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 vacuo* to get a residue. The residue was suspended in H₂O and then adjusted to pH 6 with 5% HCl. The acidic suspension was partitioned with CHCl₃ to remove the neutral component. The aqueous layer was basified with NH₃·H₂O and extracted with CHCl₃ 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 fluggeainol (**8**),^{6,7)} respectively.

1 was obtained as yellow oil, and its molecular formula was determined as C₁₃H₁₅NO₃ on the basis of HR-electrospray ionization (ESI)-MS at *m/z*: 234.1127 [M+H]⁺ (Calcd for C₁₃H₁₆NO₃, 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 ¹³C-NMR spectrum. Accordingly, the ¹H-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 ¹H- and ¹³C-NMR data of **1** (Table 1) with those of securinine⁵⁾ (**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 ($\Delta\epsilon$ -3.34) and 312 nm ($\Delta\epsilon$ -17.37), which were similar to those of securinine (**2**) [λ_{max} 227 nm ($\Delta\epsilon$ -1.13) and 302 nm ($\Delta\epsilon$ -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 4 α -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 C₁₄H₂₀NO₄, 266.1387) for the molecular formula of C₁₄H₁₉NO₄. The presence of α,β -unsaturated γ -lactone ring

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

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Table 1. NMR Data of **1**, **3** and **5** (CDCl₃, *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)} 1.35 ^{a)}	34.0	1.62 ^{a)} 1.06 (m)	33.8	2.14 (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) 1.53 (m)	35.5	1.69 ^{a)} 1.61 ^{a)}	32.1	1.80 (m) 1.27 ^{a)}
6	43.7	2.78 (m) 2.56 (m)	47.5	3.04 (m) 2.79 ^{a)}	48.9	3.06 ^{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) 1.91 (d, 10.0)	36.2	2.34 (dd, 10.8, 4.6) 1.99 (d, 10.8)	33.6	2.51 (dd, 11.2, 4.8) 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) 2.74 ^{a)}	30.6	2.84 ^{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	—	—	58.1	3.27 (s)	56.6	3.35 (s)
15-OCH ₃	—	—	—	—	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 ¹³C 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 ¹³C-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 B⁸⁾ (**4**) showed that the NMR signals of the two compounds were similar, except that **3** only had a methoxyl group as well as the ¹³C-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 OCH₃ (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 ($\Delta\epsilon$ +2.87) and 277 nm ($\Delta\epsilon$ +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 4 α -hydroxy-15 α -methoxy-14,15-dihydroallosecurinine.

5 showed an [M+H]⁺ ion peak at *m/z*: 280.1540 (Calcd for C₁₅H₂₂NO₄, 280.1543) in the HR-ESI-MS spectrum, consistent with a molecular formula of C₁₅H₂₁NO₄. The IR spectrum indicated the presence of α,β -unsaturated γ -lactone ring (1758, 1644 cm⁻¹). The ¹H- and ¹³C-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** (CDCl₃, *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 OCH₃ (δ_H 3.35) and C-4 (δ_C 77.4) as well as between OCH₃ (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 4 α -methoxy-15 α -methoxy-14,15-dihydrosecurinine.

7 was obtained as yellow oil. The molecular formula of **7** was determined as C₁₂H₁₅NO₃ according to the HR-ESI-MS at *m/z*: 222.1126 [M+H]⁺ (Calcd for C₁₂H₁₆NO₃, 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 ¹H-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 ¹³C-NMR spectrum displayed twelve carbon signals including five methylenes, four methines and three quaternary carbons. Furthermore, in the ¹³C-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 flugeainol (**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 ($\Delta\epsilon$ -2.76), 238 nm ($\Delta\epsilon$ +1.74), and 265 nm ($\Delta\epsilon$ -0.85)] were also similar to those of flugeainol (**8**) [λ_{max} 218 nm ($\Delta\epsilon$ -1.37), 231 nm ($\Delta\epsilon$ +2.67), and 261 nm ($\Delta\epsilon$ -0.27)]. Hence, the absolute configuration of **7** was assigned as 2*R*, 6*S*, 8*S*, and 14*R*. **7** was identified as 14 β -hydroxy-13,14-dihydronorsecurinine.

