confirmed by application of the modified Mosher's method.[16)] Differences of proton chemical shift ($\Delta\delta$ values, $\delta_S-\delta_R$) 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 2*S*, 4*S*, 7*S*, and 9*S*. **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 $C_{14}H_{20}NO_4$, 266.1387) for the molecular formula of $C_{14}H_{19}NO_4$. The presence of α,β-unsaturated γ-lactone ring

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Table 1. NMR Data of **1**, **3** and **5** ($CDCl_3$, *J* in Hz)

Position	**1**		**3**		**5**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
2	57.0	3.95 (dd, 12.8, 4.0)	61.5	3.51[a)]	60.2	2.96[a)]
3	32.5	1.39[a)]	34.0	1.62[a)]	33.8	2.14 (m)
		1.35[a)]		1.06 (m)		1.28[a)]
4	64.9	4.19 (m)	66.0	4.14 (t, 2.8)	77.4	3.23[a)]
5	34.4	2.18 (m)	35.5	1.69[a)]	32.1	1.80 (m)
		1.53 (m)		1.61[a)]		1.27[a)]
6	43.7	2.78 (m)	47.5	3.04 (m)	48.9	3.06[a)]
		2.56 (m)		2.79[a)]		2.95[a)]
7	60.1	3.89 (br t, 4.8)	60.1	3.35 (br t, 5.2)	62.9	3.33[a)]
8	44.1	2.67 (dd, 10.0, 4.0)	36.2	2.34 (dd, 10.8, 4.6)	33.6	2.51 (dd, 11.2, 4.8)
		1.91 (d, 10.0)		1.99 (d, 10.8)		1.90 (d, 11.2)
9	92.9	—	91.0	—	92.3	—
11	174.0	—	174.1	—	174.6	—
12	110.7	5.71 (br s)	114.6	5.68 (d, 2.4)	112.6	5.64 (br s)
13	168.8	—	172.2	—	174.1	—
14	124.3	6.64 (d, 9.2)	32.1	2.95 (m)	30.6	2.84[a)]
		—		2.74[a)]		2.82[a)]
15	150.1	6.77 (dd, 9.2, 5.2)	81.0	3.55[a)]	81.0	3.56 (d, 2.8)
4-OCH_3					56.6	3.35 (s)
15-OCH_3			58.1	3.27 (s)	58.2	3.30 (s)

a) Overlapped signals were reported without designating multiplicity.

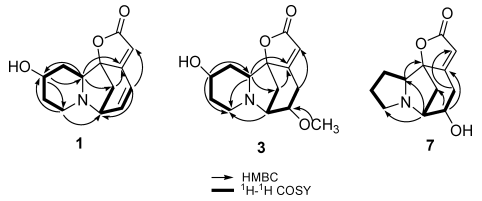

Fig. 2. Key HMBC and ^1H–^1H COSY Correlations of **1**, **3** and **7**

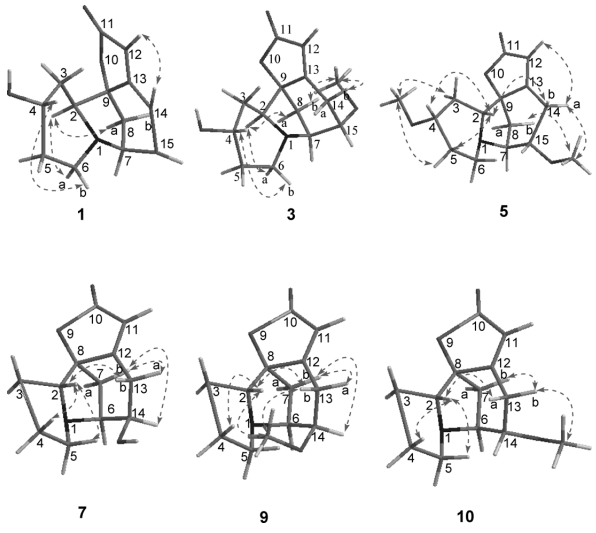

Fig. 3. Key NOESY Correlations of **1**, **3**, **5**, **7**, **9** and **10**

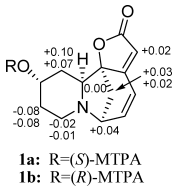

Fig. 4. $\Delta\delta$ Values ($\delta_S-\delta_R$) of the MTPA Esters **1a** and **1b**

