Six New Alkaloids from the Purple Red Sea Tunicate *Eudistoma* sp.

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Six novel alkaloids, segoline A (**1**), segoline B (**2**), isosegoline A (**3**), norsegoline (**4**), debromoshermilamine A (**5**), and eilatin (**6**), possessing the benzo-1,6-diazaphenanthroline ring system have been isolated from the Red Sea tunicate *Eudistoma* sp. Eilatin (**6**) is unusual in having a symmetrical heptacyclic structure. The structures of all compounds were elucidated on the basis of spectroscopic data and, in the cases of compounds **1** and **2**, with chemical transformations. The relative configurations of the chiral compounds **1–3** is suggested on the basis of circular dichroism measurements.

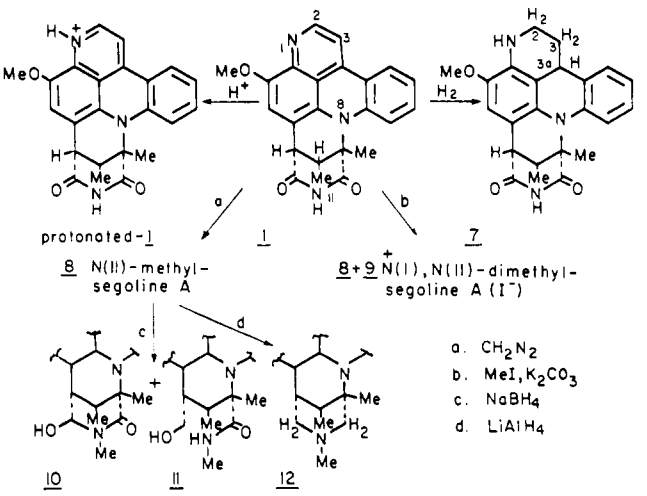
Unique antiviral and antitumor bioactive compounds from tunicates have very recently attracted considerable attention to these sessile marine invertebrates.[1]

Eudistoma sp. is a purple tunicate found in the Gulf of Eilat and the straits of the Gulf of Suez, the Red Sea. In the course of a survey of chemical constituents of tunicates we have isolated from this organism by intensive chromatographies of the concentrated CH_2Cl_2 extract six new alkaloids (**1–6**). The structures of four of the compounds (**1**, **3**, **4**, and **6**) have already been described by us in two short communications.[2,3]

This report describes the structure and spectral features of the two new compounds (**2** and **5**), the comprehensive spectral data of all the compounds, and the stereochemistry and relative configurations of the three chiral compounds **1–3**. The report also includes chemical reactions performed on compounds **1** and **2**.

The major alkaloid, segoline A (**1**),[4] $C_{23}H_{19}N_3O_3$, was isolated in ca. 0.4% (dry weight). Intensive 1D and 2D NMR studies conducted on **1**, including COSY, d-NOE, short- and long-range CH correlations, COLOC, and HETCOSY experiments, led to the proton and carbon spectral assignments (Table I). The NMR data suggested a trisubstituted benzo-3,6-diazaphenanthroline ring system for **1** (Figure 1). The pyridine ring of the latter system was characterized by the H-2 and -3 signals with their mutual coupling constant of 6 Hz (Table I). The ease by which the pyridine moiety was hydrogenated to the 1,2,3,3a-tetrahydro derivative **7** suggested that it was part of a quinoline ring. NOEs between H-2 and -3, H-3 and -4, H-5 and -6, and H-6 and -7 (Figure 2) were in full agreement with the suggested heterocyclic system. Three substituents of the latter system, namely, an OCH_3, a $-C-CH_3$, and a $-CHCHCH_3$ group at C-15, N(8), and C-13a, respectively, suggested by the NMR data, were fully confirmed by NOE enhancements (Figure 2). To complete the structure of **1** and fulfill the 16 elements of unsaturation, two more rings, incorporating the two remaining carbonyls and one NH group, had to be defined. More than a single structure could have been suggested because of the many long-range C–H correlations (up to ^7J in case of C-3/H-16). On the basis of the 1710-cm^{-1} absorption in the IR spectrum and the easy methylation of the exchangeable NH group with CH_2N_2 to afford the monomethyl derivative **8** (Figure 1), a glutarimide could have been proposed. Segoline A was found to be highly resistant to acid and base and did not afford any identifiable $LiAlH_4$ or $NaBH_4$ reduction products. The unequivocal structure of **1** was ultimately achieved by single-crystal X-ray analysis.[2] Very characteristic for segoline A was its UV spectrum, and even more diagnostic was the change in color from yellow–orange to deep purple on acidification (Table III). We propose that protonation of **1** occurs at the nitrogen at position 1, on the basis of the downfield shift of H-2 in a TFA-d_1/$CDCl_3$ solution relative to $CDCl_3$ ($\Delta\delta$ 0.59 ppm). The change in the chromophore of **1** with acid is shown in Scheme I. The nitrogen at position 1 was also the heterocyclic one which was methylated, in addition to N(11), with MeI in acetone (compound **9**). The location of the latter ammonium methyl was confirmed by an NOE measured between the N(1)-Me group of **9** and H-2.

Scheme I. Chemical Transformations of Segoline A (1)



Reduction of N-alkylated imides, like **8**, is known to be preferred over the reduction of the free imides (vide supra).[5] Indeed, reduction of **8** with $NaBH_4$ afforded two major compounds, **10** and **11** (Scheme I) in which the imide group was reduced either to the acylaminal or to the open hydroxymethyl *N*-methylamide, respectively. Lithium aluminum hydride reduction of **8**, on the other hand, afforded the piperidine derivative **12**.

Closely related in its structure to segoline A was compound **2**, $C_{23}H_{19}N_3O_3$, *m/e* 385, designated segoline B. The almost identical ^1H and ^{13}C NMR data of a great part of **1** and **2** (Table I) suggested that segoline B had the same

(1) (a) Rinehart, Jr., K. L.; et al. *J. Am. Chem. Soc.* **1987**, *109*, 6846, and references therein. (b) Rinehart, Jr., K. L.; et al. *J. Am. Chem. Soc.* **1987**, *109*, 3378, and references therein. (c) Kobayashi, J.; et al. *Tetrahedron Lett.* **1988**, *29*, 1177.

(2) Rudi, A.; Benayahu, Y.; Goldberg, I.; Kashman, Y. *Tetrahedron Lett.* **1988**, *29*, 3861.

(3) Rudi, A.; Benayahu, Y.; Goldberg, I.; Kashman, Y. *Tetrahedron Lett.* **1988**, *29*, 6655.

(4) Segole in Hebrew means purple—the color of the tunicate and the compound in acidic media.

(5) Zabicky, J. *The chemistry of amides*, in the series of the chemistry of functional groups; Patai, S., Ed.; Interscience Publishers: New York, 1970; p 335.

0022-3263/89/1954-5331$01.50/0

Table I. Proton and Carbon-13 Data for Segolines A and B (1 and 2)

	1					2	
C	δ_C[a]	δ_H at C[a]	long-range CH coupling with proton no.	δ_C[b]	δ_H[c]	δ_C[b]	δ_H[c]
2	143.9 (d)	8.73 (d, 6.0)	3	149.6	8.63	144.0	8.64
3	107.6 (d)	7.66 (d, 6.0)	2, 4, 16	108.6	7.72	107.6	7.74
3a	147.5 (s)		2, 4	147.2		146.9	
3b	118.4 (s)		5, 6, 7	120.5		118.5	
4	126.2 (d)	8.02 (d, 7.9)	6	124.4	7.66	125.8	7.80
5	123.8 (d)	7.25 (t, 7.9)	7	121.9	7.42	123.6	7.43
6	133.9 (d)	7.55 (c, 7.9)	4	130.1	7.12	133.5	7.13
7	119.7 (d)	7.94 (d, 7.9)	5, 6	118.6	8.10	119.8	8.12
7a	140.5 (s)		4, 6	139.2		140.1	
8a	127.5 (s)		13, 14	138.8		130.5	
9	63.2 (s)		NH-11, 13, 16, 17, 18	61.6		61.6	
10	162.1 (s)		13, 16	170.6		170.4	
NH-11					11.50		11.47
12	171.3 (s)		16	172.9		171.6	
13	48.1 (d)	3.96 (d, 1.8)	NH-11, 14	49.9	4.10	47.9	3.80
13a	114.1 (s)		13, 16	111.2		110.5	
14	114.6 (d)	7.47 (s)	13	109.2	7.14	115.4	7.14
15	141.5 (s)		14, 19	139.9		141.8	
15a	128.9 (s)		2, 14	127.3		128.8	
15b	120.8 (s)		3, 16	120.9		120.0	
16	36.8 (d)	2.20 (dq, 1.8, 6.9)	17, 18	37.1	2.20	34.5	2.83
17	19.2 (q)	1.87 (s, 3 H)		18.5	1.72	18.2	1.77
18	15.5 (q)	1.36 (d, 3 H, 6.9)	16	15.1	1.17	12.4	0.68
19	56.7 (q)	4.09 (s, 3 H)		55.6	3.91	56.4	3.92

[a] $CDCl_3$; TFA (9:1). [b] $CDCl_3$. [c] d_6-DMSO.

