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SERAGADINE A, A β -CARBOLINE ALKALOID FROM MARINE SPONGE

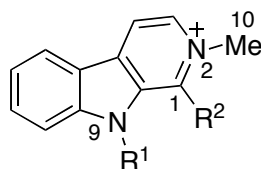
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Abstract - A new quaternary β -carboline alkaloid, seragadine A (**1**), and three known related alkaloids (**2** ~ **4**) have been isolated from an Okinawan Haplosclerida sponge (SS-1022). The structure of **1** was determined by the spectroscopic data and its synthesis.

INTRODUCTION

Many β -carboline alkaloids from natural sources are known to have a variety of bioactivity¹. In our search for bioactive substances from marine invertebrates, some different types of β -carboline alkaloids have been isolated from a tunicate *Eudistoma glaucus*², and sponges of *Amphimedon* sp.³ and family Thorectidae⁴. In our continuing search for new metabolites from marine sponges, we have recently isolated a new β -carboline alkaloid with 2-*N*-methyl and 1-ethyl group, seragadine A (**1**), together with three known related alkaloids⁵⁻⁹ (**2** ~ **4**) from an Okinawan Haplosclerida marine sponge (SS-1022). Here we describe the isolation and structure elucidation of **1** and its synthesis.



- 1** : R¹=H, R²=Et
2 : R¹=R²=H
3 : R¹=Me, R²=H
4 : R¹=H, R²=Me

RESULTS AND DISCUSSION

The sponge (SS-1022) collected off Seragaki, Okinawa, was extracted with MeOH. The MeOH extract was partitioned between EtOAc and H₂O, and subsequently the aqueous layer was extracted with *n*-BuOH. *n*-BuOH-soluble materials of the extract were subjected to SiO₂ gel and C₁₈ column chromatographies followed by C₁₈ HPLC to afford seragadine A (**1**, 0.00004%, wet weight) together with known related alkaloids, 2-methyl-9*H*- β -carbolin-2-ium^{5,6} (**2**), 2,9-dimethyl-9*H*- β -carbolin-2-ium^{6,7} (**3**), and melinonine F^{8,9} (**4**).

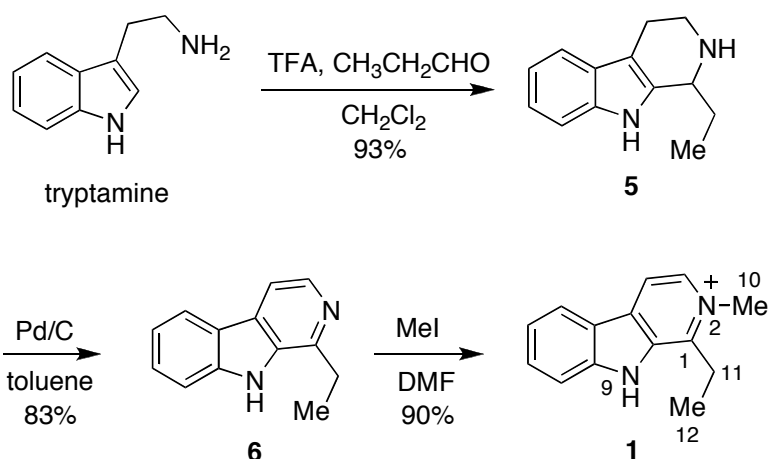
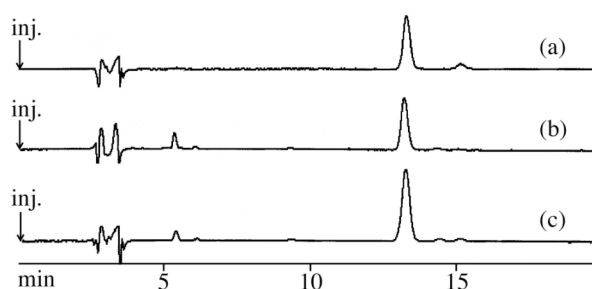
The molecular formula, C₁₄H₁₅N₂, of **1** was established by HRESIMS [*m/z* 211.1241 (M)⁺, Δ +0.5 mmu]. IR absorptions indicated the existence of OH and/or NH (3243 cm⁻¹) functionalities. The ¹H NMR (Table 1) spectrum of **1** was similar to that of **4**, except for the presence of a methylene (δ_{H} 3.55, q) and methyl (δ_{H} 1.51, t) resonances in **1**. The molecular weight of **1** was larger than that of **4** by 14 mass units, indicating that a methyl group at C-1 in **4** was replaced by an ethyl one in **1**, which was also supported by the COSY correlation for H₂-11/H₃-12. Thus, seragadine A (**1**) was elucidated to be 1-ethyl-2-methyl-9*H*- β -carbolin-2-ium.

