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Secu'amamines E–G, new alkaloids from *Securinega suffruticosa* var. *amamiensis*

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ABSTRACT

One new *ent*-neosecurinane-type alkaloid, secu'amamine E (**1**), and two novel alkaloids consisting of a *ent*-neosecurinane and a piperidine unit, secu'amamine F (**2**), and secu'amamine G (**3**), were isolated from *Securinega suffruticosa* var. *amamiensis*. Their structures and stereochemistry were elucidated on the basis of spectroscopic analyses.

In a search for biologically active and structurally unique compounds in subtropical and tropical medicinal plants,[1] we investigated the minor constituents of *Securinega suffruticosa* (Pall.) var. *amamiensis* Hurusawa (Euphorbiaceae),[2] which grows in the Ryukyu Islands, the subtropical region of Japan. Its main alkaloid, securinine,[3] has been reported to be a $GABA_A$ receptor antagonist with significant in vivo CNS activity.[4] Securinine also has been reported to exhibit antimalarial[5] activity and antibacterial[6] activity, as well as apoptotic activity in human leukemia HL-60 cells.[7] Previously, we reported the structure of secu'amamines A–D.[8,9] Secu'amamine A has a novel structural framework, which was investigated in order to achieve total synthesis.[10]

In this Letter, we describe the isolation and elucidation of the structure of a new *ent*-neosecurinane alkaloid,[11] secu'amamine E (**1**), and novel alkaloids, secu'amamines F (**2**) and G (**3**), consisting of a *ent*-neosecurinane and a piperidine unit.

The leaves and twigs of *S. suffruticosa* var. *amaminensis* (dry weight, 366 g) were extracted with MeOH. The MeOH extracts (57.0 g) were partitioned between petroleum ether, EtOAc and 3% aqueous tartaric acid. Water-soluble materials were adjusted to pH 10 with Na_2CO_3 and successively partitioned with $CHCl_3$, EtOAc, and *n*-BuOH.

The alkaloidal $CHCl_3$-soluble materials (1.18 g) were subjected to amino-silica gel column chromatography (hexane/EtOAc, 100:0→0:100, and then EtOAc/MeOH, 100:0→20:80, Chromatorex-NH, Fuji Silysia, Japan) to obtain eight fractions (F1-8). The fifth fraction (F-5, hexane/EtOAc, 1:1, 35.3 mg) was separated on a silica-gel column (hexane/EtOAc, 100:0→0:100, and then EtOAc/MeOH, 100:0→0:100, Wako-gel, C-300, Wako) and purified using TLC plates (Et_2O/CH_2Cl_2, 1:1, NH_2-F254S, Merck) to isolate secu'amamine E (2.5 mg). The seventh fraction (F-7, hexane/EtOAc, 3:7, 598 mg) was separated by using LH-20 (MeOH, Sephadex), and then silica gel ($CHCl_3$/MeOH, 100:0→0:100) followed by reversed phase HPLC (MeOH/30 mM, $(NH_4)_2CO_3$, 6:4, CAPCEL PAK C18, Shiseido, Japan) to isolate secu'amamines F (**2**, 2.0 mg) and G (**3**, 2.9 mg).

The molecular formula, $C_{13}H_{17}NO_3$, of secu'amamine E (**1**), {$[\alpha]_D^{25}$ −43.8 (*c* 0.08, MeOH)} was established by HREIMS [*m*/*z* 235.1186, M^+, Δ −2.2 mmu]. The IR spectrum suggested the presence of a hydroxyl group (3437 cm^{-1}) and an α,β-unsaturated γ-lactone group (1739, 1651 cm^{-1}). The gross structure of secur'amamine E (**1**) was deduced from extensive analyses of the ^1H, ^{13}C NMR, and DEPT experiments and 2D NMR data (^1H–^1H COSY, HSQC, HMBC, and TOCSY). These data for **1** indicated one ester carbonyl, one sp^3 oxy quaternary, one sp^3 oxymethine, one sp^2 methine, one sp^2 quaternary, two sp^3 methine, and six sp^3 methylene carbons. With two of six unsaturations thus accounted for, it was concluded that **1** contains four rings (rings A–D). The ^1H–^1H COSY spectrum revealed connectivities of C-2 to C-6, C-7 to C-9, and C-7 to C-15 (Fig. 2A). HMBC correlations were observed from H-2 to C-4, C-6, and C-9, from H-3 to C-2, C-4, and C-10, from H-5 to C-4, and C-6, from H-9 to C-2, C-7, C-8, C-10, and C-14, from H-7 to C-2, from