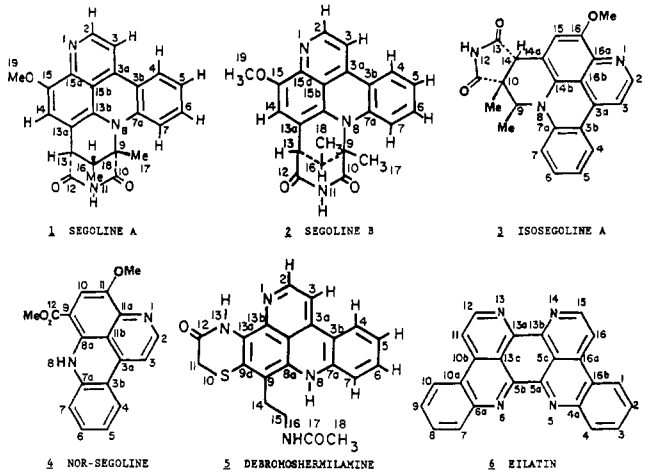
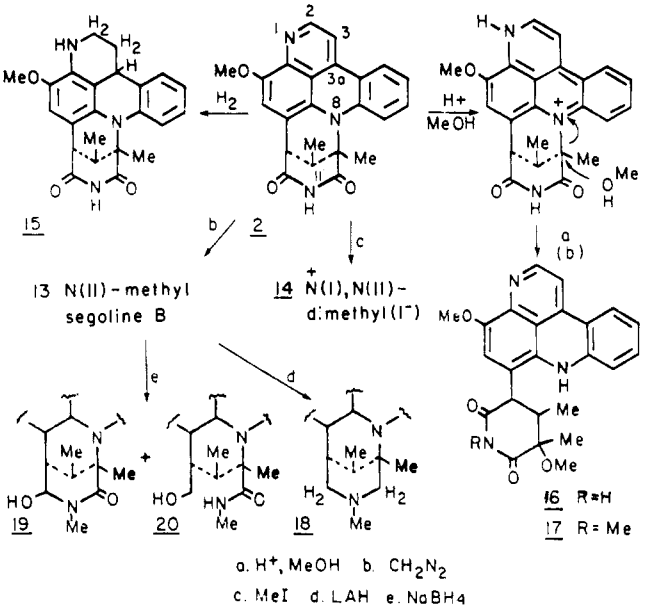


Figure 1. Structure of compounds 1–6.

substituted benzo-3,6-diazaphenanthroline ring system. The almost identical UV spectrum of **2**, including the bathochromic acid shift (Table II), further confirmed the latter heterocycle. That the glutarimide moiety was also present in **2** was suggested from the 1710-cm^{-1} adsorption in the IR spectrum, the exchangeable NH signal at δ 8.04 ppm, and the two carbonyls at δ 171.6 and 170.4. As in the case of **1**, the latter NH could have readily been

Scheme II. Chemical Transformations of Segoline B (2)



methylated with CH_2N_2 to afford the N(11)-methyl derivative **13** (Scheme II). Furthermore, upon reaction with MeI in the presence of base in acetone, segoline B fur-

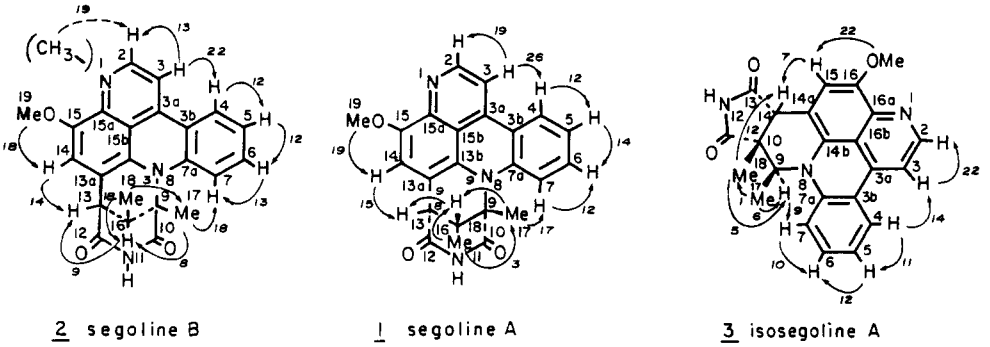


Figure 2. Measured NOEs of compounds 1–3.

Table II. UV Spectra of Compounds 1–6 (λ, ε)

1		2		3		4		5		6	
460	3100	462	4200	470	3500	440	1200	460	3700	434	27000
383	2600	383	3700	386	3100	400	2800	390	4100	408	30400
368	1600	368	2500	360	2300	367	1500	365	6000	388	21000
320	5300	320	7400	330	9400	340	3000			360	11500
308	5100	310	7700					284	16700	286	36700
274	16200	265	24900	274	19100	264	12700	270	20000		
236	9000	242	11300	242	13900	225	17300	264	27800	242	48200
					Changing upon Addition of H^+						
545	2500	544	4800	550	7800	515	850	535	2000	440	21500
382	2500	383	3600	388	7700	402	2300	385	4100		
366	1400	368	2100	368	6100	385	2300	320	20000	305	99800
298	21300	298	31300	297	25000	366	1500	300	27000		
278	15600	280	24000			295	10900	283	27000		
245	7400	245	11300	248	13400	264	12000	268	27000		
						225	18900				

Table III. Proton and Carbon-13 NMR Data for Debromoshermilamine A

C	δ_C[a]	δ_H at C	long-range CH coupling with proton no.
22	151.0 (d)	8.51	
3	107.3 (d)	7.40	2
3a	139.7		2, 4
3b	115.5		5
4	124.2 (d)	8.06	
5	121.1 (d)	7.45	
6	132.1 (d)	7.03	4
7	116.5 (d)	7.35	5, 8
7a	140.2		4, 6
8		10.2	
8a	131.3		14
8b	116.7		3
9	108.9		11, 15
9a	121.7		14
11	29.3 (t)	3.57 (s, 2 H)	
12	163.7		11
13		9.20	
13a	121.6		
13b	137.0		2
14	27.3 (t)	2.97 (m, 2 H)	
15	37.1 (t)	3.11 (m, 2 H)	
16		8.61	
17	171.7		15, 18
18	22.3 (q)	1.93 (s, 3 H)	

[a] d_6-DMSO.

nished the *N*(1),*N*(11)-dimethyl iodide derivative (**14**) and following hydrogenation over PtO_2 the 1,2,3,3a-tetrahydro derivative **15**. The major differences in the NMR data of **2**, in comparison to those of **1** (Table I), were in the chemical shifts and coupling constants of the alicyclic portion of the molecule (H-13, H-16, Me-17, and Me-18, Table I), suggesting that **2** was a stereoisomer of **1**. Indeed, the almost equal NOEs measured for **2** (Figure 2) confirmed the same planar structure for **1** and **2**. Also interesting were the almost mirror image specific rotations of compounds **1** and **2** (−325° and +375°, respectively).