9 was isolated as yellow oil. Its molecular formula was determined as C₁₃H₁₇NO₃ by the HR-ESI-MS at *m/z*: 236.1286 [M+H]⁺ (Calcd for C₁₃H₁₈NO₃, 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 ¹H- and ¹³C-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 OCH₃ (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 ($\Delta\epsilon$ -2.56), 234 nm ($\Delta\epsilon$ +2.25), and 284 nm ($\Delta\epsilon$ -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 14 β -methoxy-13,14-dihydronorsecurinine.

10 was obtained as yellow oil. The molecular formula of **10** was established as C₁₃H₁₇NO₃ by the HR-ESI-MS at *m/z*: 258.1093 [M+Na]⁺ (Calcd for C₁₃H₁₇NO₃Na, 258.1101), which had the same molecular formula as **9**. The IR spectrum showed the presence of α,β -unsaturated γ -lactone ring (1756, 1642 cm⁻¹). The ¹H- and ¹³C-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 14 α -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. ¹H-, ¹³C- 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 MarinerTM 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 C₁₈ 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 vacuo* 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% $\text{NH}_3 \cdot \text{H}_2\text{O}$ 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.

4 α -Hydroxyallosecurinine (1): Yellow oil; $[\alpha]_{\text{D}}^{20}$ –102 ($c=0.07$, MeOH); UV (MeOH) λ_{max} (log ϵ) 218 (1.65), 257 (1.97) nm; CD (MeOH) λ_{max} ($\Delta\epsilon$) 216 (–3.34), 312 (–17.37) nm; IR (KBr) ν_{max} : 3436, 2948, 1746, 1632, 1464, 1286 cm^{-1} ; ¹H- and ¹³C-NMR data see Table 1; ESI-MS m/z : 234 [M+H]⁺; HR-ESI-MS m/z : 234.1127 [M+H]⁺ (Calcd for C₁₃H₁₆NO₃, 234.1125).

4 α -Hydroxy-15 α -methoxy-14,15-dihydroallosecurinine (3): Yellow oil; $[\alpha]_{\text{D}}^{20}$ –32 ($c=0.12$, MeOH); UV (MeOH) λ_{max} (log ϵ) 212 (1.85) nm; CD (MeOH) λ_{max} ($\Delta\epsilon$) 226 (+2.87), 277 (+0.76) nm; IR (KBr) ν_{max} : 3447, 2936, 1756, 1643, 1438, 1268 cm^{-1} ; ¹H- and ¹³C-NMR data see Table 1; ESI-MS m/z : 266 [M+H]⁺; HR-ESI-MS m/z : 266.1387 [M+H]⁺ (Calcd for C₁₄H₂₀NO₄, 266.1387).

4 α -Methoxy-15 α -methoxy-14,15-dihydrosecurinine (5): Yellow oil; $[\alpha]_{\text{D}}^{20}$ +37 ($c=0.11$, MeOH); UV (MeOH) λ_{max} (log ϵ) 220 (1.89) nm; CD (MeOH) λ_{max} ($\Delta\epsilon$) 218 (–1.39), 240 (+2.62), 279 (+0.77), 334 (–0.50) nm; IR (KBr) ν_{max} : 3035, 2928, 1738, 1644, 1453, 1204 cm^{-1} ; ¹H- and ¹³C-NMR data see Table 1; ESI-MS m/z : 280 [M+H]⁺; HR-ESI-MS m/z : 280.1540 [M+H]⁺ (Calcd for C₁₅H₂₂NO₄, 280.1543).

14 β -Hydroxy-13,14-dihydronorsecurinine (7): Yellow oil; $[\alpha]_{\text{D}}^{20}$ –45 ($c=0.13$, MeOH); UV (MeOH) λ_{max} (log ϵ) 216 (2.15) nm; CD (MeOH) λ_{max} ($\Delta\epsilon$) 219 (–2.76), 238 (+1.74), 265 (–0.85) nm; IR (KBr) ν_{max} : 3442, 2929, 1755, 1631, 1460, 1274 cm^{-1} ; ¹H- and ¹³C-NMR data see Table 2; ESI-MS m/z : 222 [M+H]⁺; HR-ESI-MS m/z : 222.1126 [M+H]⁺ (Calcd for C₁₂H₁₆NO₃, 222.1125).

