Bengacarboline, a New β-Carboline from a Marine Ascidian *Didemnum* sp.

Tommaso A. Foderaro,† Louis R. Barrows,‡ Piotr Lassota,§ and Chris M. Ireland*,†

Department of Medicinal Chemistry, University of Utah, Salt Lake City, Utah 84112, Department of Pharmacology and Toxicology, University of Utah, Salt Lake City, Utah 84112, and Wyeth Ayerst Research, Pearl River, New York

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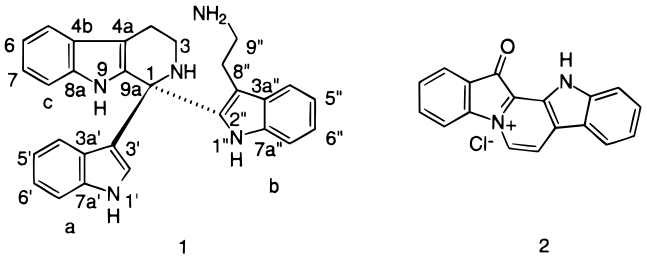
Introduction

Chemical studies of marine organisms have resulted in the discovery of a variety of new natural products with unique structures and activities.[1] Among these are several families of β-carboline alkaloids. Most notable are the eudistomins and manzamines from sponges[2−5] and eudistomin U from an ascidian.[6] We now report the isolation and structure elucidation of bengacarboline (**1**), a new tetrahydro-β-carboline with two indole units attached to C1 of the carboline nucleus from the Fijian ascidian *Didemnum* sp.[7] Fascaplysin (**2**)[8] was also isolated from the extract. Bengacarboline (**1**) was cytotoxic toward a 26 cell line human tumor panel *in vitro* with a mean IC_{50} of 0.9 μg/mL and inhibited the catalytic activity of topoisomerase II at 32 μM.



1

2

Results and Discussion

The *Didemnum* sp. ascidian was collected by SCUBA in 1986 and January 1994, from Pratt Reef, Fiji Islands. The colonies appeared blood red underwater and were quite small with an average diameter of 5−10 mm. The organisms were kept frozen until work up. The ascidian was extracted with MeOH followed by standard solvent partitioning with hexanes and $CHCl_3$. The cytotoxicity of the extract was tracked to the $CHCl_3$ fraction. Size exclusion column chromatography (LH20−MeOH, 0.1% TFA) gave two brightly colored purple and red bands. The red band contained primarily fascaplysin. Reversed phase gravity chromatography (C-18, 55% MeOH, 45% H_2O, 0.1% TFA) of the purple band gave pure bengacarboline (**1**) as a TFA salt.[9]

Table 1. NMR Assignments for Bengacarboline (1) in Pyridine-d_5[a]

atom no.	δ ^{13}C	mult[b]	δ ^{1}H	mult, *J* (Hz)	HMBC[c]
1	58.16	s			
2					
3	39.66	t	3.08	m	1, 4, 4a
			3.20	m	1
4	22.89	t	2.81	m	
			3.03	m	
4a	107.86	s			
4b	127.87	s			
5	118.68	d	7.71	d (7.0)	7, 8a
6	118.89	d	7.22	m	
7	121.13	d	7.16	m	
8	111.92	d	7.40	d (7.5)	4b, 6
8a	137.07	s			
9			13.34		4a, 4b, 9a
9a	137.77	s			
1′			12.09	bs	3′, 3a′, 7a′
2′	126.60	d	7.08	d (1.5)	3′, 3a′, 7a′
3′	120.01	s			
3a′	126.17	s			
4′	120.66	d	7.47	d (8.0)	6′, 7a′
5′	119.48	d	6.94	t (8.0)	3a′, 7′
6′	121.94	d	7.22	m	
7′	112.28	d	7.58	d (8.0)	3a′, 5′
7a′	138.59	s			
1″			11.33	bs	2″,3″, 3a″, 7a″
2″	140.08	s			
3″	110.49	s			
3a″	130.25	s			
4″	119.26	d	7.63	d (8.0)	6″, 7a″
5″	118.76	d	7.22	m	
6″	121.65	d	7.27	t (8.0)	4″, 7a″
7″	111.86	d	7.63	d (8.0)	3a″, 5″
7a″	135.88	s			
8″	27.48	t	2.69	m	2″, 3″, 3a″, 9″
9″	41.39	t	2.86	m	
			3.14	m	3″, 8″

[a] Proton and carbon data were acquired at 500 and 125 MHz, respectively. [b] From a DEPT experiment. [c] The HMBC experiment was optimized to obsereve $^{n}J_{CH}$ coupling of 8.0 Hz.