Both segoline A and B possess three chiral centers, of which two, C-9 and -13, have to be either *S*,*S* or *R*,*R* to allow the closure of the imide ring. As segoline A and B (judged from the NMR data) are diastereomers, they can either differ in the chiralities of C-9 and -13 or in the configuration of C-16 (change of all three centers would result in an enantiomer). The distinction between the two possibilities was achieved from the measured CD curves (Figure 3). It is well-known that absolute stereochemistry of organic compounds exhibiting typical split CD Cotton effects due to chiral exciton coupling are assignable from the positive or negative chirality of the CD curve.[6] In case

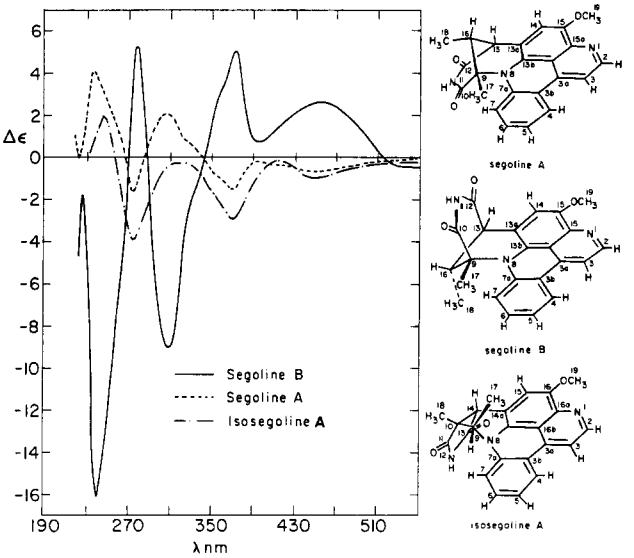


Figure 3. Measured CD Cotton effects of compounds **1–3**.

of segolines A and B, the coupling between the imide (which by itself is expected to give rise to a Cotton effect in the 230–270-nm region)[7] and the heterocycle, of **1** and **2**, resulted in several split CD cotton effects (Figure 3). Because of the complexity of the heterocycle chromophore further study is required before the absolute configuration of the segolines can be assigned from the CD curves. Nevertheless, the almost mirror image CD curves of **1** and **2**, in shape and in wavelengths, clearly indicates that they both are of opposite chiralities, that is, if **1** has the 9*S**,13*S**,16*R** stereochemistry, compound **2** has to be 9*R**,13*R**,16*R** (in the absence of the 18-methyls both compounds would have been enantiomers).

In spite of the marked similarity in the structures of **1** and **2**, they differ in their chemical behavior as well as in their bioactivity.[8] Compound **2** was found to be more reactive, it was transformed more rapidly into compounds **13–15**, and it was also found to be acid sensitive. Upon reaction with 5% H_2SO_4 in MeOH segoline B afforded product **16**. In the ^1H NMR spectrum of **16**, two exchangeable NH groups could be observed (δ 7.29 and 7.88 ppm); one, the original imide –N*H* which, as in the case of the other above-mentioned imides, could have been

(6) Harada, N.; Nakanishi, K. *CD Spectroscopy*; University Science Books: New York, 1979.
(7) Potonski, T. *J. Chem. Soc., Perkin Trans. 1* **1988**, 629, 639.
(8) The antiviral and antitumor activities of the various compounds, tested by Harbor Branch Oceanographic Institution, will be reported elsewhere.

methylated with CH_2N_2 to afford compound **17**, and the second a newly introduced N*H*. A NOE enhancement of 12% between the latter NH (which was not methylated by CH_2N_2) and H-16 suggested this NH to be at position 8. The most plausible explanation, the cleavage of the N(8)–C(9) bond under the acidic conditions, with simultaneous addition of methanol, is shown in Scheme II. As expected, the ^{13}C NMR resonance of C-9 shifted downfield, from 61.6 ppm in **2** to 65.4 ppm, in **16**. In analogy to the reactions of **8**, the mono-*N*(11)-methyl derivative of segoline B, compound **13**, also afforded with $LiAlH_4$ mainly one compound, the reduced piperidine derivative **18** and upon $NaBH_4$ reduction the aminal **19** together with the hydroxymethyl monomethylated amide **20** (Scheme II and Experimental Section).

In addition to segolines A and B we have isolated another isomeric, more polar compound, $C_{23}H_{19}N_3O_3$, *m/e* 385, designated isosegoline A (**3**).

The ^1H and ^{13}C NMR data of compound **3** (Experimental Section) pointed clearly, on the basis of the same arguments as discussed above for **2**, to the identical trisubstituted benzo-3,6-diazaphenanthroline ring system as in segolines A and B. Furthermore, the heterocycle system was in full agreement with the measured neutral and acidic UV spectra (Table II). Isosegoline A differed, however, from **1** and **2**, in the spectral data of the alicyclic portion of the molecule. That an imide functionality still existed in the molecule was clear from the two carbonyls (δ 180.5 and 175.2) and the exchangeable NH (δ 11.20) which, as before, could have been methylated with CH_2N_2 to afford compound **21**. The 1710-cm^{-1} IR absorption of **1** and **2** was, however, replaced by a 1730-cm^{-1} line, suggesting for **3** a succinimide rather than the glutarimide ring of **1** and **2**. Furthermore, the methyl-carrying methine C-9 (the counterpart of CH-16 in compounds **1** and **2** which resonances at δ_C 36.8 and 34.5 and δ_H 2.10 and 2.58, respectively), was prominently shifted downfield to δ_C 49.3 and δ_H 5.06 (q, J = 6.5 Hz), suggesting that this methine was vicinal to the N(8) atom. The suggested structure of isosegoline A in which the alicyclic portion forms a bicyclo[4.3.0] system rather than the bicyclo[3.3.1] system of **1** and **2** (Figure 1) was based, in addition to the above data, on the measured NOEs between H-7 and -9, H-14 and Me-18, Me-17 and -18 and H-14 and -15 (Figure 2).

It could have been expected that, in analogy to compounds **1** and **2**, compound **3** will also show similar split CD Cotton effects due to a chiral exciton coupling between the benzodiazaphenanthroline and the succinimide chromophores. Furthermore, because of the almost equal chromophores, the expected Cotton effects should be very similar to those of either segoline A or B. In the event, the observed Cotton effects of compound **3** were close to those of segoline A (Figure 3), suggesting a similar spatial relationship between the chromophores of **1** and **3** and hence to the 10*R**,14*R** relative configuration.[9] The stereochemistry of the third chiral center of **3**, C-9, was suggested to be 9*S** on the basis of the proximity of H-9 and H-7, a spatial closeness that is required for the explanation of the observed NOE between the latter two atoms (9%, Figure 2).

Compound **4**, which was found to be less polar than **1** and **2**, $C_{18}H_{14}N_2O_3$, *m/e* 306, was obtained in small amounts only (ca. 0.001%, dry weight). The ^1H and ^{13}C NMR spectra (Experimental Section) together with the UV spectra (Table II) clearly suggested for **4** the same benzodiazaphenanthroline heterocycle as in compounds **1–3**. It was also obvious from the NMR data that compound **4** lacked the alicyclic portion of the above compounds. Furthermore, the spectroscopic data proposed a methoxycarbonyl (δ_H 4.06 (s, 3 H), δ_C 168.8, and ν 1670 cm^{-1}) attached to the aromatic core of the molecule. The position of the latter ester group was determined to be at C-9 following a NOE experiment in which irradiation of either the 11-OMe or the esteric OMe group resulted in enhancements of 4 and 5%, respectively, of the singlet of the intermediate H-10 (the reverse irradiation of H-10 also affected the two OMe groups, however, to a lesser extent). Furthermore, a 10% enhancement of H-7 upon irradiation of the NH group established the 8 position for the latter functionality. Methylation of N(8) was achieved by MeI in acetone (but not with CH_2N_2), a reaction that furnished the *N*(1),*N*(8)-dimethylammonium iodide **22**. A 6% enhancement of H-7 while irradiating one of the two methyls, i.e., the N(8) methyl of **22**, confirmed the position of the NH.

