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Secu'amamine A, a novel indolizidine alkaloid from *Securinega suffruticosa* var. *amamiensis*

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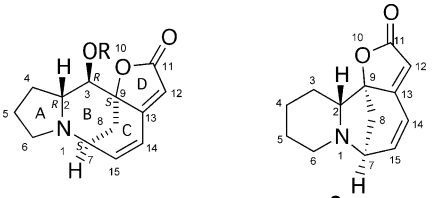
Abstract—A novel indolizidine alkaloid, secu'amamine A (**1**), was isolated from the leaves and twigs of the medicinal plant, *Securinega suffruticosa* var. *amamiensis* together with securinine (**2**). The structure and relative stereochemistry of **1** was elucidated by spectroscopic data and its absolute configuration was assigned on the basis of the *OMe*-madelate method.

More than 20 *Securinega* alkaloids[1] have been isolated from several genera belonging to the family Euphorbiaceae. Securinine (**2**),[2] a major alkaloid isolated from the leaves of *Securinega suffruticosa*, is a unique indolizidine alkaloid containing an α,β-unsaturated-γ-lactone ring, and a stereospecific $GABA_A$ receptor antagonist with a significant in vivo CNS activity,[3] while **2** exhibits antimalarial and antibacterial activity, and causes apoptosis against human leukaemia HL-60 cells.[4]

In our search for biologically active and structurally unique compounds from the subtropical and tropical plants,[5] investigation of minor alkaloidal constituents of *S. suffruticosa* var. *amamiensis* resulted in the isolation of a novel indolizidine alkaloid, secu'amamine A (**1**). In this paper we describe the isolation and structure elucidation of **1**.

The leaves and twigs of *S. suffruticosa* var. *amamiensis* collected at a herb garden in Osaka were extracted with MeOH. MeOH extracts were partitioned between hexane and 90% aqueous MeOH. MeOH-soluble materials were partitioned between EtOAc and 3% aqueous tartaric acid. Water-soluble materials, adjusted at pH 10 with saturated Na_2CO_3, were partitioned with EtOAc, and EtOAc-soluble materials were subjected to a silica gel column ($CHCl_3$/BuOH/AcOH/H_2O, 1.5:6:1:1) to give an alkaloidal fraction. This fraction was separated with a silica gel column ($CHCl_3$/*n*-C_6H_{14}, 1:1→$CHCl_3$/MeOH, 100:0→0:100), in which fractions eluted with $CHCl_3$/MeOH (95:5) were purified by a silica gel column ($CHCl_3$/MeOH, 98:2, and then EtOAc/MeOH, 99:1) to afford a novel alkaloid, secu'amamine A (**1**) (0.013%), together with known related alkaloids, securinine (**2**, 0.14%), securinol A (0.0021%),[6] allosecurinine (0.0094%),[7] and 4-epiphyllanthine (0.0026%).[6]



1: R=H
1a: R=Ac

2

The molecular formula, $C_{13}H_{15}O_3N$, of secu'amamine A (**1**), colourless oil, $[\alpha]_D^{23}$ −479° (*c* 0.149, $CHCl_3$), was established by HREIMS [*m*/*z* 233.1046, M^+, Δ −0.5 mmu]. IR absorptions implied the presence of α,β-unsaturated-γ-lactone (1736 and 1637 cm^{-1}) and hydroxy group (3448 cm^{-1}), while the UV spectrum also indicated the presence of α,β-unsaturated-γ-lactone chromophore (254 nm, log ε 4.06, MeOH). The gross structure of **1** was deduced from detailed analysis of ^1H

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Table 1. ^{1}H and ^{13}C NMR data for secu'amamine A (**1**)

Position	^{1}H ($CDCl_3$)[a]	^{1}H (CD_3OD)[a]	^{13}C ($CDCl_3$)[a]	H coupled with C ($CDCl_3$)[b]
2	2.57 dt (J=9.8, 6.7)	2.81m	59.63	H-3, H-4b, H_2-6a, H-7
3	3.74 d (J=9.8)	3.74 d (J=9.9)	74.45	H-2, H-4b, H_2-8
4a	2.08 m	2.13 m	28.11	H-3, H_2-5, H-6a
4b	1.65 m	1.77 m		
5a	1.93 m	2.05 m	22.14	H_2-4, H_2-6
5b	1.77 m	1.90 m		
6a	3.02 dt (J=3.7, 8.5)	3.20 m	48.53	H-2, H-4a
6b	2.61 q (J=8.5)	2.78 m		
7	3.94 dt (J=6.1, 3.7)	4.13 m	52.04	H-6b, H_2-8, H-14, H-15
8a	2.38 dd (J=11.6, 2.4)	2.48 dd (J=12.0, 1.2)	36.55	H-3, H-15
8b	2.01 dd (J=11.6, 3.7)	2.11 dd (J=11.8, 3.1)		
9			87.10	H-3, H-7, H_2-8, H-12, H-14
11			172.57	H-12
12	5.86 s	6.00 s	114.13	H-14
13			162.07	H-3, H-8a, H-12, H-14, H-15
14	6.78 d (J=9.2)	6.97 d (J=9.6)	124.18	H-7, H-12
15	6.19 dd (J=9.8, 6.1)	6.33 dd (J=9.6, 5.6)	134.00	H-7, H-8a

