Securinega Alkaloids from the Wood of *Securinega suffruticosa* var. *amamiensis*

Ayumi Ohsaki,*[,†] Yukino Kobayashi,[†] Kaisuke Yoneda,[‡] Akio Kishida,[†] and Haruaki Ishiyama[§]

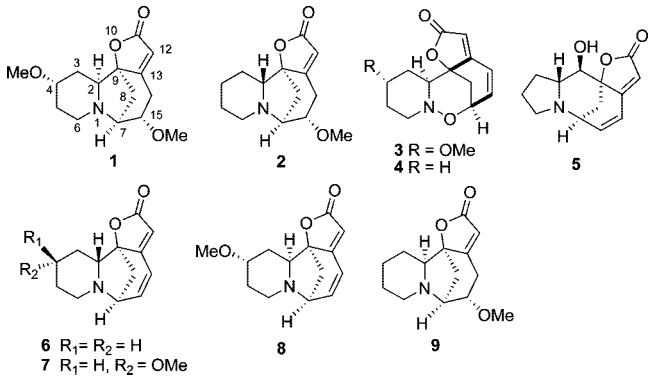
Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University, Chiyoda-ku, Tokyo 101-0062, Japan, Graduate School of Pharmaceutical Sciences, Osaka University, Suita 565-0871, Japan, and Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo 060-0812, Japan

Received June 16, 2007

Three new *Securinega* alkaloids, secu'amamines B−D (**1**–**3**), were isolated from the wood of the Japanese medicinal plant *Securinega suffruticosa* var. *amamiensis*, together with five known analogues (**4**, **6**–**9**). The structures **1**−**3** were elucidated by spectroscopic methods including 2D NMR, and all eight compounds were evaluated for cytotoxicity against two cancer cell lines.

In a search for biologically active and structurally unique compounds from subtropical and tropical medicinal plants,[1] we have studied the minor constituents of *Securinega suffruticosa* (Pall.) Rehd. var. *amamiensis* Hurusawa (Euphorbiaceae),[2] commonly known as "Amami-hitotsubahagi", which occurs in the Ryukyu Islands in the subtropical area of Japan. This plant has been used to treat the aftereffects of infantile paralysis.[3] Its main alkaloid, securinine (**6**),[4] was reported to be a $GABA_A$ receptor antagonist with significant in vivo CNS activity.[5,6] Securinine has been reported to have antimalarial[7] and antibacterial activity,[8] in addition to apoptotic activity in human leukemia HL-60 cells.[9] Previously, we reported the structure of secu'amanine A (**5**)[10] as a new indolizidine alkaloid, isolated from the leaves of *S. suffruticosa* var. *amamiensis*. In the present study, we describe the isolation and structural elucidation of three new *Securinega* alkaloids, secu'amamines B−D (**1**–**3**), and cytotoxicity results for all of the *Securinega* alkaloids obtained.

The wood of *S. suffruticosa* var. *amamiensis* was extracted with MeOH. The MeOH extract was partitioned between EtOAc and 3% aqueous tartaric acid. Water-soluble materials were adjusted to pH 10 with Na_2CO_3 and partitioned with $CHCl_3$ and then EtOAc. The $CHCl_3$-soluble materials were subjected to silica gel column chromatography [*n*-hexane−$CHCl_3$ (1:1) → $CHCl_3$−MeOH (100:0) → (0:100)], and the fractions obtained were purified by passage over silica gel [$CHCl_3$−MeOH (100:0) → (1:1) and/or EtOAc−MeOH (100:0) → (1:1)], to isolate the new *Securinega* alkaloids secu'amamines B (**1**), C (**2**), and D (**3**), together with the known related *Securinega* alkaloids phyllantidine (**4**),[11] securinine (**6**), 4-epiphyllanthine (**7**),[12] securitinine (**8**),[12] and 15α-methoxy-14,15- dihydrophyllochrysine (**9**).[13]