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 α,β-unsaturated γ-lactone ring (δ_C 174.1, 172.2, 114.6, 91.0), two oxygenated methines (δ_C 66.0, 81.0) and a methoxyl group (δ_C 58.1) were displayed in the ^{13}C-NMR spectrum. In the ^1H-NMR spectrum, an olefinic proton (δ_H 5.68, d, J=2.4 Hz), two oxygenated protons [δ_H 3.55 (m) and 4.14 (t, J=2.8 Hz)] and a methoxy singlet (δ_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.[8)]

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 δ_C 76.2 in **4** to δ_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 (δ_H 3.27) and C-15 (δ_C 81.0) (Fig. 2). The NOESY correlations between H-2 and H-6a, between H-6b and H-4, between H-2 and H-8a, between H-8b and 15-OMe, as well as between 15-OMe and H-14b (Fig. 3) established the relative configuration of **3**. The Cotton effects at λ_{max} 226 nm ($\Delta\varepsilon$+2.87) and 277 nm ($\Delta\varepsilon$+0.76) in CD spectrum of **3** were similar to those of **4**.[8)] Therefore, the absolute configuration of **3** was assigned as 2*S*, 4*S*, 7*S*, 9*S*, and 15*S*. The structure of **3** was elucidated as 4α-hydroxy-15α-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 $C_{15}H_{21}NO_4$. The IR spectrum indicated the presence of α,β-unsaturated γ-lactone ring (1758, 1644 cm^{-1}). The ^1H- 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

Table 2. NMR Data of **7**, **9** and **10** ($CDCl_3$, *J* in Hz)

Position	**7**		**9**		**10**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
2	65.6	3.14[a)]	66.7	3.22[a)]	66.9	3.15 (dd, 8.8, 6.4)
3	28.9	1.89[a)]	29.1	1.91[a)]	30.3	1.90[a)]
		1.81[a)]		1.75[a)]		1.71[a)]
4	26.6	1.97[a)]	26.5	1.96[a)]	27.9	1.95[a)]
		1.79[a)]		1.79[a)]		1.71[a)]
5	57.2	3.36 (m)	57.6	3.47 (m)	58.6	3.36 (m)
		2.63 (m)		2.62[a)]		2.62 (m)
6	66.0	3.10[a)]	63.3	3.24[a)]	64.7	3.22 (m)
7	29.5	2.33 (dd, 11.6, 6.4)	31.5	2.51 (dd, 11.6, 6.4)	31.2	2.27 (dd, 11.6, 6.4)
		1.96 (d, 6.4)		1.31 (d, 6.4)		1.81 (d, 6.4)
8	91.8	—	91.4	—	92.5	—
10	172.9	—	172.8	—	173.9	—
11	111.1	5.66 (d, 2.0)	110.6	5.64 (d, 2.4)	112.3	5.59 (d, 2.0)
12	172.9	—	171.5	—	173.9	—
13	32.2	2.93[a)]	29.4	3.22[a)]	30.1	2.88 (d, 12.8)
		2.80[a)]		2.64[a)]		2.77 (m)
14	69.1	4.24 (t, 4.4)	79.8	3.34 (dd, 6.4, 2.0)	79.1	3.67 (dd, 4.8, 4.4)
14-OCH_3			56.6	3.41 (s)	58.1	3.26 (s)

a) Overlapped signals were reported without designating multiplicity.

two methoxyl groups. The HMBC spectrum showed the correlations between OCH_3 (δ_H 3.35) and C-4 (δ_C 77.4) as well as between OCH_3 (δ_H 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 2*R*, 4*S*, 7*S*, 9*S*, and 15*S* by comparison of the CD spectrum of **5** with that of secu'amamine C (**6**).[8] Therefore, the structure of **5** was elucidated as 4α-methoxy-15α-methoxy-14,15-dihydrosecurinine.

