HETEROCYCLES, Vol. 74, 2007, pp. 849 - 853. © The Japan Institute of Heterocyclic Chemistry
Received, 26th September, 2007, Accepted, 15th November, 2007, Published online, 16th November, 2007. COM-07-S(W)77

SERAGADINE A, A β-CARBOLINE ALKALOID FROM MARINE SPONGE

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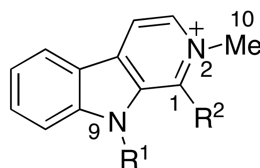
Abstract - A new quaternary *β*-carboline alkaloid, seragadine A (**1**), and three known related alkaloids (**2** ~ **4**) have been isolated from an Okinawan Haplosclerida sponge (SS-1022). The structure of **1** was determined by the spectroscopic data and its synthesis.

INTRODUCTION

Many *β*-carboline alkaloids from natural sources are known to have a variety of bioactivity[1]. 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 Thorectidae[4]. 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 alkaloids[5-9] (**2** ~ **4**) from an Okinawan Haplosclerida marine sponge (SS-1022). Here we describe the isolation and structure elucidation of **1** and its synthesis.



1 : R^1=H, R^2=Et
2 : R^1=R^2=H
3 : R^1=Me, R^2=H
4 : R^1=H, R^2=Me

This paper is dedicated to Professor Dr. Ekkehard Winerfeldt on the occasion of his 75th birthday.

RESULTS AND DISCUSSION

The sponge (SS-1022) collected off Seragaki, Okinawa, was extracted with MeOH. The MeOH extract was partitioned between EtOAc and H_2O, and subsequently the aqueous layer was extracted with *n*-BuOH. *n*-BuOH-soluble materials of the extract were subjected to SiO_2 gel and C_{18} column chromatographies followed by C_{18} 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**).

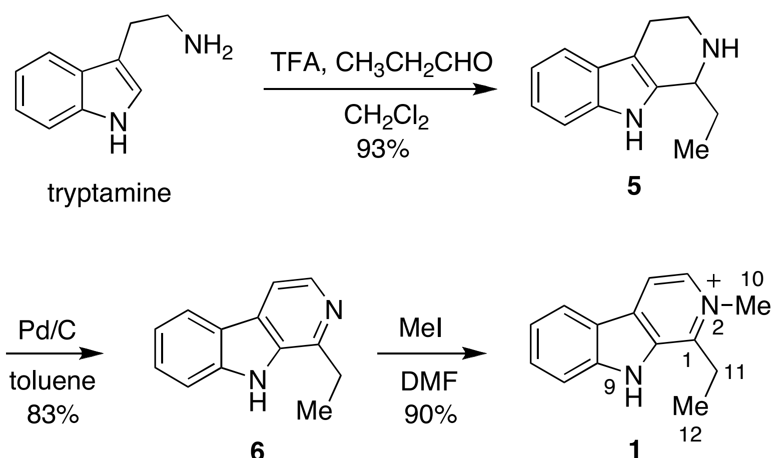
The molecular formula, $C_{14}H_{15}N_2$, of **1** was established by HRESIMS [*m/z* 211.1241 $(M)^+$, Δ +0.5 mmu]. IR absorptions indicated the existence of OH and/or NH (3243 cm^{-1}) functionalities. The ^1H NMR (Table 1) spectrum of **1** was similar to that of **4**, except for the presence of a 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 H_2-11/H_3-12. Thus, seragadine A (**1**) was elucidated to be 1-ethyl-2-methyl-9*H*-*β*-cabolin-2-ium.

Table 1. ^1H NMR Data for Seragadine A (**1**) and Synthetic **1** in CD_3OD

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 Picter–Spengler reaction[10] 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 product[11] (**6**) in 83% yield. The quaternary iminium (**1**) was prepared from **6** by using CH_3I in DMF at room temperature for 12 h[12]. The ^1H NMR data (Table 1) and C_{18} 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**)

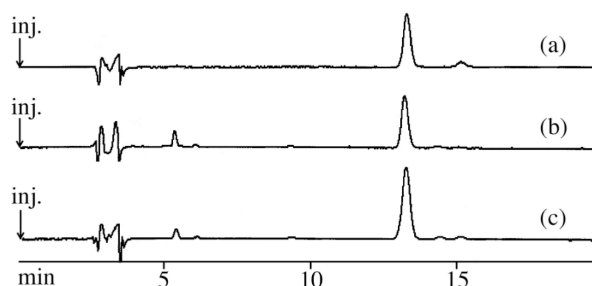


Figure 1. HPLC profiles of seragadine A (**1**) (a), synthetic **1** (b), and co-injection of them (c).

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 $CHCl_3$/MeOH. The fraction eluted with $CHCl_3$/MeOH (3:2) was subjected to a C_{18} 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 /H_2O/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) ν_{max} 3243 cm^{-1}; UV (MeOH) λ_{max} 308 nm (17000); ^{1}H NMR (see Table 1); ESIMS *m/z* 211 $(M)^+$, HRESIMS *m/z* 211.1241 [calcd. for $C_{14}H_{15}N_2$, $(M)^+$, 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 ($CHCl_3$/MeOH, 9 : 1) to give 1-ethyl-2-methyl-*β*-carboline (140 mg, 0.66 mmol, 90%). : colorless amorphous solid; IR (film) ν_{max} 3156, 3075, 2981, 2902, 2787, 2682, 1683, 1633, 1198, 1126 cm^{-1}; UV (MeOH) λ_{max} 308 (ε 17000); ^{1}H NMR (see Table 1); ^{13}C NMR (CD_3OD, 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 $C_{14}H_{15}N_2$, $(M)^+$, 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/H_2O (20:80)). The retention times of synthetic **1** and seragadine A (**1**) were both 13.3 min (Figure 1).

ACKNOWLEDGEMENTS

Authors thank Ms. S. Oka, Center for Instrumental Analysis, Hokkaido University, for measurements of ESIMS. This work was partly supported by a grant from the Yakult Bio-Science Foundation and a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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