Several of the collections of the *Eudistoma* sp. resulted in small amounts of another compound, **5**. The ^1H and ^{13}C NMR data (Table III) and the UV spectra (Table II) of **5** ($C_{21}H_{18}N_4O_2S$, *m/e* 390, HR EI MS 390.1156 (mmu 0.6)) suggested once again the benzo-3,6-diazaphenanthroline ring system.[10] The full ^1H and ^{13}C spectral assignments for compound **5** were achieved from COSY, CH correlations and HETCOSY experiments (Table III). As before, the benzodiazaphenanthroline portion of the molecule was confirmed by NOE enhancements between H-2 and -3 (10%), H-3 and -4 (15%), H-4 and -5 (9%), and H-7 and N(8)-H (16%). The latter experiment also established the position of an exchangeable NH at the 8-position as in norsegoline (**4**).

In addition to the heterocycle system of **5**, the IR (ν_{max} 1660 cm^{-1}) and NMR spectra (Table III) suggested a $CH_2CH_2NHCOCH_3$ group. The location of the latter functionality was determined at the 9-position based on a 9% enhancement between H-14 and N(8)-H in a NOE experiment. Furthermore, NOEs between H-15 and N-(16)-H, 5%, and between the latter NH and Me-18, 7%, unequivocally confirmed the acetamide side chain. Still to be assembled were one carbonyl, one NH, and a CH_2S group, which according to the molecule's degree of unsaturation had to build a ring sited on carbons 9a and 13a—the remaining nonprotonated carbons of the heterocycle. As exhaustive methylations and hydrogenations of **5** failed to give defined isolated compounds, more than one single structure could have been suggested.

Most recently Scheuer's group reported the structure of shermilamine A (**23**) isolated from the tunicate *Tridiemnum* sp., which was determined by X-ray diffraction analysis.[11] Comparison of the NMR data and especially the ^{13}C resonance lines of **5** (Table III) with those reported for **23**[11] has shown great similarity.[12] The only difference between the two compounds being the absence of the bromine in **5**, which must therefore be 6-debromoshermilamine A. As our NMR spectral assignments are based on various 2D NMR experiments (vide supra), we suggest the reassignment of several of the shermilamine

(9) The 10*R**,14*R** relative configuration of **3** gives the same chirality as the 9*S**,13*S** of **1** the change in the terminology stemming from the difference in the numbering of the atoms in **1** and **3** and the change in the priority of the groups.

(10) The presence of sulfur was first determined qualitatively.

(11) Cooray, N. M.; Scheuer, P. J.; Parakanyi, L.; Clardy, J. *J. Org. Chem.* **1988**, *53*, 4619.

(12) We would expect to see a greater influence of substituting an aromatic proton by a bromine atom, e.g., see: Tsujii, S.; Rinehart, K. L.; Gunasekara, S. P.; Kashman, Y.; Cross, S. S.; Lui, M. S.; Pomponi, S. A.; Diaz, M. C. *J. Org. Chem.* **1988**, *53*, 5446. It might be that in case of **23**, because of long relaxation time, the C-6 signal was overlooked.

A resonances as given in Table III.

The sixth compound was isolated from the tunicate, compound **6**, mp > 310 °C, was designated eilatin after the place of the collection. The molecular formula $C_{24}H_{12}N_4$ for **6** implying 21 degrees of unsaturation was determined by high-resolution EI MS. The ^1H NMR spectrum showed only six aromatic protons that could agree with the common six protons of the benzodiazaphenanthroline system of compounds **1–5** (Experimental Section); however, the UV spectrum of **6** (Table II) was different—changes were also observed in the presence of Ni^{2+}.[15] The almost overlapping signals of H-1 and -16 (δ 8.68 and 8.70, respectively) prevented the determination of the relative position of the pyridine and benzene rings by NMR measurements (NOE and COSY). The ^{13}C NMR spectrum exhibited only 12 carbon lines (6 methines and 6 nonprotonated carbons) suggesting a symmetrical dimeric structure. As various 2D NMR experiments (e.g., CH correlations and a HETCOSY experiment) failed to solve the structure, it was determined by a single-crystal X-ray analysis of the monoclinic crystals obtained from a mixture of chloroform–methanol–water.[3] Following the structure determination of eliatin, which possesses the unique highly symmetrical heptacyclic structure, the complete NMR line assignment could be made (Experimental Section).

All six alkaloids discussed above have in common the tetracyclic benzo-3,6-diazaphenanthroline ring system. The same or isomeric heterocycles have most recently been found to construct several other marine natural products.[13] Preliminary tests using a variety of common tunicates revealed several that had body fluids and tunics that were of neutral pH while several other species gave immediate reactions to indicator paper, down to pH 1.[14] Whether compounds **1–5**, which turn purple in acidic media, are responsible for the purple color of our tunicate has to be studied. Most intriguing are the biosynthetic pathways of alkaloids **1–6** and the many other benzodiazaphenanthroline-containing metabolites, and it is not clear whether they should be classed together.

Experimental Section

General Procedures. Melting points were measured by using a Thomas-Hoover capillary melting-point apparatus and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer Model 177 spectrophotometer. Ultraviolet spectra were recorded on a Varian Cary 219 spectrophotometer in methanol solutions. Optical rotations were measured on a Perkin-Elmer 243B polarimeter in chloroform solutions, and circular dichroism by using a JASCO 500C spectropolarimeter in methanol solutions.

^1H and ^{13}C NMR spectra were obtained on a Bruker AM 360 spectrometer. All chemical shifts are reported relative to Me_4Si, δ 0 ppm. Mass spectra were recorded on a Finnigan-4021 mass spectrometer. The numbering of the atoms in the various derivatives within this report is the same as in the parent compounds. Chemical shifts with an asterisk may be interchanged.

The tunicate was collected either in the Gulf of Eilat or in the entrance to the Gulf of Suez in July 1985. The samples were deep-frozen immediately after collection, freeze-dried, and then extracted with 20% MeOH–80% $CHCl_3$ solution. The latter extract (ca. 2 g from 100 g of dry tunicate) was separated by chromatography on a Silica gel column eluted with $CHCl_3$–hexane (7:3), $CHCl_3$, and $CHCl_3$ with increasing percentages of methanol, up to 15%. From repeated chromatographies we have obtained (percent dry weight; R_f on silica gel plates eluted with 5% methanol, 95% $CHCl_3$; spots color, without spraying) the following: **4** (0.001, 0.6, brown–orange spot), **1** (0.4, 0.34, purple spot), **2** (0.1, 0.30, brown–purple spot), **3** (0.01, 0.28, brown spot), **5** (0.05, 0.25, brown-orange spot); **6** (0.001, 0.10, yellow spot), all as amorphous powders.

Segoline A (1): mp > 276 °C; IR ($CHCl_3$) 2840, 1710, 1585, 1520, 1400, 1290, 1140 cm^{-1}; UV, see Table II; ^1H and ^{13}C NMR, see Table I; $[\alpha]^{24}_D$ −322° (c = 1, $CHCl_3$); CI MS (methane), m/z (rel intensity) 386 (MH^+, $C_{23}H_{20}N_3O_3$, 100); X-ray analysis see ref 2.

Segoline B (2): IR ($CHCl_3$) 3700, 3620, 3040, 1710, 1520, 1220, 1050, 940, 880 cm^{-1}; UV see Table II; ^1H and ^{13}C NMR, see Table I; $[\alpha]^{24}_D$ +355° (c 1, $CHCl_3$); CI MS (methane), m/z (rel intensity) 386 (MH^+, $C_{23}H_{20}N_3O_3$, 100). Anal. Calcd for $C_{23}H_{19}N_3O_3$: C, 71.68; H, 4.97. Found: C, 71.51; H, 5.05.

