



A new alkaloid from the purple Indian Ocean tunicate *Eudistoma bituminis*

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Abstract—A new alkaloid, segoline C **1** possessing the benzo 1,6-diazaphenanthroline ring system has been isolated from the Indian Ocean tunicate *Eudistoma bituminis* with the known segoline A **2** isolated from the Red Sea tunicate *Eudistoma* sp. The structure of all compounds was elucidated on the basis of spectroscopic data. The relative configuration of the chiral compound **1** is proposed on the basis of comparative circular dichroism (CD) measurements of segolines A **2** and B **3**. © 2001 Elsevier Science Ltd. All rights reserved.

Eudistoma bituminis is a purple tunicate found on the rocks at 15 m depth in the Mayotte lagoon, in the Comoros Islands, northwest of Madagascar. In the course of a survey of chemical constituents of tunicates from Mayotte,^{1,2} we have isolated from this organism (52 g of dry weight after extraction) a mixture of segolines A and C (158 mg, 8.3%) by column chromatography on silica gel and RP-18 of the 1:2 methanol–chloroform extract (1.9 g) (Fig. 1).

This mixture was subjected to HPLC by using an RP-18 column, yielding segolines A (20%) and C (80%)

and also pure norsesoline⁴ (3 mg, 0.2%). Only segoline C (2 mg) was separated by chiral column Cyclobond I 2000 from the mixture of segolines.

The major alkaloid, segoline C **1** showed a negative specific rotation (-374°) and a pseudomolecular ion at m/z 386 (MH^+ , 100) in the ESI mass spectrum, which indicated a formula of $C_{23}H_{19}N_3O_3$ with 16 degrees of unsaturation. Intensive 1D and 2D NMR studies, including HMQC, HMBC, COSY and NOE experiments led to the protons and carbons spectral assignment (Table 1).

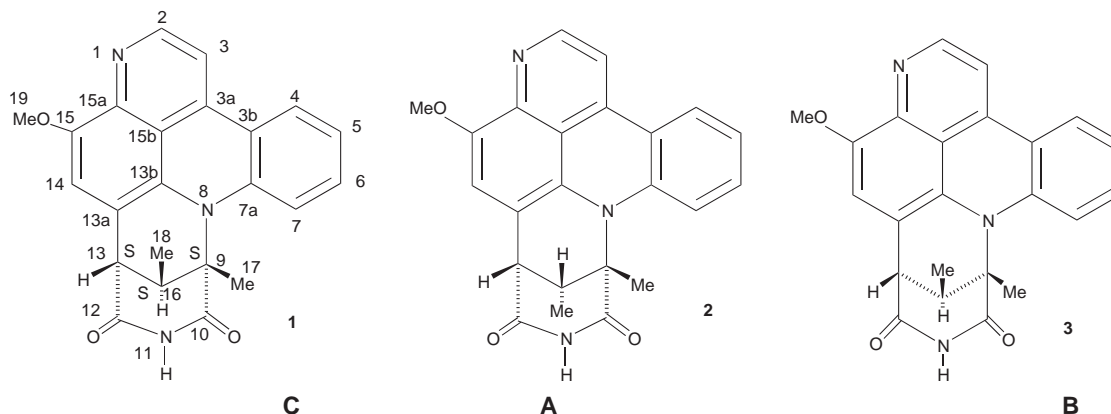


Figure 1. Structure of compounds 1–3.

Keywords: tunicate; *Eudistoma bituminis*; alkaloids; Indian Ocean.

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Table 1. Proton and ^{13}C NMR data for segolines C **1** and A **2**

Number	Segoline C 1		Segoline A 2	
	$\delta_{\text{C}}^{\text{a}}$	$\delta_{\text{H}}^{\text{a}}$	$\delta_{\text{C}}^{\text{a}}$	$\delta_{\text{H}}^{\text{a}}$
C				
2	150.8 d	8.76 d, 4.9	149.6	8.75 d, 5
3	109.2 d	7.50 d, 5	108.6	7.48 d, 4.8
3a	138.4 s		138.8	
3b	121.2 s		120.9	
4	124.5 d	7.91 dd, 8.0; 1.4	124.4	7.93 d, 8.5
5	122.5 d	7.10 brt, 7.8	121.9	7.11 dt, 7.9
6	130.6 d	7.35 ddd, 8.8; 7.2; 1.6	130.1	7.34 dt, 7.8
7	119.2 d	7.82 dbr, 8.7	118.6	7.70 d, 8.6
7a	140.0 s		139.2	
9	60.4 s		61.6	
10	172.3 s		172.9	
NH-11		11.5		Signal not observed
12	170.4 s		170.6	
13	48.5 d	3.69 d, 3.0	49.9	3.75 d, 2
13a	106.4 s		111.2	
13b	128.3 s		127.3	
14	110.2 d	6.97 s	109.2	6.95 s
15	148.3 s		147.2	
15a	141.1 s		139.9	
15b	120.6 s		120.5	
16	35.7 q	2.54 dq, 6.7; 3.1	37.1	2.0 dq, 7.3; 2.2
17	18.6 q	1.92 s	18.5	1.88 s
18	13.4 q	0.87 d, 6.7	15.1	1.31 d, 7
19	56.3 q	4.06 s	55.6	4.05 s

^a 400 MHz, CDCl_3 .