The molecular formula, $C_{15}H_{21}O_4N$, of secu'amamine B was established by HREIMS, and the IR spectrum implied the presence of an α,β-unsaturated γ-lactone group (1760, 1644 cm^{-1}). The gross structure of **1** was deduced from detailed analysis of the ^1H and ^{13}C NMR data aided by 2D NMR experiments ($^1H-^1H$ COSY, HMQC, and HMBC). The ^1H and ^{13}C NMR and HMQC data for **1** indicated the presence of one ester carbonyl, one sp^3 oxyquaternary carbon, two sp^3 oxymethines, one sp^2 quaternary carbon, one sp^2 methine, two sp^3 methines, five sp^3 methylenes, and two methoxy groups. With two of the six unsaturations thus accounted for, it was concluded that **1** contains four rings. The $^1H-^1H$ COSY spectrum revealed connectivities of C-2 to C-6, C-7 to C-8, and C-7 to C-14. HMBC correlations (Figure 1) were observed from H-2 to C-4, C-6, and C-9 (δ_C 92.1 s), from H-8 to C-2, C-9, from H-7 (δ_H 3.39 d) to C-6, and from H-6 to C-4 (δ_C 76.2 d) and C-5 and indicated the presence of an indolizine skeleton (rings A and B). Additionally, the correlations of H-8 to C-13 (δ_C 174.9 s) and C-15 (δ_C 81.7 d) (ring C), H-14 to C-7, C-13, and C-15, and an olefinic proton H-12 (δ_H 5.75, d , $J = 2.4$ Hz) of the α,β-unsaturated γ-lactone to C-9, C-11, and C-14 suggested that compound **1** has a securinine skeleton type without a $\Delta^{14,15}$-olefin.[13] Two methoxy groups were attached to C-4 and C-15 as deduced from the cross-peaks of H-4 and H-15 to the methoxy goups, respectively. Thus, the gross structure of secu'amamine B (**1**) was elucidated as **1**. NOESY correlations (Figure 2) of H-2/H-6a, H-2/H-8a, H-14a/H-3b, and H-7/H-6b suggested that ring A was in a half-chair form, and the angular proton (H-2) between rings A and B was in the α-orientation. The cross-peaks of H-2/OMe-4, H-8b/OMe-15, H-7/OMe-15, and H-14b/15-OMe indicated β-orientations for H-4 and H-15, respectively. Therefore, the relative configuration of **1** was assigned as shown in Figure 2. The CD spectrum of **1** showed Cotton effects at λ_{max} 217 nm ($\Delta\varepsilon$ +3.61), 238 nm (sh, $\Delta\varepsilon$ +2.37), and 284 nm ($\Delta\varepsilon$ +0.32), which were similar to those of 15α-methoxy-14,15-dihydrophyllochrysine (**9**) [λ_{max} 227 nm ($\Delta\varepsilon$ +3.44), 256 nm ($\Delta\varepsilon$ +1.56), and 272 ($\Delta\varepsilon$ +1.63)], and established the absolute configuration by X-ray crystal structure analysis.[13] Therefore, the absolute configuration of **1** was assigned as depicted.

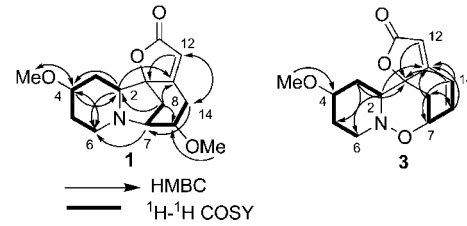


Figure 1. HMBC and $^1H-^1H$ COSY correlations of compounds **1** and **3**.