The molecular formula of **1** was established as $C_{29}H_{27}N_5$ (19 unsaturations) by HRFAB MS and ^{13}C NMR data. The presence of three substituted indolic units was apparent from cursory inspection of the ^{1}H and ^{13}C NMR spectra. For example, the ^{1}H NMR spectrum of the free base in pyridine-d_5 revealed three broad singlets at δ 13.34, 12.09, and 11.33, as well as 13 aromatic and eight aliphatic protons. Pyridine-d_5 gave the greatest dispersion in the proton domain; however, after 48 h the ^{1}H NMR spectrum revealed additional resonances, implying the compound was not stable in pyridine-d_5. The free base was stable in $CDCl_3$. Interestingly, the ^{1}H and ^{13}C NMR spectra of the TFA salt in DMSO-d_6 gave more complicated spectra than the free base; in fact, most signals were doubled. NMR assignments for **1** (free base) are reported in Tables 1 and 2 for pyridine-d_5 and $CDCl_3$, respectively. The ^{13}C NMR spectrum in pyridine-d_5 contained resonances for 24 sp^2 carbons, 13 of which were protonated, four methylenes and one quaternary sp^3

† Department of Medicinal Chemistry.
‡ Department of Pharmacology and Toxicology.
§ Wyeth Ayerst Research.

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(9) Bengacarboline is presumed to form a bis TFA salt on the basis of the presence of two aliphatic amines.

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Table 2. NMR assignments for Bengacarboline (1) in $CDCl_3$[a]

atom no.	δ ^{13}C	(mult)[b]	δ ^{1}H	mult., J (Hz)	HMBC[c]
1	57.17	s			
2					
3	39.27	t	3.00	m	1, 4a
4	22.27	t	3.04	m	
			2.78	m	4a, 9a
4a	107.55	s			
4b	126.84	s			
5	118.22	d	7.52	d, (6.5)	7, 8a
6	118.55	d	7.03	m	
7	120.94	d	7.03	m	
8	111.01	d	7.12	m	
8a	136.13	s			
9			12.23	bs	
9a	135.81	s			
1′			8.10	bs	
2′	125.38	d	6.66	bs	3′, 3a′, 7a′
3′	119.90	s			
3a′	125.02	s			
4′	119.99	d	7.10	d, (8.5)	6′, 7a′
5′	119.79	d	6.88	t, (8.0)	3a′, 7′
6′	122.20	d	7.14	m	
7′	111.38	d	7.37	d, (8.0)	3a′, 5′
7a′	137.14	s			
1″			9.45	bs	2″, 3″, 3a″, 7a″
2″	138.45	s			
3″	109.49	s			
3a″	129.27	s			
4″	118.70	d	7.48	d, (8.0)	6″, 7a″
5″	118.40	d	7.02	m	
6″	121.56	d	7.14	m	
7″	110.99	d	7.32	d, (7.5)	3a″, 5″
7a″	134.25	s			
8″	26.17	t	2.23	m	3a″
			2.45	m	2″, 3″
9″	40.40	t	2.88	m	3″
			3.18	m	

[a] Proton and carbon data were acquired at 500 and 125 MHz, respectively. [b] From a Dept experiment. [c] The HMBC experiment was optimized to obsereve $^{n}J_{CH}$ coupling of 8.0 Hz.

carbon. Bengacarboline contained six exchangeable protons. COSY, TOCSY,[10] HMQC[11] and HMBC[12] data summarized in Tables 1 and 2 readily allowed assignment of a 3-substituted indole (**a**), a 2-substituted tryptamine (**b**), and a tetrahydro-β-carboline (**c**). For unit **a**, the HMBC of the well-resolved doublet at δ 7.58, correlated to a quaternary carbon at δ 126.17(C-3a′) and a protonated carbon at δ 119.48(C-5′). H4′ at δ 7.47 correlated to a methine carbon at δ 121.94(C-6′) and a quaternary carbon at δ 138.59(C-7a′). The indole NH at δ 12.09 correlated to δ 138.59 (C-7a′), 126.17 (C-3a′), and 120.01 (C-3′). A key COSY correlation between the proton at δ 7.09 to the NH proton at δ 12.09 places the vinyl proton at H2′.