Isosegoline A (3): IR ($CHCl_3$) 2850, 1730, 1580, 1520, 1280, 1150 cm^{-1}; UV see Table II; ^1H NMR (d_6-DMSO) δ 11.20 (br s, N(12)-H), 8.57 (d, 1 H, J = 4.8 Hz, H-2), 8.08 (d, 1 H, J = 7.9 Hz, H-7), 7.63 (d, 1 H, J = 4.8 Hz, H-3), 7.48 (t, 1 H, J = 7.9 Hz, H-6), 7.47 (d, 1 H, J = 7.9 Hz, H-4), 7.18 (s, 1 H, H-15), 7.06 (t, 1 H, J = 7.9 Hz, H-5), 5.06 (q, 1 H, J = 6.5 Hz, H-9), 3.89 (s, 3 H, Me-19), 3.88 (s, 1 H, H-14), 1.45 (s, 3 H, Me-18), 1.04 (d, 3 H, J = 6.5 Hz, Me-17); ^{13}C NMR ($CDCl_3$) δ 180.5 (s, C-11), 175.2 (s, C-13), 150.9 (d, C-2), 147.8 (s, C-3a), 143.1 (s, C-16), 140.5 (s, C-7a), 138.9 (s, C-14b), 132.4 (d, C-6), 127.0 (s, C-16a), 124.4 (d, C-4), 121.4 (d, C-5), 120.4 (s, C-16b), 118.9 (s, C-3b), 114.5 (d, C-7), 109.9 (d, C-3), 108.8 (d, C-15), 107.4 (s, C-14a), 56.5 (q, C-19), 52.7 (s, C-10), 51.1 (d, C-9), 49.3 (d, C-14), 23.6 (q, C-18), 12.3 (q, C-17); $[\alpha]^{24}_D$ −660° (c = 0.001, $CHCl_3$); CI MS (methane), m/z (rel intensity) 386 (MH^+, $C_{23}H_{20}N_3O_3$, 100). Anal. Calcd for $C_{23}H_{19}N_3O_3$: C, 71.68; H, 4.97. Found: C, 71.61; H, 5.11.

Norsegoline (4): IR ($CHCl_3$) 2940, 1670, 1620, 1520, 1310, 1130, 1070 cm^{-1}; UV see Table II; ^1H NMR (d_6-DMSO) δ 11.6 (br s, 1 H, N(8)-H), 8.86 (d, 1 H, J = 4.8 Hz, H-2), 8.03 (d, 1 H, J = 7.9 Hz, H-7), 7.61 (d, 1 H, J = 4.8 Hz, H-3), 7.51 (s, 1 H, H-10), 7.48 (dt, 1 H, J = 7.9, 1.2 Hz, H-5), 7.18 (dd, 1 H, J = 7.9, 1.2 Hz, H-4), 7.17 (dt, 1 H, J = 7.9, 1.2 Hz, H-6), 4.00* (s, 3 H, OMe-11), 3.99* (s, 3 H, OMe-12); ^{13}C NMR ($CDCl_3$) δ 168.8 (s, C-12), 151.9 (d, C-2), 145.2 (s, C-3a), 141.1 (s, C-11), 137.9 (s, C-7a), 137.3 (s, C-8a), 132.3 (d, C-6), 130.8 (d, C-4), 123.9 (d, C-5), 123.5 (s, C-11a), 122.3 (d, C-7), 119.3 (s, C-11b), 117.1 (s, C-9), 116.5 (s, C-3b), 109.1 (d, C-10), 108.7 (d, C-3), 56.2 (q, OCH_3-11), 51.9 (q, OCH_3-12); CI MS (methane), m/z (rel intensity) 307 (MH^+, $C_{18}H_{15}N_2O_3$, 100). Anal. Calcd for $C_{18}H_{14}N_2O_3$: C, 70.58; H, 4.6.. Found: C, 70.35; H, 4.80.

Debromoshermilamine (5): IR ($CHCl_3$) 3920, 2860, 1660, 1630, 1440, 1350 cm^{-1}; UV, see Table II; ^1H and ^{13}C NMR, see Table III; CI MS (methane), m/z (rel intensity) 391 (MH^+, $C_{21}H_{19}N_4O_2S$, 100). Anal. Calcd for $C_{21}H_{18}N_4O_2S$: C, 64.61; H, 4.65; S, 8.20. Found: C, 64.72; H, 4.58; S, 8.08.

Eilatin (6): mp > 310 °C; IR ($CHCl_3$) 3000, 1240, 1200, 1120, 970 cm^{-1}; UV, see Table II; UV spectrum in the presence of Ni^{2+} ions, λ_{max} (ϵ) 296 (38700), 368 (7300), 404 (18300), 426 (22500), 450 (19900); ^1H NMR ($CDCl_3$) δ 9.32 (d, 1 H, J = 5.5 Hz, H-12), 8.70 (d, 1 H, J = 8.0 Hz, H-4), 8.68 (d, 1 H, J = 7.2 Hz, H-1), 8.57 (d, 1 H, J = 5.5 Hz, H-11), 8.00 (t, 1 H, J = 8.0 Hz, H-3), 7.87 (t, 1 H, J = 7.2 Hz, H-2); ^{13}C NMR ($CDCl_3$) δ 150.2 (s, C-5a), 149.7 (d, C-12), 148.8 (s, C-10b), 146.1 (s, C-4a), 138.8 (s, C-13a), 132.1 (d, C-1), 131.7 (d, C-3), 129.3 (d, C-2), 122.5 (d, C-4), 122.3 (s, C-10a), 118.7 (s, C-5c), 117.1 (d, C-11); HR EI MS, m/e (rel intensity), 356.1 (M^+, $C_{24}H_{12}N_4$, 100), 178.1 ($M^+/2$, 75); X-ray analysis, see ref 3.

1,2,3,3a-Tetrahydrosegoline A (7). A solution of **1** (10 mg) in absolute ethanol (5 mL) was hydrogenated over PtO_2 at 60 psi for 24 h. The catalyst was then removed by filtration, and the solution evaporated under vacuum to afford compound **7** (8 mg) as an oil; IR ($CHCl_3$) 2840, 1710, 1560, 1280, 1070 cm^{-1}; UV λ_{max} (ϵ) 460 (1500), 380 (1700), 340 (1700), 365 (1500), 320 (4100), 275 (12100), changing upon H^+ addition to 382 (1300), 292 (12600), 245 (5600); ^1H NMR ($CDCl_3$) δ 7.83 (br s, 1 H, N(1)-H), 7.58 (d, 1 H, J = 8.6 Hz, H-7), 7.27 (d, 1 H, J = 8.0 Hz, H-4), 7.15 (t, 1 H, J = 8.6 Hz, H-6), 6.99 (t, 1 H, J = 8.0 Hz, H-5), 6.76 (s, 1 H, H-14), 3.80 (s, 3 H, Me-19), 3.76 (m, 1 H, H-3a), 3.57 (dt, 1 H, J = 11.2, 3.5 Hz, H-2′), 3.47 (br s, 1 H, H-13), 3.40 (dd, 1 H, J = 11.2, 2.0 Hz, H-2), 2.68 (ddd, 1 H, J = 6.8, 4.0, 2.0 Hz, H-3),

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2.35 (dq, 1 H, J = 6.9, 1.5 Hz, H-16), 2.18 (ddd, 1 H, J = 6.8, 4.0, 1.3 Hz, H-3'), 1.80 (s, 3 H, Me-17), 1.23 (d, 3 H, J = 6.9 Hz, Me-18); ^{13}C NMR ($CDCl_3$) δ 175.5 (s, C-10), 174.9 (s, C-12), 134.4 (s, C-15), 133.5 (s, C-7a), 131.7 (s, C-8a), 128.9 (s, C-15a), 126.4 (d, C-6), 124.6 (s, C-3b), 124.5 (d, C-4), 121.5 (d, C-5), 118.9 (s, C-15b), 115.8 (d, C-7), 107.9 (s, C-13a), 107.8 (d, C-14), 61.3 (s, C-9), 56.1 (q, C-19), 48.5 (d, C-13), 40.6 (d, C-3a), 39.7 (d, C-16), 31.7 (t, C-2), 23.6 (t, C-3), 19.1 (q, C-17), 15.4 (q, C-18); EI MS, m/e (rel intensity) 389 (M^+, $C_{23}H_{23}N_3O_3$, 100).