The molecular formula, $C_{14}H_{19}O_3N$, of secu'amamine C (**2**) was established by HREIMS, and the IR spectrum implied the presence of an α,β-unsaturated γ-lactone group (1760, 1644 cm^{-1}). The ^1H and ^{13}C NMR (Tables 1 and 2) and HMQC data of **2** resembled those of **1** except for the lack of one methoxy group. Detailed

* To whom correspondence should be addressed. Tel & Fax: +81-3-5280-8153. E-mail: a-ohsaki.fm@tmd.ac.jp.
† Tokyo Medical and Dental University.
‡ Osaka University.
§ Hokkaido University.

10.1021/np0780102 CCC: $37.00 © 2007 American Chemical Society and American Society of Pharmacognosy
Published on Web 12/01/2007

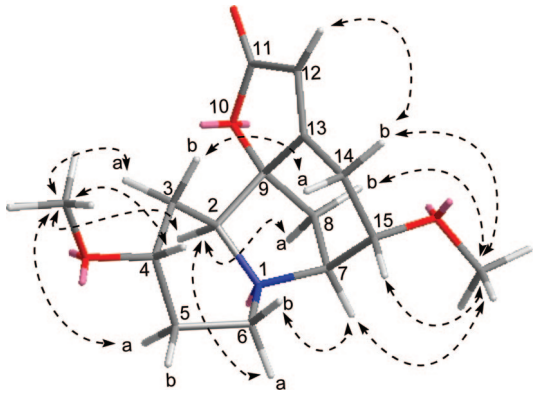


Figure 2. Key NOESY correlations of compound **1**.

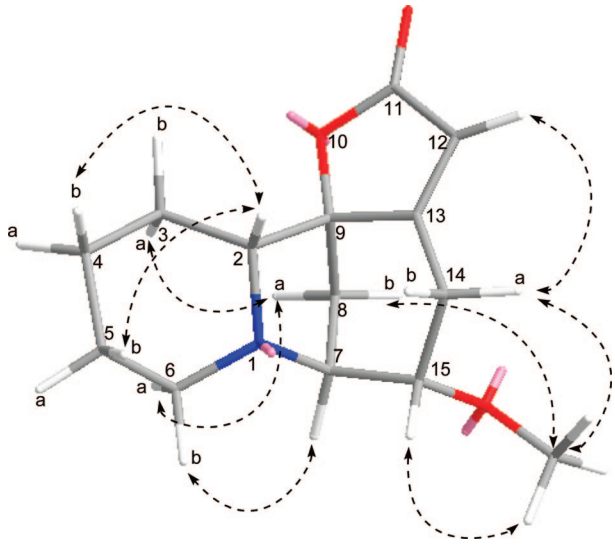


Figure 3. Key NOESY correlations of compound **2**.

analysis using the 2D NMR (HMQC, HMBC) spectra revealed that the gross structure of **2** lacks one methoxy group at the C-4 position of **1**. The NOESY spectrum of **2** showed cross-peaks of H-8a/H-3a, H-8a/ H-6a, H-7/H-6b, H-12/H-14a, and H-8b/15-OMe (Figure 2). Thus, the structure and relative configuration of secu'amamine C was elucidated as **2**.