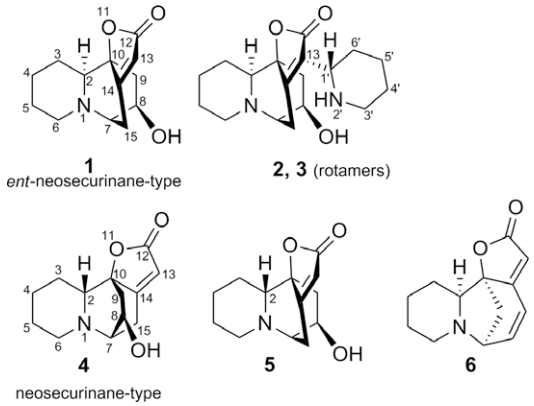

Figure 1. Chemical structure of compounds **1**–**6**.

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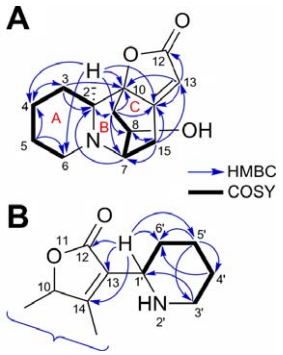

Figure 2. Key HMBC correlations of secu'amamine E (**1**) and the piperidine unit of secu'amamine F (**2**) and G (**3**).

H-8 to C-15, and from H-15 to C-7 and C-14. These correlations indicated the presence of a 1,4-ethano-quinolizine skeleton with one hydroxy group at C-8 (rings A, B, and C). Further, the cross peaks of HMBC observed from H-15 to C-13 (sp^2 olefinic carbon of α,β-unsaturated γ-lactone), from H-13 to C-10, C-12, and C-14 and from H-2 to C-10 and C-14 (Fig. 2A). These correlations suggested that compound **1** has a neosecurinane skeleton.[11] Therefore, the gross structure of secu'amamine E (**1**) was elucidated as **1** (Fig. 1).

The NMR data of compound **1** were compared with the NMR data of securinol A (**4**),[11] which was newly isolated by us from this plant. Their ^{13}C NMR data differed at C-2, 8, and 14, and the coupling pattern of the oxygenated proton H-8 (δ_H 4.33, dddd) was different from that of **4**. These findings indicated that compound **1** has differing combinations of chiral centers at C-2, C-7, C-8, and C-10. (Tables 1 and 2) NOESY correlations of H-2/H-4α, H-2/H-6α, H-2/H-8, H-6β/H-7, and H-2/H-9a suggested that ring A had a chair-like form, the angular proton (H-2) between rings A and B was in an α-orientation, and the proton of H-7 was in a β-orientation, and in addition, a hydroxyl group at C-8 extended to the outside of its B/C ring cage. The *W*-coupling (J = 3.0 Hz) was observed between

Table 1
^{1}H NMR data of secu'amamines E (**1**) to G (**3**)[14]

No.	**1**[a] δ_H (mult, *J*, Hz)	**2**[a] δ_H (mult, *J*, Hz)	**3**[a] δ_H (mult, *J*, Hz)
2	2.79, dd, 12.0, 1.8	2.72, dd, 12.0, 1.8	2.71, dd, 12.1, 2.1
3α	1.50, m	1.38, m	1.38, m
3β	0.86, qd, 12.0, 4.0	0.82, qd, 12.0, 3.9	0.80 qd, 12.1, 3.7
4α	1.35, m	1.26, m	1.25, m
4β	1.81, m	1.73, m	1.73, m
5a,b	1.53m, 1.58m	1.42–1.50, m, 2H	1.42–1.50, m, 2H
6α	2.75, td, 11.0, 3.2	2.68, m	2.67, m
6β	2.94m	2.86, m	2.86, m
7	2.88, ddd, 5.7, 1.4, 1.3	2.81, m	2.81, m
8	4.30, dddd	4.21, dddd	4.27, dddd
	9.5, 5.0, 3.0, 1.4	9.5, 5.0, 3.0, 1.4	9.5, 5.0, 3.0, 1.4
9a	2.66, dd, 12.2, 9.5	2.58, dd, 12.2, 9.5	2.57, dd, 12.2, 9.5
9b	1.38, dd, 12.2, 5.0	1.29, dd, 12.2, 5.0	1.29, dd, 12.2, 5.0
13	5.74, t, 1.9		
15a	2.82, m	2.83–2.96, m, 2H	2.78–2.96, m, 2H
15b	2.97, ddd, 18.4, 1.9, 1.3		
1′		3.49, dd, 11.5, 2.6	3.44, dd, 11.4, 2.5
3′α		3.04, br d, 12.4	2.61, m
3′β		2.68, m	3.02, br d, 12.3
4′a		1.44, m	1.42, m
4′b		1.60, m	1.58, m
5′α		1.82, m	1.81, m
5′β		1.45, m	1.44, m
6′a		1.61, m	1.56, m
6′b		1.73, m	1.72, m

[a] In CD_3OD at 500 MHz.