N(11)-Methylsegoline A (8). To a solution of segoline A (**1**, 15 mg) in methanol (5 mL) a solution of CH_2N_2 in ether (5 mL) was added. After 18 h, the solvents were removed under reduced pressure to afford compound **8** (15 mg) as an amorphous powder: ^{1}H NMR ($CDCl_3$) δ 8.76 (d, 1 H, J = 4.9 Hz, H-2), 7.92 (dd, 1 H, J = 7.6, 1.2 Hz, H-7), 7.73 (d, 1 H, J = 8.6 Hz, H-4), 7.48 (d, J = 4.9 Hz, H-3), 7.39 (dt, 1 H, J = 7.6, 1.2 Hz, H-6), 7.11 (t, 1 H, J = 8.6 Hz, H-5), 7.02 (s, 1 H, H-14), 4.07 (s, 3 H, Me-19), 3.85 (d, 1 H, J = 1.5 Hz, H-13), 3.20 (s, 3 H, N(11)-Me), 2.16 (dq, 1 H, J = 6.9, 1.5 Hz, H-16), 1.90 (s, 3 H, Me-17), 1.26 (d, 3 H, J = 6.9, Me-18); ^{13}C NMR ($CDCl_3$) δ 173.6 (d, C-12), 170.0 (s, C-10), 150.6 (d, C-2), 147.9 (s, C-3a), 140.6 (s, C-7a), 140.0 (s, C-6), 138.3 (s, C-8a), 130.3 (d, C-6), 127.3 (s, C-15a), 124.6 (d, C-4), 122.0 (d, C-5), 121.0 (s, C-15b), 120.1 (s, C-3b), 113.7 (d, C-7), 111.3 (s, C-15), 108.9 (d, C-14), 108.6 (d, C-3), 62.2 (s, C-9), 56.1 (q, C-19), 49.2 (d, C-13), 36.7 (d, C-16), 27.8 (q, N-Me), 19.8 (q, C-17), 15.9 (q, C-18); EI MS, m/e (rel intensity) 399 (M^+, $C_{24}H_{21}N_3O_3$, 100).

N(1),N(11)-Dimethylsegoline A (9) and Compound 8. To a solution of segoline A (10 mg) in acetone (3 mL) in the presence of anhydrous K_2CO_3 (50 mg), methyl iodide (0.5 mL) was added, and the solution was refluxed for 72 h. After filtration and evaporation of the solvent, the residue was chromatographed on a short silica gel column. Elution with chloroform:petrol ether 8:2 afforded the monomethyl derivative **8** (5 mg), and chloroform:methanol 9:1 furnished the dimethyl derivative **9** (3 mg) as an amorphous powder: ^{1}H NMR ($CDCl_3$) δ 9.72 (d, 1 H, J = 5.7 Hz, H-2), 8.15 (d, 1 H, J = 5.7 Hz, H-3), 7.98 (d, 1 H, J = 8.0 Hz, H-7), 7.95 (d, 1 H, J = 7.5 Hz, H-4), 7.67 (t, 1 H, J = 8.0 Hz, H-5), 7.54 (s, 1 H, H-14), 7.37 (t, 1 H, J = 7.5 Hz, H-5), 4.73 (s, 3 H, N(1)-Me), 4.06 (s, 3 H, Me-19), 4.04 (br s, 1 H, H-13), 3.22 (s, 3 H, N(11)-Me), 2.25 (br q, 1 H, J = 6.9 Hz, H-16), 1.96 (s, 3 H, Me-17), 1.34 (d, 3 H, J = 6.9 Hz, Me-18); EI MS, m/e (rel intensity) 414 (M^+, $C_{25}H_{24}N_3O_3$, 100).

Sodium Borohydride Reduction of 8 To Afford Compounds 10 and 11. To a solution of compound **8** (10 mg) in methanol (10 mL) $NaBH_4$ (5 mg) was added. After 1 h at room temperature the reaction was over (TLC), a few drops of acetone were added, and then $CHCl_3$ (50 mL) and the solution was washed once with brine (10 mL). The residue after evaporation was chromatographed on a short silica gel column affording with $CHCl_3$:MeOH (9:1) compound **10** and with (8:2) compound **11**. **10**: an oil; ^{1}H NMR ($CDCl_3$) δ 8.71 (d, 1 H, J = 4.9 Hz, H-2), 8.05 (d, 1 H, J = 8.0 Hz, H-7), 7.87 (d, 1 H, J = 7.3 Hz, H-4), 7.42 (d, 1 H, J = 4.9 Hz, H-3), 7.35 (t, 1 H, J = 8.0 Hz, H-6), 7.05 (t, 1 H, J = 7.3 Hz, H-5), 6.86 (s, 1 H, H-14), 6.26 (br s, 1 H, O*H*), 5.07 (dd, 1 H, J = 6.4, 3.2 Hz, H-12), 4.04 (s, 3 H, Me-19), 3.38 (s, 3 H, N(11)-Me), 3.34 (br dd, 1 H, J = 6.6, 2.6 Hz, H-13), 2.23 (dq, 1 H, J = 7.1, 2.6 Hz, H-16), 1.80 (s, 3 H, Me-17), 1.32 (d, 3 H, J = 7.1 Hz, Me-18); EI MS, m/e (rel intensity) 401 (M^+, $C_{24}H_{23}N_3O_3$, 100). **11**: an oil; ^{1}H NMR (d_6-DMSO) δ 8.12 (d, 1 H, J = 4.8 Hz, H-2), 7.63 (d, 1 H, J = 8.0 Hz, H-7), 7.26 (br s, 1 H, N(11)-H), 7.19 (d, 1 H, J = 4.8 Hz, H-3), 7.00 (t, 1 H, J = 8.0 Hz, H-6), 7.12 (s, 1 H, H-14), 6.98 (t, 1 H, J = 8.0 Hz, H-5), 6.80 (d, 1 H, J = 8.0 Hz, H-4), 5.02 (br s, 2 H, H-12, 12'), 4.21 (br s, 1 H, OH), 4.03 (s, 3 H, Me-19), 3.83 (br s, 1 H, H-13), 2.65 (d, 3 H, J = 4.0 Hz, N(11)-Me), 2.49 (br q, 1 H, J = 7.0 Hz, H-16), 2.10 (s, 3 H, Me-17), 1.03 (d, 3 H, J = 7.0 Hz, Me-18); ^{13}C NMR ($CDCl_3$) δ 172.1 (s), 149.3 (d), 146.6 (s), 140.0 (s), 138.9 (s), 138.2 (s), 129.6 (d), 128.1 (s), 124.4 (d), 120.5 (d), 120.4 (s), 119.9 (s), 119.4 (s), 117.1 (d), 109.6 (d), 107.9 (d), 66.2 (t, C-12), 62.1 (s), 56.1 (q), 38.4 (d), 36.9 (d), 26.1 (q, N(11)-Me), 25.8 (q), 10.7 (q); EI MS, m/e (rel intensity) 403 (M^+, $C_{24}H_{25}N_3O_3$, 100), 367 (53).