7 was obtained as yellow oil. The molecular formula of **7** was determined as $C_{12}H_{15}NO_3$ according to the HR-ESI-MS at *m*/*z*: 222.1126 $[M+H]^+$ (Calcd for $C_{12}H_{16}NO_3$, 222.1125). The IR spectrum showed the presence of hydroxyl group (3442 cm^{-1}) and α,β-unsaturated γ-lactone ring (1755, 1631 cm^{-1}). The ^{1}H-NMR spectrum showed an olefinic proton at δ_H 5.66 (d, J=2.0 Hz) and an oxygenated proton at δ_H 4.24 (t, J=4.4 Hz). The ^{13}C-NMR spectrum displayed twelve carbon signals including five methylenes, four methines and three quaternary carbons. Furthermore, in the ^{13}C-NMR spectrum, an α,β-unsaturated γ-lactone ring at δ_C 172.9, 172.9, 111.1 and 91.8 as well as an oxygenated methine group at δ_C 69.1 were observed. The above data were very similar to those of fluggeainol (**8**),[6,7] indicating that **7** was a dihydronorsecurinine type alkaloid with a hydroxyl group. The position of hydroxyl group was assigned by the HMBC correlations between H-14 (δ_H 4.24) and C-6 (δ_C 66.0), C-7 (δ_C 29.5) and C-12 (δ_C 172.9) (Fig. 2). The relative configuration of **7** was assigned by the NOE correlations between H-2 and H-13b, between H-7b and H-13a, as well as between H-7b and H-14 (Fig. 3). The Cotton effects of **7** [λ_{max} 219 nm ($\Delta\varepsilon$−2.76), 238 nm ($\Delta\varepsilon$+1.74), and 265 nm ($\Delta\varepsilon$−0.85)] were also similar to those of fluggeainol (**8**) [λ_{max} 218 nm ($\Delta\varepsilon$−1.37), 231 nm ($\Delta\varepsilon$+2.67), and 261 nm ($\Delta\varepsilon$−0.27)]. Hence, the absolute configuration of **7** was assigned as 2*R*, 6*S*, 8*S*, and 14*R*. **7** was identified as 14β-hydroxy-13,14-dihydronorsecurinine.

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 $C_{13}H_{18}NO_3$, 236.1281). The IR spectrum of **9** implied the presence of α,β-unsaturated γ-lactone ring (1755, 1649 cm^{-1}). Except for an additional methoxyl group (δ_H 3.41/δ_C 56.6), the ^{1}H- and ^{13}C-NMR data of **9** were similar to those of **7** (Table 2), suggesting **9** was a dihydronorsecurinine type alkaloid with a methoxyl group. The HMBC correlations between OCH_3 (δ_H 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 [λ_{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 2*R*, 6*S*, 8*S*, and 14*R*. Thus, the structure of **9** was elucidated as 14β-methoxy-13,14-dihydronorsecurinine.

10 was obtained as yellow oil. The molecular formula of **10** was established as $C_{13}H_{17}NO_3$ by the HR-ESI-MS at *m*/*z*: 258.1093 $[M+Na]^+$ (Calcd for $C_{13}H_{17}NO_3Na$, 258.1101), which had the same molecular formular as **9**. The IR spectrum showed the presence of α,β-unsaturated γ-lactone ring (1756, 1642 cm^{-1}). The ^{1}H- and ^{13}C-NMR spectra of **10** were also very similar to those of **9**, suggesting that **10** was the isomeride of **9**. In the NOESY spectrum of **10**, the correlations between H-7b and H-13b, as well as between H-13b and 14-OMe (Fig. 3) were observed, indicating that the configuration at C-14 of **10** was opposite to that of **9**. The absolute configuration of **10** was finally determined as 2*R*, 6*S*, 8*S*, and 14*S* by comparing the CD spectrum of **10** with those of **7**, **8** and **9**. Therefore, the structure of **10** was elucidated as 14α-methoxy-13,14-dihydronorsecurinine.

Experimental

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 FT/IR-480 plus spectrometer. ^{1}H-, ^{13}C- and 2D-NMR spectra were determined on a Bruker-

AV-400 spectrometer in $CDCl_3$. ESI-MS spectra were run on a HP-1100 HPLC/EST spectrometer. HR-ESI-MS spectra were obtained on a Biosystems Mariner^{TM} 5140 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 C_{18} preparative column (5 μm, 20×250 mm).