Table 2
^{13}C NMR data of secu'amamines E (**1**) to G (**3**) and securinol A (**4**)

No.	**1**[a] δ_C	**2**[a] δ_C	**3**[a] δ_C	**4**[a] δ_C
2	66.50	66.44	66.37	62.84
3	26.80	26.66	26.60	24.20
4	25.11	25.02	25.96	22.41
5	27.71	27.59	27.52	25.62
6	53.55	53.42	53.35	52.49
7	60.39	60.19	60.19	59.24
8	65.37	65.25	65.32	69.76
9	41.57	41.54	41.60	41.14
10	86.29	84.37	84.36	84.48
12	176.31	175.44	175.51	173.63
13	112.04	125.12	125.22	112.66
14	177.25	168.77	168.60	171.78
15	30.32	30.12	30.02	30.17
1′		54.44	54.41	
3′		47.25	47.73	
4′		26.52	26.60	
5′		25.48	25.56	
6′		31.40	31.41	

[a] In CD_3OD at 125 MHz.

H-8 and H-15a deduced from the crosspeaks of COSY, long-range COSY, and TOCSY, respectively (Fig. 2A). Therefore, the relative stereochemistry of **1** was assigned as shown in Figure 1. The CD spectrum of **1** yielded a Cotton curve that was completely opposite that of securinol A (**4**), whose absolute configuration was determined by the X-ray method described by Arbain et al.[11] (Fig. 4). The absolute configuration of **1** was therefore assigned as depicted (2*S*, 7*R*, 8*R*, 10*S*). Secu'amamine E (**1**) differed from virosine B (**5**),[12] isolated from *Flueggea virosa*, in the configuration of C-2. Thus, secu'amamine E (**1**) may be a biosynthetically related compound of allosecurinine (**6**)[11] derived from the combination of chiral centers at C-2, C-7, and C-10.

The molecular formula, $C_{18}H_{26}O_3N_2$, of secu'amamine F (**2**), {$[\alpha]_D^{25}$ −20.8 (*c* 0.096, MeOH)} was established by HREIMS [*m*/*z*

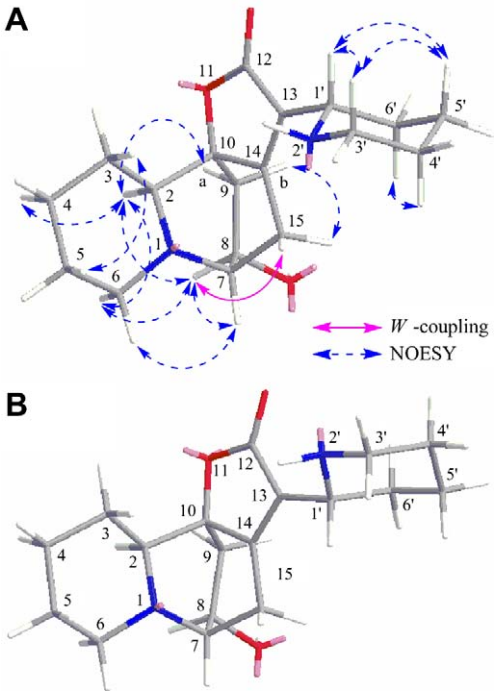

Figure 3. NOESY correlations of secu'amamine F (**2**) [A] and secu'amamine G (**3**) [B].

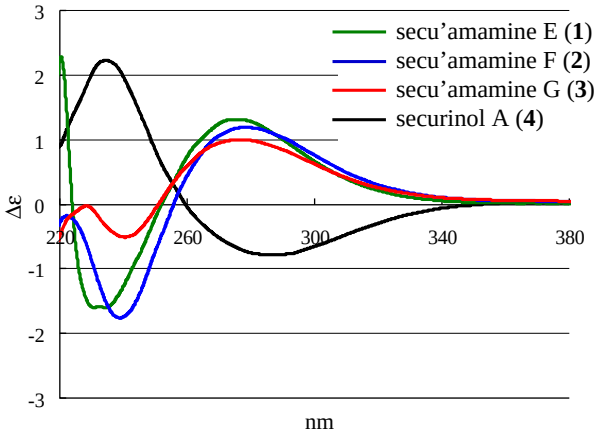

Figure 4. CD spectra of secu'amaines E (**1**) to G (**3**) and securinol A (**4**).[13]