10,10',12,12'-Tetrahydrosegoline A (12). To a solution of compound **8** (10 mg) in ether (10 mL) $LiAlH_4$ (2 mg) was added, and the solution was stirred for 1 h at room temperature. Ethyl acetate (0.1 mL) followed by a few drops of a saturated solution of Na_2SO_4 were added, the solution was filtered and evaporated to afford compound **12** (6 mg) as an amorphous powder. The crude material was purified by chromatography on silica gel, eluded with $CHCl_3$:MeOH (8:2): IR ($CHCl_3$) 2940, 1650, 1580, 1410, 1300, 1070 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.61 (d, 1 H, J = 4.8 Hz, H-2), 7.87 (d, 1 H, J = 7.9 Hz, H-7), 7.33 (d, 1 H, J = 4.8 Hz, H-3), 7.28 (d, 1 H, J = 7.9 Hz, H-4), 7.21 (t, 1 H, J = 7.9 Hz, H-6), 6.97 (t, 1 H, J = 7.9 Hz, H-5), 6.83 (s, 1 H, H-14), 4.01 (s, 3 H, Me-19), 3.62 and 2.48 (AB quartet, 2 H, J = 12.3 Hz, H = 10, 10'), 2.94 and 2.73 (AB quartet, 2 H, J = 12.2 Hz, H-12, 12'), 2.71 (br s, 1 H, H-13), 2.25 (s, 3 H, N(11)-Me), 1.97 (dq, 1 H, J = 6.9, 2.0 Hz, H-16), 1.69 (s, 3 H, Me-17), 1.34 (d, 3 H, J = 6.9 Hz, Me-18); EI MS, m/e (rel intensity) 371 (M^+, $C_{24}H_{25}N_3O$, 100).

N(11)-Methylsegoline B (13). Compound **13** was prepared in the same manner as described for **8**: an oil; IR ($CHCl_3$) 2940, 1700, 1585, 1400, 1280 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.77 (d, 1 H, J = 4.7 Hz, H-2), 7.94 (dd, 1 H, J = 8.0, 1.5 Hz, H-7), 7.87 (d, 1 H, J = 8.6 Hz, H-4), 7.51 (d, 1 H, J = 4.7 Hz, H-3), 7.39 (dt, 1 H, J = 8.0, 1.5 Hz, H-6), 7.12 (t, 1 H, J = 8.6 Hz, H-5), 7.03 (s, 1 H, H-14), 4.09 (s, 3 H, Me-19), 3.80 (d, 1 H, J = 3.1 Hz, H-13), 3.20 (s, 3 H, N(11)-Me), 2.53 (dq, 1 H, J = 6.9, 3.1 Hz, H-16), 1.96 (s, 3 H, Me-17), 0.85 (d, 3 H, J = 6.9 Hz, Me-18); EI MS, m/e (rel intensity) 399 (M^+, $C_{24}H_{21}N_3O_3$, 100).

N(1),N(11)-Dimethylsegoline B (14). Compound **14** was prepared in the same manner as described for **9** as an amorphous powder: ^{1}H NMR ($CDCl_3$) δ 9.08 (d, 1 H, J = 6.7 Hz, H-2), 8.15 (d, 1 H, J = 8.1 Hz, H-7), 8.04 (d, 1 H, J = 7.9 Hz, H-4), 7.62 (d, 1 H, J = 6.7 Hz, H-3), 7.58 (t, 1 H, J = 8.1 Hz, H-6), 7.57 (s, 1 H, H-14), 7.32 (t, 1 H, J = 7.9 Hz, H-5), 4.51 (s, 3 H, N(1)-Me), 4.01 (s, 3 H, Me-19), 3.98 (d, 1 H, J = 3.1 Hz, H-13), 3.10 (s, 3 H, N(11)-Me), 2.67 (dq, J = 6.9, 3.1 Hz, H-16), 1.94 (s, 3 H, Me-17), 0.80 (d, 3 H, J = 6.9 Hz, Me-18); EI MS, m/e (rel intensity) 414 (M^+, $C_{25}H_{24}N_3O_3$, 100).

1,2,3,3a-Tetrahydrosegoline B (15). Compound **15** was prepared in the same manner as described for **7** as an oil: ^{1}H NMR ($CDCl_3$) δ 7.65 (d, 1 H, J = 8.0 Hz, H-7), 7.30 (d, 1 H, J = 7.6 Hz, H-4), 7.15 (t, 1 H, J = 8.0 Hz, H-6), 7.00 (t, 1 H, J = 7.6 Hz, H-5), 6.55 (s, 1 H, H-14), 3.81 (s, 3 H, Me-19), 3.76 (m, 1 H, H-3a), 3.57 (dt, 1 H, J = 12.0, 3.5 Hz, H-2'), 3.48 (dd, 1 H, J = 12.0, 2.0 Hz, H-2), 3.41 (d, 1 H, J = 3.0 Hz, H-13), 2.68 (ddd, 1 H, J = 9.4, 5.6, 1.9 Hz, H-3), 2.50 (dq, 1 H, J = 6.9, 3.0 Hz, H-16), 2.19 (dq, 1 H, J = 11.8, 1.8 Hz, H-3'), 1.84 (s, 3 H, Me-17), 1.01 (d, 3 H, J = 6.9 Hz, Me-18); EI MS, m/e (rel intensity) 389 (M^+, $C_{23}H_{23}N_3O_3$, 100).

Acid Hydrolysis of Compound 2 To Afford Compound 16. Segoline B (15 mg) in a solution of 5% concentrated H_2SO_4 in MeOH (10 mL) was refluxed for 24 h. The acid was then neutralized with NH_4OH, and chloroform (40 mL) was added. The solution was washed with brine (5 mL), dried over $MgSO_4$, and evaporated. The residue was purified by chromatography on silica gel eluted with chloroform: IR ($CHCl_3$) 2940, 1729, 1580, 1120 cm^{-1}; UV λ_{max} (ϵ) 465 (7500), 382 (6000), 367 (5000), 322 (12000), 310 (10000), 275 (28500), 240 (18000), changing upon H^+ addition to 545 (9000), 383 (7200), 368 (7200), 268 (33500), 270 (31000), 248 (18500); ^{1}H NMR (d_6-DMSO) δ 8.56 (d, 1 H, J = 4.9 Hz, H-2), 8.31 (br s, 1 H, N(11)-H)*, 8.11 (d, 1 H, J = 7.9 Hz, H-7), 7.88 (br s, 1 H, N(8)-H)*, 7.68 (d, 1 H, J = 4.9 Hz, H-3), 7.34 (d, 1 H, J = 7.2 Hz, H-4), 7.29 (t, 1 H, J = 7.9 Hz, H-6), 7.05 (t, 1 H, J = 7.2 Hz, H-5), 6.64 (s, 1 H, H-14), 3.88 (d, 1 H, J = 12.1 Hz, H-13), 3.84 (s, 3 H, Me-19), 3.82 (s, 3 H, Me-20), 2.45 (dq, 1 H, J = 12.1, 6.6 Hz, H-16), 1.52 (s, 3 H, Me-17), 0.97 (d, 3 H, J = 6.6 Hz, Me-18); ^{13}C NMR ($CDCl_3$) δ 176.2 (s, C-12), 173.9 (s, C-10), 149.9 (d, C-2), 146.6 (s, C-3a), 140.1 (s, C-15), 139.4 (s, C-7a), 139.3 (s, C-8a), 131.2 (d, C-6), 127.7 (s, C-15a), 124.4 (d, C-4), 121.5 (d, C-5), 120.3 (s, C-15b), 119.1 (s, C-3b), 116.1 (d, C-7), 109.5 (s, C-13a), 108.7 (d, C-3), 108.2 (d, C-14), 66.4 (s, C-9), 55.6 (q, C-19), 52.3 (C(9)-O*C*H$_3$), 47.5 (d, C-16), 38.3 (d, C-13), 14.7 (q, C-17), 14.2 (q, C-18); CI MS, m/z (rel intensity) 418 (MH^+, $C_{24}H_{23}N_3O_4$, 100), 319 (100).

Methylation of 16 To Afford the N(8)-Methyl Derivative 17. Compound **17** was prepared in the same manner as described for **8** as an oil: ^{1}H NMR ($CDCl_3$) δ 8.77 (d, 1 H, J = 5.1 Hz, H-2), 7.93 (d, 1 H, J = 7.9 Hz, H-7), 7.86 (d, 1 H, J = 7.9 Hz, H-4), 7.51 (d, 1 H, J = 5.1 Hz, H-3), 7.36 (t, 1 H, J = 7.9 Hz, H-6), 7.12 (t, 1 H, J = 7.9 Hz, H-5), 6.73 (s, 1 H, H-14), 4.09 (s, 3 H, Me-19), 4.07 (s, 3 H, C(9)-OCH$_3$), 3.80 (d, 1 H, J = 3.3 Hz, H-13), 3.20 (s, 3 H, N(11)-Me), 2.40 (dq, 1 H, J = 6.7, 3.3 Hz, H-16), 1.97 (s, 3 H, Me-17), 0.85 (d, 3 H, J = 6.7 Hz, Me-18); EI MS, m/e (rel intensity) 431 (M^+, $C_{25}H_{25}N_3O_4$, 100).