Table 1. ¹H NMR Data for Seragadine A (**1**) and Synthetic **1** in CD₃OD

position	Seragadine A (1) δ_{H} [ppm]	synthetic 1 δ_{H} [ppm]
3	8.53 (d, 6.9)	8.48 (d, 6.6)
4	8.48 (d, 6.9)	8.47 (d, 6.6)
5	7.80 (d, 6.9)	7.80 (d, 8.4)
6	7.51 (t, 7.5)	7.50 (t, 7.4)
7	7.85 (t, 8.1)	7.83 (t, 8.3)
8	8.42 (d, 8.1)	8.40 (d, 8.1)
10	4.47 (s) 3H	4.50 (s)
11	3.55 (q, 7.6) 2H	3.58 (q, 7.7)
12	1.51 (t, 7.7) 3H	1.54 (t, 7.7)

Because of a small amount of isolated sample of **1**, we planned to prepare **1** by synthesis from tryptamine (Scheme 1). A TFA-catalyzed Pictet–Spengler reaction¹⁰ was utilized to form the tetrahydro- β -carboline (**5**). Treatment of **5** with palladium black in boiling toluene for 2 h produced the ring *C*-dehydrogenated product¹¹ (**6**) in 83% yield. The quaternary iminium (**1**) was prepared from **6** by using CH₃I in DMF at room temperature for 12 h¹². The ¹H NMR data (Table 1) and C₁₈ HPLC profile (Figure 1) of synthetic **1** were identical with those of natural specimen. Thus, the structure of seragadine A (**1**) was confirmed to be 1-ethyl-2-methyl- β -carbolin-2-ium.

Seragadine A (**1**) showed antibacterial activity and a weak cytotoxicity against L1210 murine leukemia cells *in vitro*.

Scheme 1. Synthesis of seragadine A (**1**)Figure 1. HPLC profiles of seragadine A (**1**) (a), synthetic **1** (b), and co-injection of them (c).

EXPERIMENTAL

General Experimental Procedures

IR and UV spectra were recorded on JASCO FT/IR-230 and Shimadzu UV-1600PC spectrophotometer, respectively. NMR spectra were recorded on a Bruker AMX-600 spectrometer using 2.5 mm micro cells (Shigemi Co., Ltd). The 3.35 and 49.8 ppm resonances of residual CD_3OD were used as internal references for ^1H and ^{13}C NMR spectra, respectively. Positive-mode ESIMS was obtained on a JEOL JMS 700-TZ spectrometer using a sample dissolved in MeOH.

Collection, Extraction, and Isolation

The dark brown Haplosclerida sponge (SS-1022) was collected off Seragaki, Okinawa Island and kept frozen until used. The sponge (2.6 kg, wet weight) was extracted three times with MeOH (2 L) and then evaporated to give a residue (66.12 g). A portion of the residue (24.78 g) was partitioned between *n*-hexane (500 mL X 2) and 90% aqueous MeOH (500 mL). The 90% aqueous MeOH-soluble portion was evaporated under reduced pressure and partitioned between EtOAc (500 mL) and H_2O (500 mL).

Water-soluble portion was extracted with *n*-BuOH (500 mL). A part (257 mg) of the *n*-BuOH-soluble fraction (513 mg) was subjected to a silica gel column chromatography (Wako gel C-300, Wako Pure Chemical, 2 X 30 cm) with CHCl₃/MeOH. The fraction eluted with CHCl₃/MeOH (3:2) was subjected to a C₁₈ Sep-Pak column with 30 % aqueous MeCN and then reversed-phase HPLC (Mightysil RP-18, KANTO CHEMICAL CO., INC., 10 X 250 mm; flow rate 2.5 mL/min; UV detection at 240 nm; eluent MeCN /H₂O/TFA, 18:82:0.1) to afford seragadine A (**1**, 0.2 mg, 0.00004%, wet weight, *t_R* 25.2 min), 2-methyl-9*H*-β-carbolin-2-ium (**2**, 0.6 mg, 0.00012%, wet weight, *t_R* 13.6 min), 2,9-dimethyl-9*H*-β-carbolin-2-ium (**3**, 0.4 mg, 0.00008%, *t_R* 20.4), and melinonine F (**4**, 0.5 mg, 0.00010%, *t_R* 21.0)

Seragadine A (1): colorless oil; IR (neat) $\nu_{\max}$ 3243 cm⁻¹; UV (MeOH) $\lambda_{\max}$ 308 nm (17000); ¹H NMR (see Table 1); ESIMS *m/z* 211 (M)⁺, HRESIMS *m/z* 211.1241 [calcd. for C₁₄H₁₅N₂, (M)⁺, 211.1236].

Synthesis of Seragadine A (1): To a stirred solution of 1-ethyl-β-carboline (**6**, 145 mg, 0.74 mmol) in DMF (2 mL) was added methyl iodide (120 mL) under an argon atmosphere. After stirring at rt for 48 h, the reaction mixture was concentrated *in vacuo*. The residue was purified by an amino silica gel column chromatography (CHCl₃/MeOH, 9 : 1) to give 1-ethyl-2-methyl-β-carboline (140 mg, 0.66 mmol, 90%). : colorless amorphous solid; IR (film) $\nu_{\max}$ 3156, 3075, 2981, 2902, 2787, 2682, 1683, 1633, 1198, 1126 cm⁻¹; UV (MeOH) $\lambda_{\max}$ 308 (ε 17000); ¹H NMR (see Table 1); ¹³C NMR (CD₃OD, 500 MHz) δ 146.9, 146.4, 136.8, 134.7, 133.9, 125.0, 123.9, 122.3, 120.2, 114.6, 97.2, 45.6, 24.1, 12.8. ESIMS *m/z* 211 (M)⁺, HRESIMS *m/z* 211.1231 [calcd. for C₁₄H₁₅N₂, (M)⁺, 211.1236].

HPLC Analysis of Synthetic 1 and Seragadine A (1). The synthetic **1** and seragadine A (**1**) were subjected to C18 HPLC analyses (Mightysil RP-18, KANTO CHEMICAL CO., INC., 10 X 250 mm; flow rate 1.0 mL/min; UV detection at 240 nm; eluent MeCN/H₂O (20:80)). The retention times of synthetic **1** and seragadine A (**1**) were both 13.3 min (Figure 1).

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