The molecular formula, $C_{14}H_{17}O_4N$, of secu'amamine D (**3**) was determined by positive-ion HRFABMS [*m*/*z* 264.1227, $(M + H)^+$, Δ –0.8 mmu], and its IR spectrum indicated the presence of an α,β-unsaturated γ-lactone group (1759, 1635 cm^{-1}). The ^1H and ^{13}C NMR data of **3** (Tables 1 and 2) showed a deshielded shift at the C-7 position (δ_C 70.9 d, δ_H 4.74 dt, J = 5.8, 3.4 Hz), which suggested the presence of an oxygen atom between a nitrogen and the C-7 position. The chemical shifts of the ^1H NMR spectrum were similar to those of phyllantidine (**4**). The EIMS fragmentation was also similar to those of phyllantidine (**4**).[11] A methoxy group attached to C-4 was deduced from the HMBC cross-peak of the methoxy group (δ_H 3.29 s) to C-4 (δ_C 72.0 d). HMBC and $^1H-^1H$ COSY correlations for **3** are shown in Figure 4. NOESY cross-peaks of H-2/H-8a and H-2/H-6a suggested an α-orientation for H-2 (Figure 4). The configuration of the methoxy group at C-4 was revealed to be in the α-orientation as deduced from the coupling constant of H-4 (δ_H 3.43, brdt, J = 5.5, 2.7 Hz) and the NOESY cross-peaks of H-14/OMe-4, H-3a/OMe-4, and H-5a/OMe-4. Therefore, the structure and relative configuration of secu'amamine D was elucidated as **3**. The CD spectrum of **3** showed Cotton effects at λ_{max} 208 nm ($\Delta\varepsilon$ −2.66) and λ_{max} 264 nm ($\Delta\varepsilon$ −20.84), which were similar to those of phyllantidine (**4**) [λ_{max} 212 nm ($\Delta\varepsilon$ −1.09) and 262 nm ($\Delta\varepsilon$ −15.94)]. Therefore, the absolute configuration of **3** was assigned as depicted.

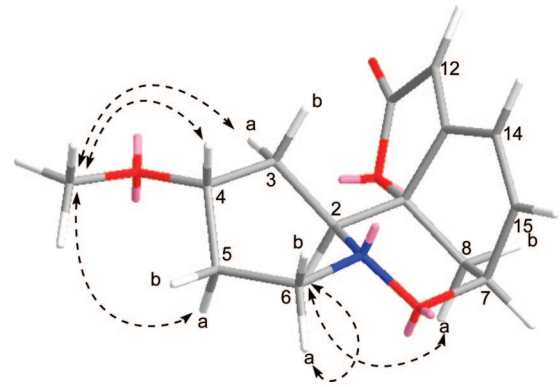


Figure 4. Key NOESY correlations of compound **3**.

Securinine (**6**) and 4-epiphyllanthine (**7**) both exhibited cytotoxicity against human epidermoid carcinoma KB cells (IC_{50}, **6**, 2.2; **7**, 3.0 μg/mL) and murine lymphoma L1210 cells (IC_{50}, **6**, 1.9; **7**, 3.7 μg/mL) in vitro, while secu'amamines B−D (**1**−**3**) and phyllantidine (**4**) did not show such activity (>5 μg/mL).

Experimental Section

General Experimental Procedures. Optical rotations were determined on a JASCO DIP-370 digital polarimeter. UV spectra were

Table 1. ^{1}HNMR Data of Compounds **1**–**3**

position	**1**[a] δ_H mult. (*J*)	**2**[a] δ_H mult. (*J*)	**3**[b] δ_H mult. (*J*)
2	3.38 dd (12.3, 1.5)	2.95 m	3.19 dd (12.0, 2.5)
3a	1.73 br d (12.4)	1.51 m, 2H	1.02 ddd (14.1, 12.0, 2.6)
3b	1.03 dt (2.7, 12.4)		2.04 m
4a	3.58 m, 2H	1.88 m	3.43 brdt (5.5, 2.7)
4b		1.40 m	
5a	1.85 br d (12.8)	1.49 m, 2H	1.67 tq (10.9, 2.8)
5b	1.62 m		1.91 ddt (14.2, 5.8, 2.9)
6a	2.97 dt (2.5, 12.3)	2.96 m, 2H	2.96 m, 2H
6b	2.84 m		
7	3.39 d (2.3)	3.43 m	4.74 dt (5.8, 3.4)
8a	2.33 dd (10.3, 6.2)	2.53 dd (10.4, 5.8)	2.05 m
8b	1.95 d (10.3)	1.85 d (10.4)	2.54 dd (11.4, 3.4)
12	5.75 d (2.4)	5.57 d (1.4)	5.85 s
14a	3.03 ddd (17.0, 6.2, 2.4)	2.87 d (16.8)	6.88 d (9.4)
14b	2.85 d (17.0)	2.83 br d (16.8)	
15	3.62 dd (5.5, 4.9)	3.59 m	6.30 dd (9.4, 5.8)
OMe-4	3.27 s		3.29 s
OMe-15	3.29 s	3.30 s	

[a] In CD_3OD at 600 MHz. [b] $CDCl_3$ at 500 MHz.