[a] δ in ppm.
[b] HMBC correlations.

and ^{13}C NMR data (Table 1) aided with 2D NMR (^{1}H–^{1}H COSY, HOHAHA, HMQC, and HMBC). The ^{1}H and ^{13}C NMR data indicated that the molecule possessed one carboxylic carbon (δ_C 172.57), one trisubstituted olefin (δ_C 162.07, δ_C 114.13; δ_H 5.86 s), one disubstituted olefin (δ_C 124.18; δ_H 6.78 d, δ_C 134.00; δ_H 6.19 dd), one oxygenated sp^3 quaternary carbon (δ_C 87.10), one sp^3 oxymethine (δ_C 74.45; δ_H 3.74 d), two sp^3 methines, and four sp^3 methylenes.

Since three out of seven unsaturations were thus accounted for, it was concluded that **1** contained four rings. The ^{1}H–^{1}H COSY spectrum revealed connectivities (Fig. 1) of C-2 to C-3, C-2∼C-6, C-7 to C-8, C-7 to C-15, and C-15 to C-14. HMBC correlations (Fig. 1) of H-2 to C-6, H-6b to C-7, H-3 to C-8 and C-9, H_2-8 to C-3 and C-9, and H-7 to C-2, and C-9 indicated the presence of an indolizidine skeleton (rings A and B), in which a secondary hydroxy group and a lactonyl oxygen were attached to C-3 and C-9, respectively. On the other hand, HMBC correlations of H-3 to C-13, H-12 to C-9, C-11, C-13, and C-14, H-14 to C-9 and C-13, and H-8a to C-13 suggested the presence of a cyclohexene ring (C-7∼C-9 and C-13∼C-15) and an α,β-unsaturated γ-lactone ring (C-9, O-10, and C-11∼C-13). Thus, the gross structure of secu'amaminine A (**1**) was elucidated to be **1**. Secu'amamine A (**1**) was treated with Ac_2O and pyridine to give its monoacetate (**1a**), which was supported by the molecular formula, $C_{15}H_{17}O_4N$, of **1a**, established by HREIMS [m/z 275.1172, M^+, Δ +1.4 mmu]. The ^{1}H and ^{13}C NMR data[8] of **1a** indicated that the acetoxy group was attached to C-3.

NOESY correlations (Fig. 2) of H_2-6 to H-7, H-6a to H-8a, H-3 to H-4b and H-8a, and H-2 to H-15 of **1** indicated a chair-like form of ring B, a *trans*-relationship between H-2 and H-3 ($J_{H\text{-}2,H\text{-}3}$= 9.8 Hz), a β-orientation of H-2, and α-orientations of H-3 and H-7. Therefore, the relative stereochemistry of **1** was assigned as shown in Figure 2.

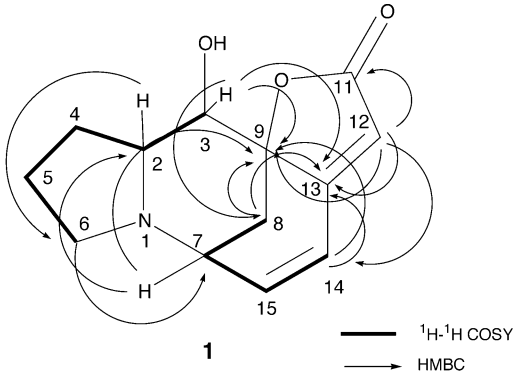


Figure 1. Selected ^{1}H–^{1}H COSY and HMBC correlations for secu'amamine A (**1**).

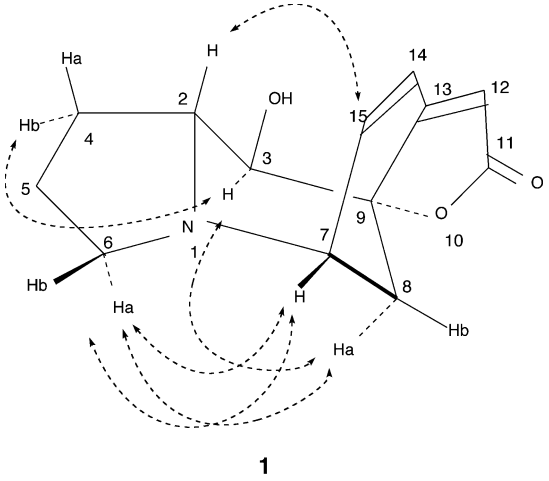
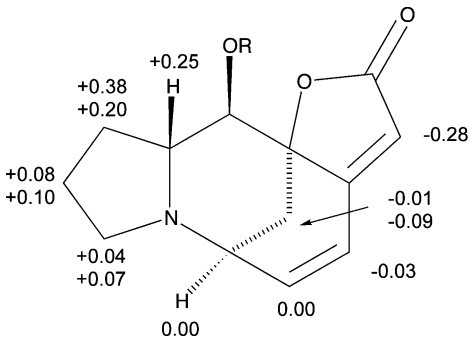


Figure 2. Selected NOESY correlations for Secu'amamine A (**1**).