318.1946, M^+, Δ +0.3 mmu], and the IR spectrum suggested the presence of a hydroxyl group (3437 cm^{-1}) and α,β-unsaturated γ-lactone group (1751, 1637 cm^{-1}). The ^1H and ^{13}C NMR (Tables 1 and 2) and HSQC data of **2** closely resembled those of secu'amamine E (**1**); however, the data indicated the lack of an H-13 proton and the presence of four sp^3 methylenes and one sp^3 methine (δ_C 54.44, δ_H 3.49 dd). The molecular formula deduced from HREIMS and its seven unsaturations suggested the presence of a piperidine ring unit. The HMBC cross peaks of H-1′ to C-12, C-13, and C-14 indicated that the structure of compound **2** comprised a segment of secu'amamine E (**1**) and one piperidine unit, and that these segments connected between C-13 and C-1′. The HMBC correlations of **2** in the piperidine unit were observed from H-1′ to C-5′ and C-6′, from H-3′ to C-1′, from H-4′ to C-6′, and from H-5′ to C-3′, C-4′, and C-6′ (Fig. 2B). These correlations verified the presence of a piperidine unit. Therefore, the gross structure of secu'amamine F (**2**) was elucidated as **2** (Fig. 1). NOESY correlations of H-2/H-4α, H-2/H-6α, H-2/H-8, H-2/H-9a, H-6β/H-7, H-6α/H-8, and H-15b/H-9b suggested that secu'amamine F (**2**) has the same stereochemistry as secu'amamine E (**1**) in regard to rings A–C. Further, the NOESY cross peaks of H-1′/H-3′β, H-1′/H-5′β, H-5′β/H-3′β and H-4′α/H-6′α indicated that the piperidine unit was the chair-like form (Fig. 3A).

The molecular formula, $C_{18}H_{26}O_3N_2$, of secu'amamine G (**3**), {$[\alpha]_D^{25}$ −30.6 (*c* 0.049, MeOH)}was established by HREIMS [*m*/*z* 318.1935, M^+, Δ −0.9 mmu] and the IR spectrum suggested the presence of a hydroxyl group (3437 cm^{-1}) and α,β-unsaturated γ-lactone group (1760, 1644 cm^{-1}). The ^1H and ^{13}C NMR (Tables 1 and 2) and HSQC data of **3** closely resembled those of secu'amamine F (**2**); however, these data were slightly different from those of secu'amamine F (**2**) around the H-1′, H-3′ and H-6′ protons. The chemical shifts of protons shifted to the upfield in comparison with those of secu'amamine F (**2**). Therefore, secu'amamine F (**2**) (Fig. 3A) and G (**3**) (Fig. 3B) were rotamers each other between C-13 and C-1′ linkage.[15] The relative stereochemistry of **2** and **3** was assigned as shown in Figure 1, and the H-1′ of secu'amamines F (**2**) and G (**3**) were in a α-orientation. The CD spectra of **2** and **3** showed close similarity to Cotton curves as compared with that of secu'amamine E (**1**) (Fig. 4). Thus, the absolute configuration were assigned as 1′*S*.

Secu'amamines E (**1**)–G (**2**) were first isolation as *ent*-neosecurinane-type alkaloids.

Secu'amamines E (**1**)–G (**3**) did not display cytotoxicity against P388 cells (>10 μg/mL).

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13. *Secu'amamine E* (**1**): colorless amorphous solid; UV (MeOH) λ_{max} (log ε) 227 (3.52) nm; CD (MeOH) λ_{max} (log ε) 231 (−3.29), 275 (+3.21), *Secu'amamine F* (**2**): colorless amorphous solid; UV (MeOH) λ_{max} (log ε) 218 (3.88) nm; CD (MeOH) λ_{max} (log ε) 222 (+2.80), 239 (−3.79), 278 (+3.62) nm, *Secu'amamine G* (**3**): colorless amorphous solid; UV (MeOH) λ_{max} (log ε) 220 (3.84) nm; CD (MeOH) λ_{max} (log ε) 228 (+1.86), 240 (−3.12), 276 (+3.41) nm.
14. The hydrogen peaks corresponding to O*H* (C-8) and N*H* (C-2′) of secu'amamines E (**1**) to G (**3**) were failed in the application of acetone-d_6 and DMSO-d_6.
15. The purified secu'amamines F (**2**) and G (**3**) were mixed to **2** and **3** in the equal proportions within two months at room temperature.