Plant Material The twigs and leaves of *F. leucopyra* were collected in Nujiang country, Yunnan province of China, in September of 2006. The plant was authenticated by Prof. Guang-xiong Zhou of Jinan University. A voucher specimen (No. 060914) 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 *F. leucopyra* (8.5 kg) were extracted with 95% EtOH and the solution was evaporated *in vacuum* to get a residue (1045 g). The residue was dissolved in H_2O to form a suspension and then adjusted to pH 6 with 5% HCl. The acidic suspension was partitioned with $CHCl_3$ to remove the neutral components. The aqueous phase was basified with 2% $NH_3 \cdot H_2O$ to pH 8 and then extracted with $CHCl_3$ to obtain a total alkaloid part (42 g), which was subjected to silica gel column chromatography (CC) ($CHCl_3$–CH_3OH, 100 : 0→0 : 100) to give 10 fractions (1—10). Fraction 3 (3.86 g) was subjected to silica gel CC (*n*-hexane–EtOAc, 10 : 1→1 : 1) to afford 8 subfractions (3-1—3-8). Subfraction 3-4 (346 mg) was purified by HPLC [CH_3OH–H_2O–$(CH_3CH_2)_3N$, 85 : 15 : 0.02] to afford **8** (12 mg), **9** (11 mg) and **10** (9 mg). Fraction 4 (2.76 g) was purified by Sephadex LH-20 column (CH_3OH) and silica gel CC ($CHCl_3$–CH_3OH, 100 : 0→80 : 20) to yield **4** (21 mg) and **5** (12 mg). Fraction 5 (3.34 g) was subjected to reversed-phase silica gel (ODS) CC (CH_3OH–H_2O, 60 : 40→90 : 10) to afford 9 subfractions (5-1—5-9). Subfraction 5-3 (569 mg) was purified by preparative HPLC [CH_3OH–H_2O–$(CH_3CH_2)_3N$, 70 : 30 : 0.02] to obtain **1** (22 mg), **3** (19 mg) and **7** (15 mg), respectively. Subfraction 5-4 (369 mg) was purified by preparative HPLC [CH_3OH–H_2O–$(CH_3CH_2)_3N$, 75 : 25 : 0.02] to obtain **2** (22 mg) and **6** (19 mg), respectively.

4α-Hydroxyallosecurinine (**1**): Yellow oil; $[\alpha]_D^{20}$ −102 (c=0.07, MeOH); UV (MeOH) λ_{max} (log ε) 218 (1.65), 257 (1.97) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 216 (−3.34), 312 (−17.37) nm; IR (KBr) ν_{max}: 3436, 2948, 1746, 1632, 1464, 1286 cm^{-1}; ^{1}H- and ^{13}C-NMR data see Table 1; ESI-MS *m*/*z*: 234 $[M+H]^+$; HR-ESI-MS *m*/*z*: 234.1127 $[M+H]^+$ (Calcd for $C_{13}H_{16}NO_3$, 234.1125).

4α-Hydroxy-15α-methoxy-14,15-dihydroallosecurinine (**3**): Yellow oil; $[\alpha]_D^{20}$ −32 (c=0.12, MeOH); UV (MeOH) λ_{max} (log ε) 212 (1.85) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 226 (+2.87), 277 (+0.76) nm; IR (KBr) ν_{max} 3447, 2936, 1756, 1643, 1438, 1268 cm^{-1}; ^{1}H- and ^{13}C-NMR data see Table 1; ESI-MS *m*/*z*: 266 $[M+H]^+$; HR-ESI-MS *m*/*z*: 266.1387 $[M+H]^+$ (Calcd for $C_{14}H_{20}NO_4$, 266.1387).

4α-Methoxy-15α-methoxy-14,15-dihydrosecurinine (**5**): Yellow oil; $[\alpha]_D^{20}$ +37 (c=0.11, MeOH); UV (MeOH) λ_{max} (log ε) 220 (1.89) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 218 (−1.39), 240 (+2.62), 279 (+0.77), 334 (−0.50) nm; IR (KBr) ν_{max}: 3035, 2928, 1758, 1644, 1453, 1204 cm^{-1}; ^{1}H- and ^{13}C-NMR data see Table 1; ESI-MS *m*/*z*: 280 $[M+H]^+$; HR-ESI-MS *m*/*z*: 280.1540 $[M+H]^+$ (Calcd for $C_{15}H_{22}NO_4$, 280.1543).