3a:R=(*S*)-MPA
3b: R=(*R*)-MPA

Figure 3. $\Delta\delta$ values [$\Delta\delta$ (in ppm) $=\delta_R-\delta_S$] obtained for (*S*)- and (*R*)-MPA esters (**3a** and **3b**) at C-3 of secu'amamine A (**1**). (in CD_3OD).

The absolute configuration of the hydroxy group at C-3 of **1** was determined by the *OMe*-mandelate method[9] as follows. Treatment of **1** with (*S*)-(+)- and (*R*)-(−)-methoxyphenyl acetic acid (MPA) afforded the corresponding (*S*)- and (*R*)-MPA esters (**3a** and **3b**, respectively). $\Delta\delta$ [δ(*R*-MPA ester)-δ(*S*-MPA ester)] values obtained from the ^{1}H NMR (Fig. 3) spectra[10] suggested that the absolute configuration of the hydroxy group at C-3 was *R* and the remaining three chiral centres were 2*R*, 7*S* and 9*S*.

Secu'amamine A (**1**) possesses a novel fused tetracyclic ring system consisting of an indolizidine ring, a cyclohexene ring, and an α,β-unsaturated γ-lactone ring. Biological activity of **1** is currently investigated.

Acknowledgements

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8. ^{1}H NMR data of **1a** ($CDCl_3$): δ 2.65 (m, H-2), 5.10 (d, $J=10.4$, H-3), 1.90 (m, H-4a), 1.70 (m, H-4b), 1.93 (m, H-5a), 1.73 (m, H-5b), 3.03 (dt, $J=8.5$, 4.3, H-6a), 2.63 (m, H-6b), 3.94 (m, H-7), 2.45 (brd, $J=11.6$, H-8a), 2.01 (m, H-8b), 5.84 (s, H-12), 6.79 (d, $J=9.2$, H-14), 6.20 (dd, $J=9.2$, 5.5, H-15), 2.00 (s, 3H, 3-OAc); ^{13}C NMR data of **1a** ($CDCl_3$): δ 58.17 (C-2), 73.58 (C-3), 27.78 (C-4), 22.27 (C-5), 48.56 (C-6), 51.95 (C-7), 36.81 (C-8), 84.83 (C-9), 171.51 (C-11), 114.07 (C-12), 161.48 (C-13), 123.92 (C-14), 134.17 (C-15), 169.67 (3-OAc) and 20.66 (3-OAc).
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10. Compound **3a**: ^{1}H NMR data (CD_3OD): 2.46 (m, H-2), 5.11 (d, $J=9.8$, H-3), 1.34 (m, H_2-4), 1.85 (m, H-5a), 1.64 (m, H-5b), 3.03 (m, H-6a), 2.57 (q, $J=8.8$, H-6b), 4.01 (m, H-7), 2.46 (m, H-8a), 2.09 (dd, $J=11.8$, 3.3, H-8b), 6.02 (s, H-12), 6.94 (d, $J=9.5$, H-14), 6.30 (dd, $J=9.5$, 5.6, H-15), 7.40 (m, 5H, Ph), 3.40 (s, 3H, MeO), HR-ESIMS (positive): $C_{22}H_{23}O_5NNa$ (404.1464, Δ −1.0 mmu). Compound **3b**: ^{1}H NMR data (CD_3OD): 2.71 (m, H-2), 5.13 (d, $J=9.5$, H-3), 1.72 (m, H-4a), 1.54 (m, H-4b), 1.93 (m, H-5a), 1.74 (m, H-5b), 3.07 (m, H-6a), 2.64 (q, $J=8.1$, H-6b), 4.01 (m, H-7), 2.45 (dd, $J=11.9$, 2.6, H-8a), 2.00 (dd, $J=11.9$, 3.2, H-8b), 5.74 (s, H-12), 6.91 (d, $J=9.5$, H-14), 6.30 (dd, $J=9.5$, 5.5, H-15), 7.40 (m, 5H, Ph), 3.40 (s, 3H, MeO), HR-ESIMS (positive): $C_{22}H_{23}O_5NNa$ (404.1456, Δ −1.8 mmu).