The mixture of segoline A and C has been analyzed by ^1H and ^{13}C NMR. NMR data of segoline A has been deduced by comparison with the NMR data of pure segoline C (Table 1).

The comparison of the NMR data of C with segolines A and B^{3,4} suggested a structure closely related to segoline A or B. Significant differences are noticed between our ^{13}C NMR data for segoline A and literature data⁴ since our results were obtained using more recent techniques; especially for the ^{13}C NMR shift

inversion between C-3a and C-15, C-10 and C-12 and C-13b and C-15a. The almost identical ^1H and ^{13}C NMR data for **1** and **2** suggested that segoline C had the same benzo-3,6-phenanthroline ring system as segoline A. The UV spectrum of **1** confirmed this heterocycle. A glutarimide moiety in **1** was deduced from a 1710 cm^{-1} absorption band in the IR spectrum. An exchangeable NH signal by ^1H NMR was shown at 11.5 ppm, and two carbonyls by ^{13}C NMR at 170.3 and 172.3 ppm. The major difference in the NMR data of **1** in comparison with segoline A **2** (Table 1) was in the chemical shifts and coupling constants of the alicyclic portion of the molecule (H-16, Me-18, C-9 and C-13a, Table 1) showing that **1** was a stereoisomer of **2**.

Segolines A, B and C possess three chiral centers. Two, C-9 and C-13 have to be either *SS* or *RR* to allow closure of the imide ring.⁴ As segolines A, B and C are diastereoisomers, they can either differ in the configuration of C-9 and C-13 or in the configuration of C-16. Distinction between these two possibilities for segolines A and B was shown from the measured CD curves⁴ (Fig. 2) indicating that they were almost mirror images (specific rotations -325° and $+375^\circ$, respectively). Segoline A was proposed to be *9S,13S,16R* and segoline B *9R,13R,16R*.⁴

Segolines A and C showed almost the same optical rotation $[\alpha]_{\text{D}}^{25}$, -325° for segoline A⁴ and -374° for C. Comparison of the CD curve of segoline C with A and B from the literature⁴ (Fig. 2) showed similarities in shapes and in wavelengths of C with A.

As A and C are diastereoisomers, they can only differ in the configuration at C-16, which is *S* in C. Therefore, the configuration of segoline C is *9S,13S,16S*, in agreement with its specific rotation (-374°), and by comparison with the specific rotation of its enantiomer segoline B ($+375^\circ$).⁴

The segolines C and B being enantiomers should have the same ^{13}C NMR data. Significant differences are noticed with the ^{13}C NMR data for segoline B in the literature⁴ for the same reasons as segoline A; especially for the ^{13}C shift inversion between C-3a and C-15, C-10 and C-12 as well as for significant ^{13}C shift differences for C-2 and C-15a.

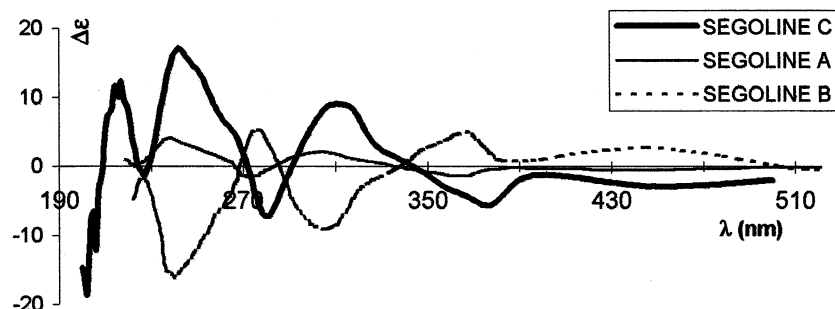


Figure 2. Measured CD cotton effects of segolines A, B and C.

All the alkaloids presented in this paper have the tetracyclic benzo-3,6 diazaphenanthroline ring system in common. Only four configurations A, B, C and D are possible for segoline because of the imide ring. A possible explanation why segoline D with the configuration 9*R*,13*R*,16*S* has not yet been isolated is the easy cleavage of the N-8–C-9 bond in the 9*R*,13*R* configuration. In the same manner, segoline B was found to be more reactive than segoline A.⁴

References

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