14β-Hydroxy-13,14-dihydronorsecurinine (**7**): Yellow oil; $[\alpha]_D^{20}$ −45 (c=0.13, MeOH); UV (MeOH) λ_{max} (log ε) 216 (2.15) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 219 (−2.76), 238 (+1.74), 265 (−0.85) nm; IR (KBr) ν_{max}: 3442, 2929, 1755, 1631, 1460, 1274 cm^{-1}; ^{1}H- and ^{13}C-NMR see Table 2; ESI-MS *m*/*z*: 222 $[M+H]^+$; HR-ESI-MS *m*/*z*: 222.1126 $[M+H]^+$ (Calcd for $C_{12}H_{16}NO_3$, 222.1125).

14β-Methoxy-13,14-dihydronorsecurinine (**9**): Yellow oil; $[\alpha]_D^{20}$ −44 (c=0.16, MeOH); UV (MeOH) λ_{max} (log ε) 216 (2.21) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 208 (−2.56), 234 (+2.25), and 284 (−0.62) nm; IR (KBr) ν_{max}: 3135, 2924, 1755, 1649, 1451, 1383 cm^{-1}; ^{1}H- and ^{13}C-NMR data see Table 2; ESI-MS *m*/*z*: 236 $[M+H]^+$; HR-ESI-MS *m*/*z*: 236.1286 $[M+H]^+$ (Calcd for $C_{13}H_{18}NO_3$, 236.1281).

14α-Methoxy-13,14-dihydronorsecurinine (**10**): Yellow oil; $[\alpha]_D^{20}$ +27 (c=0.10, MeOH); UV (MeOH) λ_{max} (log ε) 217 (2.05) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 213 (−1.71), 235 (+2.69), 263 (−1.75) nm; IR (KBr) ν_{max}: 2936, 1756, 1642, 1460, 1189 cm^{-1}; ^{1}H- and ^{13}C-NMR data, see Table 2; ESI-MS *m*/*z*: 258 $[M+Na]^+$; HR-ESI-MS *m*/*z*: 258.1093 $[M+Na]^+$ (Calcd for $C_{13}H_{17}NO_3Na$, 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 by treatment of **1** (4.3 mg) with (*S*)-(+)-α-methoxy-α-(trifluoromethyl) phenylacetyl chloride.

(*S*)-MTPA Ester (**1a**): ^{1}H-NMR ($CDCl_3$, 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, br s, H-12), 6.70 (1H, dd, J=8.8, 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 $[M+H]^+$ (Calcd for $C_{23}H_{23}F_3NO_5$, 450.1523).

(*R*)-MTPA Ester (**1b**): ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 1.56 (1H, m, H-3b), 1.86 (1H, dd, J=14.8, 2.0 Hz, H-3b), 2.18 (1H, overlapped, H-5b), 2.18 (1H, d, J=11.6 Hz, H-8b), 2.65 (1H, dd, J=16.0, 9.6 Hz, H-5a), 2.82 (1H, dd, J=14.0, 12.8 Hz, H-6b), 3.09 (1H, dd, J=11.6, 4.4 Hz, H-8a), 3.57 (1H, overlapped, H-6a), 3.60 (3H, s, OMe of MTPA), 4.08 (1H, dd, J=14.0, 3.6 Hz, H-7), 4.81 (1H, t, J=4.8 Hz, H-2), 5.35 (1H, m, H-4), 6.04 (1H, br s, H-12), 6.70 (1H, dd, J=8.8, 6.4 Hz, H-15), 7.01 (1H, d, J=9.0 Hz, H-14), 7.27 and 7.45 (phenyl protons of MTPA). ESI-MS *m*/*z*: 450 $[M+H]^+$; HR-ESI-MS *m*/*z*: 450.1529 $[M+H]^+$ (Calcd for $C_{23}H_{23}F_3NO_5$, 450.1523).

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