Table 2. ^{13}C NMR Data of Compounds **1**–**3**

position	**1**[a]	**2**[a]	**3**[b]
2	62.4	61.9	65.2
3	30.5	27.7	27.1
4	76.2	23.2	72.0
5	32.9	25.4	29.3
6	48.0	50.4	50.3
7	60.7	63.8	70.9
8	36.6	33.9	40.6
9	92.1	93.6	82.6
11	175.4	175.9	171.9
12	114.5	112.1	113.4
13	174.9	177.5	164.2
14	32.4	30.8	126.4
15	81.7	81.6	134.4
OMe-4	57.0		55.8
OMe-15	57.8	57.8	

[a] In CD_3OD at 150 MHz. [b] In $CDCl_3$ at 125 MHz.

obtained on JASCO V-560. CD spectra were measured on a JASCO J-720 spectrometer. IR spectra were obtained on a JASCO FT/IR-300E spectrometer. ^1H and ^{13}C NMR spectra were obtained on Bruker AVANCE 500 and ARX-600 spectrometers using tetramethylsilane as the internal standard. HREIMS and HRFABMS were obtained on a JEOL HX-100 spectrometer.

Plant Material. The wood of *S. suffruticosa* var. *amamiensis* was collected in a herb garden in Suita, Osaka, Japan, in 2001. The plant was identified by Dr. K. Yoneda (Osaka University), and a voucher specimen (No. J-105) with identification has been deposited at the Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University (Chiyoda-ku, Tokyo).

Extraction and Isolation. The MeOH extract (fresh weight, 1.2 kg) of the wood of *S. suffruticosa* var. *amamiensis* (102 g) was partitioned between EtOAc and 3% aqueous tartaric acid. The water layer was adjusted to pH 10 with Na_2CO_3 and partitioned with $CHCl_3$ and then EtOAc. The $CHCl_3$-soluble (2.25 g) and EtOAc-soluble materials (2.20 g) were obtained on drying. The $CHCl_3$-soluble material was subjected to silica gel column chromatography (*n*-hexane−$CHCl_3$, 1:1 → $CHCl_3$−MeOH, 100:0 → 0:100, Wako gel C-300, Wako Pure Chemical Industries Ltd.), and alkaloidal fractions were obtained. These fractions were purified by a silica gel column ($CHCl_3$−MeOH, 100:0 → 90:10, and/or EtOAc−MeOH, 100:0 → 90:10) to obtain secu'amamines B (**1**, 68.0 mg), C (**2**, 21.5 mg), and D (**3**, 3.2 mg), phyllantidine (**4**, 2.6 mg), securinine (**6**, 4.7 mg), 4-epiphyllanthine (**7**, 9.1 mg), securitinine (**8**, 44.8 mg), and 15α-methoxy-14,15-dihydroxyphyllochrysine (**9**, 84.2 mg).

Secu'amamine B (1): colorless, amorphous solid; $[\alpha]^{25}_D$ +42.6 (*c* 0.54, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 230 (3.27), 62 (sh, 2.92) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 217 (+3.61), 238, (sh, +2.37), 284 (+0.32) nm; IR (KBr) ν_{max} 1761, 1638 cm^{-1}; ^1H and ^{13}C NMR (Tables 1 and 2); HREIMS *m/z* 279.1456 $[M]^+$ (calcd for $C_{15}H_{21}O_4N$, 279.1456).