10,10',12,12'-Tetrahydrosegoline B (18). Compound 18 was prepared in the same manner as described for 12 as an amorphous powder: ^{1}H NMR ($CDCl_3$) δ 8.62 (d, 1 H, J = 4.8 Hz, H-2), 7.86 (d, 1 H, J = 7.9 Hz, H-7), 7.40 (d, 1 H, J = 4.8 Hz, H-3), 7.30 (d, 1 H, J = 7.9 Hz, H-4), 6.92 (t, 1 H, J = 7.9 Hz, H-6), 6.97 (t, 1 H, J = 7.9 Hz, H-5), 6.78 (s, 1 H, H-14), 3.72 and 2.12 (AB quartet, 2 H, J = 12.2 Hz, H-10, 10'), 3.18 and 2.45 (an ABX, 2 H, J_{AB} = 11.6, J_{AX} = 2.0, J_{BX} = 1.5 Hz, H-12, 12'), 2.69 (d, 1 H, J = 2.0 Hz, H-13), 2.20 (s, 3 H, N(11)-Me), 1.88 (q, 1 H, J = 6.9 Hz, H-16), 1.69 (s, 3 H, Me-17), 0.74 (d, 3 H, J = 6.9 Hz, Me-18); EI MS, m/e (rel intensity) 371 (M^+, $C_{24}H_{25}N_3O$, 100).

Sodium Borohydride Reduction of 13 To Afford Compounds 19 and 20. Compounds 19 and 20 were prepared in the same manner as described for compounds 10 and 11.

19: an oil; ^{1}H NMR ($CDCl_3$) δ 8.70 (d, 1 H, J = 4.8 Hz, H-2), 7.97 (d, 1 H, J = 7.9 Hz, H-7), 7.52 (d, 1 H, J = 4.8 Hz, H-3), 7.40 (d, 1 H, J = 7.8 Hz, H-4), 7.33 (t, 1 H, J = 7.9 Hz, H-6), 6.95 (t, 1 H, J = 7.9 Hz, H-5), 6.87 (s, 1 H, H-14), 5.70 (s, 1 H, H-12), 4.05 (s, 3 H, Me-19), 3.63 (d, 1 H, J = 1.5 Hz, H-13), 2.97 (s, 3 H, N(11)-Me), 2.30 (dq, 1 H, J = 6.9, 1.5 Hz, H-16), 1.65 (s, 3 H, Me-17), 0.75 (d, 3 H, Me-18); EI MS, m/e (rel intensity) 401 (M^+, $C_{24}H_{23}N_3O_3$, 4), 149 (100).

20: an oil; IR ($CHCl_3$), 2900, 1660, 1580, 1400, 1280 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.13 (br s, 1 H, N(11)-H), 7.89 (d, 1 H, J = 4.8 Hz, H-2), 7.85 (d, 1 H, J = 7.9 Hz, H-7), 7.35 (d, 1 H, J = 4.8 Hz, H-3), 7.10 (d, 1 H, J = 6.9 Hz, H-4), 7.05 (t, 1 H, J = 7.9 Hz, H-6), 7.02 (t, 1 H, J = 6.9 Hz, H-5), 6.97 (s, 1 H, H-14), 4.47 and 4.12 (AB quartet, 2 H, J = 12.0 Hz, H-12, 12'), 3.92 (s, 3 H, Me-19), 2.84 (d, 3 H, Me-19), 2.84 (d, 3 H, J = 4.6 Hz, N(11)-Me), 2.76 (d, 1 H, J = 11.5 Hz, H-13), 2.41 (dq, 1 H, J = 11.5, 6.5 Hz, H-16), 1.63 (s, 3 H, Me-17), 1.25 (d, 3 H, J = 6.5 Hz, Me-18), (assignment of H-12 and H-12' was deduced from a COSY experiment); EI MS, m/e (rel intensity) 403 (M^+, $C_{24}H_{25}N_3O_3$, 4), 149 (100).

***N*(12)-Methylisosegoline A (21).** Compound 21 was prepared in the same manner as described for 8 as an amorphous powder: IR ($CHCl_3$) 2940, 1730, 1620, 1460 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.69 (d, 1 H, J = 4.8 Hz, H-2), 7.89 (d, 1 H, J = 7.9 Hz, H-7), 7.44 (d, 1 H, J = 4.8 Hz, H-3), 7.41 (t, 1 H, J = 7.9 Hz, H-6), 7.39 (d, 1 H, J = 7.9 Hz, H-4), 7.18 (s, 1 H, H-15), 5.08 (q, 1 H, J = 6.5 Hz, H-9), 4.07 (s, 3 H, Me-19), 3.72 (s, 1 H, H-14), 2.88 (s, 3 H, N(12)-Me), 1.49 (s, 3 H, Me-18), 1.16 (d, 3 H, J = 6.5 Hz, Me-17); CI MI, m/e (rel intensity) 399 (M^+ $C_{24}H_{21}N_3O_3$, 100).

***N*(1),*N*(8)-Dimethylnorsegoline (22).** Compound 22 was prepared in the same manner as described for 9 as an amorphous powder: IR ($CHCl_3$) 2940, 1680, 1620 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 9.40 (d, 1 H, J = 5.0 Hz, H-2), 8.34 (d, 1 H, J = 8.7 Hz, H-7), 8.15 (d, 1 H, J = 5.0 Hz, H-3), 8.03 (s, 1 H, H-10), 7.87 (t, 1 H, J = 8.7 Hz, H-6), 7.54 (d, 1 H, J = 8.7 Hz, H-4), 7.50 (t, 1 H, J = 8.7 Hz, H-5), 4.73 (s, 3 H, N(1)-Me), 4.08* (s, 3 H, OMe-11), 4.06* (s, 3 H, OMe-12), 3.70 (s, 3 H, N(8)-Me); CI MI, m/e (rel intensity) 335 (M^+, $C_{20}H_{19}N_2O_3$, 100).

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Conformational Properties of Ulithiacyclamide, a Strongly Cytotoxic Cyclic Peptide from a Marine Tunicate, Determined by ^{1}H Nuclear Magnetic Resonance and Energy Minimization Calculations

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The conformational properties of ulithiacyclamide, a highly cytotoxic cyclic peptide from an ascidian, have been investigated by usine one- and two-dimensional NMR spectroscopy, molecular modeling based on the NMR data, and molecular mechanics energy minimization. The temperature dependence of the NH protons and the nuclear Overhauser enhancements between the backbone and side-chain protons indicated that ulithiacyclamide, in its major form, assumes two kinds of conformations, which are highly dependent on the solvent. While the four NH bonds of the peptide linkages are all located in the interior of the ring structure in $CDCl_3$ and C_6D_6, two, which are related to each other by C_2 symmetry, protrude to the outer part of the ring structure in $(CD_3)_2SO$. Utilizing NMR parameters in conjunction with model building, extensive energy minimization calculations essentially led to two C_2 symmetric saddle-shaped conformations that best satisfied all of the NMR criteria. The two differed primarily in the direction of the NH bonds and the sign of the disulfide helicity. The sulfide linkage affects the molecular conformation of ulithiacyclamide.

Introduction

There is increasing interest in the biochemistry of marine tunicates because a high incidence of biological activity has been ascribed to their metabolites.[1] Lipophilic cyclic peptides that contain unusual amino acid moieties involving thiazole and oxazoline rings have been isolated from marine tunicates;[2] two representative structures are shown in Figure 1. These cyclic peptides, which exhibit potent cytotoxic activities,[3] all have a common or very

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