14 β -Methoxy-13,14-dihydronorsecurinine (9): Yellow oil; $[\alpha]_{\text{D}}^{20}$ –44 ($c=0.16$, MeOH); UV (MeOH) λ_{max} (log ϵ) 216 (2.21) nm; CD (MeOH) λ_{max} ($\Delta\epsilon$) 208 (–2.56), 234 (+2.25), and 284 (–0.62) nm; IR (KBr) ν_{max} : 3135, 2924, 1755, 1649, 1451, 1383 cm^{-1} ; ¹H- and ¹³C-NMR data see Table 2; ESI-MS m/z : 236 [M+H]⁺; HR-ESI-MS m/z : 236.1286 [M+H]⁺ (Calcd for C₁₃H₁₈NO₃, 236.1281).

14 α -Methoxy-13,14-dihydronorsecurinine (10): Yellow oil; $[\alpha]_{\text{D}}^{20}$ +27 ($c=0.10$, MeOH); UV (MeOH) λ_{max} (log ϵ) 217 (2.05) nm; CD (MeOH) λ_{max} ($\Delta\epsilon$) 213 (–1.71), 235 (+2.69), 263 (–1.75) nm; IR (KBr) ν_{max} : 2936, 1756, 1642, 1460, 1189 cm^{-1} ; ¹H- and ¹³C-NMR data, see Table 2; ESI-MS m/z : 258 [M+Na]⁺; HR-ESI-MS m/z : 258.1093 [M+Na]⁺ (Calcd for

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 vacuo*. 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**): ¹H-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 C₂₃H₂₃F₃NO₅, 450.1523).

(*R*)-MTPA Ester (**1b**): ¹H-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 C₂₃H₂₃F₃NO₅, 450.1523).

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References

- 1) Snieckus V., "The Alkaloids," Vol. 14, ed. by Manske R. H. F., Academic Press, New York, 1973, pp. 425—503.
- 2) Ohsaki A., Ishiyama H., Yoneda K., Kobayashi J., *Tetrahedron Lett.*, **44**, 3097—3099 (2003).
- 3) Wang Y., Li Q., Ye W.-C., Ip C.-F., Ip Y.-R., Zhao S.-X., *Chin. Tradit. Herb Drugs*, **38**, 163—167 (2007).
- 4) Wang G.-C., Wang Y., Li Q., Liang J.-P., Zhang X.-Q., Yao X.-S., Ye W.-C., *Helv. Chim. Acta*, **91**, 1124—1129 (2008).
- 5) Arbain D., Birkbeck A. A., Byrne L. T., Sargent M. V., Skelton B. W., White A. H., *J. Chem. Soc. Perkin Trans. 1*, **1991**, 1863—1869 (1991).
- 6) Chen M. Q., Hou L. L., *Acta Bot. Sin.*, **27**, 625—629 (1985).
- 7) Dehmlow E. V., Guntenhoner M., Ree T. V., *Phytochemistry*, **52**, 1715—1716 (1999).
- 8) Ohsaki A., Kobayashi Y., Yoneda K., Kishida A., Ishiyama H., *J. Nat. Prod.*, **70**, 2003—2005 (2007).
- 9) Weenen H., Nkunya M. H. H., Bray D. H., Mwasumbi L. B., Kinabo L. S., Kilimali V. A. E. B., Wijinberg J. P. B. A., *Planta Med.*, **56**, 371—373 (1990).
- 10) Mensah J. L., Lagarde I., Ceschin C., Michel G., Gleye J., Fouraste I., *J. Ethnopharmacol.*, **28**, 129—133 (1990).
- 11) Dong N. Z., Gu Z. L., Chou W. H., Kwok C. Y., *Acta Pharm. Sin.*, **20**, 267—270 (1999).
- 12) Saito S., Kotera K., Shigematsu N., Ide A., Sugimoto N., Horii Z., Hanaoka M., Yamawaki Y., Tamura Y., *Tetrahedron*, **19**, 2085—2099 (1963).
- 13) Han G., LaPorte M. G., Folmer J. J., Werner K. M., Weinreb S. M., *J. Org. Chem.*, **65**, 6293—6306 (2000).
- 14) Rognan D., Boulanger T., Hoffmann R., Vercauteren D. P., Andre J. M., Durant F., Wermuth C. G., *J. Med. Chem.*, **35**, 1969—1977 (1992).
- 15) Galvez-Ruano E., Aprison M. H., Robertson D. H., Lapkowitz K. B., *J. Neurosci. Res.*, **42**, 666—673 (1995).
- 16) Ohtani I., Kusumi T., Kashman Y., Kakisawa H., *J. Am. Chem. Soc.*, **113**, 4092—4096 (1991).