Secu'amamine C (2): colorless, amorphous solid; $[\alpha]^{25}_D$ +82.1 (*c* 0.15, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 232 (3.41), 283 (2.53) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 216 (−0.37), 221 (−0.45), 237 (+1.54), 279 (+0.27), 332 (−0.22) nm; IR (KBr) ν_{max} 1710, 1644 cm^{-1}; ^1H and ^{13}C NMR (Tables 1 and 2); HREIMS *m/z* 249.1349 $[M]^+$ (calcd for $C_{14}H_{19}O_3N$, 249.1365).

Secu'amamine D (3): colorless, amorphous solid; $[\alpha]^{25}_D$ −303.9 (*c* 0.26, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 212 (sh, 3.33), 261 (3.62) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 208 (−2.66), 264 (−20.84) nm; IR (KBr) ν_{max} 1759, 1635 cm^{-1}; ^1H and ^{13}C NMR (Tables 1 and 2); HRFABMS *m/z* 264.1227 $[M + H]^+$ (calcd for $C_{14}H_{18}O_4N$, 264.1235).

Acknowledgment. This work was supported in part by the Kampo Science Foundation of Japan.

Supporting Information Available: The CD spectra of compounds **1**−**4** and **9**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Kobayashi, J.; Sekiguchi, M.; Shimamoto, S.; Shigemori, H.; Ishiyama, H.; Ohsaki, A. *J. Org. Chem.* **2002**, *67*, 6449–6455, and references therein.
(2) Hatsushima, S. *Ryukyu Syokubutushi; Okinawa Seibutsu kyoiku kenkyukai* **1975**, 372–373.
(3) Turov, A. D.; Aleshkina, Y. A. *Meditsinskaya Promyshlennost SSSR* **1957**, *11*, 54–55.
(4) Saito, S.; Kotera, K.; Shigematsu, N.; Ide, A.; Sugimoto, N.; Horii, Z.; Hanaoka, M.; Yamawaki, Y.; Tamura, Y. *Tetrahedron* **1963**, *19*, 2085–2099, and references therein.
(5) Rognan, D.; Boulanger, T.; Hoffmann, R.; Vercauteren, D. P.; Andre, J.-M.; Durant, F.; Wermuth, C.-G. *J. Med. Chem.* **1992**, *35*, 1969–1977.
(6) Galvez-Ruano, E.; Aprison, M. H.; Robertson, D. H.; Lapkowitz, K. B. *J. Neurosci. Res.* **1995**, *42*, 666–673.
(7) Weenen, H.; Nkunya, M. H. H.; Bray, D. H.; Mwasumbi, L. B.; Kinabo, L. D.; Kilimali, V. A. E. B.; Wijinberg, J. B. *Planta Med.* **1990**, *56*, 371–373.
(8) Mensah, J. L.; Lagarde, I.; Ceschin, C.; Michel, G.; Gleye, J.; Fouraste, I. *J. Ethnopharmacol.* **1990**, *28*, 129–133.
(9) Dong, N.-Z.; Gu, Z.-L.; Chou, W.-H.; Kwok, C.-Y. *Zhongguo Yaoli Xuebao* **1999**, *20*, 267–270.
(10) Ohsaki, A.; Ishiyama, H.; Yoneda, K.; Kobayashi, J. *Tetrahedron Lett.* **2003**, *44*, 3097–3099.
(11) Horii, Z.; Imanishi, T.; Yamauchi, M.; Hanaoka, M. *Tetrahedron Lett.* **1972**, 1877–1880.
(12) Arabain, D.; Birkbeck, A. A.; Byrne, L. T.; Sargent, M. V.; Skeleton, B. W.; White, A. H. *J. Chem. Soc., Perkin Trans. 1* **1991**, 1863–1869.
(13) Arbain, D.; Byrne, L. T.; Cannon, J. R.; Engelhardt, L. M.; White, A. H. *Aust. J. Chem.* **1990**, *43*, 439–445.

NP0780102