Unit **b** was defined in a similar manner. HMBC correlations from the indole NH at δ 11.33 to 130.25 (C-3a″), 110.49 (C-3″), and 140.08 (C-2″) formed the five-membered ring of the indole. Linkage of the ethylamine to C-3″ of the indole was straightforward since the isolated methylene protons at δ 2.69 (H-8″) correlated to C-3″at δ 110.49, as well as δ 130.25 (C-3a″), 140.08 (C-2″), and 41.39 (C-9″). If the ethylamine were attached at C-2″, then a four-bond correlation would be required from H-8″ to C-3a″ (δ 130.25), which is not as likely as a three-bond correlation.

Unit **c** contained two well-resolved doublets at δ 7.71 (H-5) and 7.40 (H-8) which in an HMBC experiment correlated to δ 137.07 (C-8a), 121.13 (C-7) and 127.87 (C-4b), 118.89 (C-6), respectively. The indole NH at δ 13.34 also provided several key correlations to δ 137.77 (C-9a), 107.86 (C-4a), and 127.87 (C-4b) that define the indole portion of unit **c**. COSY, TOCSY, and DEPT data indicated that the remaining C_3H_5N unit was composed of two contiguous methylenes (C-4 and C-3), an NH, and a quaternary carbon at δ 58.16. The methylene protons at δ 3.08 and 3.20 (H-3) clearly showed HMBC correlation to δ 107.86 (C-4a) and 58.16 (C-1) consistent with the tetrahydro-β-carboline unit (**c**). Unfortunately, long range heteronuclear correlations between the appended indoles and C-1 of the β-carboline were not observed. Analysis of mass spectroscopic data also provided support for structure **1**. The EI and FAB spectra gave limited fragmentation data. However, the methane CI spectrum contained two major fragment ions at *m*/*z* 329 and 118 corresponding to molecular elimination of indole **a** and retention of tryptamine **b** at C1 of the β-carboline. Consistent with a chiral center at C-1 for the proposed structure **1**, bengacarboline showed a strong positive peak in the CD spectrum at 247 nm; however absolute configuration is not implied in the structure drawing.

Experimental Section

General. The ^{1}H and ^{13}C NMR spectra were obtained at 500 and 125 MHz, respectively, on a Varian Unity 500 spectrometer. Proton chemical shifts are reported in parts per million relative to residual undeuterated solvent. IR spectra were recorded on a Perkin-Elmer 1600 FT spectrophotometer. UV spectra were obtained in MeOH on a Beckman DU-8 spectrophotometer. High- and low-resolution FAB mass spectral measurements were made on a Finnigan MAT 95 or a VG 7050E spectrometer. Circular dichroism spectra were obtained on a Jasco J40A spectropolarograph.

Isolation of 1 and 2. The organism (1 kg) was extracted with MeOH (1 L) for 24 h followed by filtration. This procedure was repeated three times, and the MeOH extracts were combined. The MeOH was evaporated under vacuum to obtain an aqueous MeOH mixture (800 mL). This mixture was partitioned with hexane and then $CHCl_3$, The cytotoxicity using Human Colon Tumor (HCT 116) cells was traced to the $CHCl_3$ fraction. This fraction was subjected to LH-20 column chromatography (100% MeOH, 0.1% TFA). Two brightly colored bands were isolated, a red band **2** and a dark purple band **1**. The purple band was subjected to C-18 reversed phase gravity chromatography (55% MeOH, 45% H_2O, 0.1% TFA) to yield bengacarboline (**1**) 65.1 mg as the TFA salt.

Bengacarboline (**1**) free base, brown solid: FT-IR (neat) ν_{max} 2925, 1678, 1505, 1432, 1205, 1137 cm^{-1}; UV nm (MeOH) λ_{max} 226 (ϵ 6719), 284 (ϵ 1877); CD ($CHCl_3$) 247nm ($\Delta\epsilon$ 1.97), 228 ($\Delta\epsilon$ −1.03); CD (MeOH) TFA salt 247 ($\Delta\epsilon$ 1.77), 228 ($\Delta\epsilon$ −0.86); FABHRMS TFA salt, *m*/*z* calcd for $C_{29}H_{28}N_5$ 446.2345, found 446.2321 $[MH]^+$. ^{1}H, ^{13}C, and HMBC data: Tables 1 and 2.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra of **1** in $CDCl_3$ and pyridine-d_5, the methane CI mass spectrum of **1** along with interpretation, and the CD spectrum of **1** (8 pages). This material is contained in libraries on microfiche, immediately following this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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