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Thorectandramine, a novel β-carboline alkaloid from the marine sponge *Thorectandra* sp.

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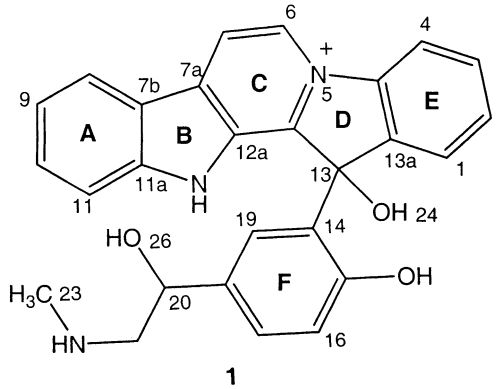
Abstract—The isolation, structure elucidation and biological activity of a novel hexacyclic quaternary alkaloid, thorectandramine (**1**), from a Palauan sponge of the genus *Thorectandra* are reported. © 2002 Published by Elsevier Science Ltd.

A number of biologically active metabolites containing a β-carboline moiety have been identified from marine sponges.[1–3] As part of our interest in marine natural products with cytotoxic activity, we investigated the organic extract of the sponge *Thorectandra* sp., which showed antiproliferative and cytotoxic activity in the NCI's 60 cell line anti-tumor screen. To date, few investigations of the genus *Thorectandra* have appeared in the chemical literature and these describe the isolation of several sesterterpenes[4] and sterols.[5] We now wish to report the isolation, structure determination and biological activity of thorectandramine (**1**), a novel hexacyclic quaternary β-carbolinium derivative from a Palauan collection of the sponge *Thorectandra* sp.



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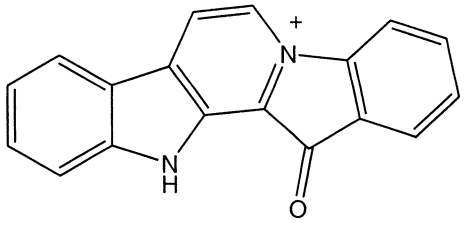
Collection and isolation: The sponge sample was collected under contract for the National Cancer Institute by P. Colin and the Coral Reef Research Foundation. It was described as 5–15 cm in diameter, with dark rusty red color both inside and outside, and a convulated, easily torn, surface. Taxonomic identification was provided by Michelle Kelly (National Institute of Water and Atmosphere) and a voucher specimen (OCDN5079) has been deposited at the Smithsonian Institution. The cytotoxic organic extract of *Thorectandra* sp. was fractionated using antiproliferative activity against melanoma (MALME-3M) and breast (MCF-7) human tumor cell lines to guide the purification. Solvent–solvent partitioning[6] provided a cytotoxic methanol–water fraction which was further purified by Sephadex LH-20 column chromatography (CH_2Cl_2–MeOH, 1:1) followed by reversed-phase HPLC (C_{18}, CH_3CN–H_2O gradient, 0.05% TFA in both mobile phases) to give thorectandramine (**1**, 2.0 mg) and the known compound fascaplysin (**2**, 0.5 mg).[2]



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Keywords: *Thorectandra*; marine natural product; β-carboline alkaloid; sponge.

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Structure elucidation: Thorectandramine (**1**) was isolated as an optically active yellow solid. Liquid chromatography–mass spectrometry (LC–MS) analysis of **1** gave a molecular ion at m/z 438 and the molecular formula of **1** was established as $C_{27}H_{24}N_3O_3$ by HRFABMS (m/z 438.1813 $[M]^+$, calcd for $C_{27}H_{24}N_3O_3$ 438.1818).[7] This formula requires 17.5 degrees of unsaturation and hence indicated that thorectandramine (**1**) is a quaternary ammonium salt. The IR spectrum of **1** showed the presence of OH and/or NH (3200–3300 cm^{-1}) functionalities.

The highly aromatic nature of **1** was established from the NMR data (Table 1) which revealed the presence of 13 hydrogens bound to sp^2 carbons (δ_H 6.24–9.63) and 23 carbons in the aromatic region (δ_C 113–153) and accounted for 12 of the 17.5 degrees of unsaturation. The ^{13}C spectrum did not provide evidence of any more unsaturations, and therefore it was concluded that **1** is hexacyclic. NMR data obtained in MeOH-d_4 showed 13 aromatic methines, one aliphatic methine, one oxymethine, one methylene and one *N*-methyl singlet. The COSY spectrum of **1** indicated the presence of two ABCD spin systems, an isolated AB system, a 1,2,4-trisubstituted benzene ring and an isolated chain (C20–C21). It was apparent from HSQC data that **1** had five exchangeable hydrogens. In order to observe and assign the exchangeable hydrogens, the whole set of NMR data were also recorded in DMSO-d_6. The detailed structure elucidation of thorectandramine (**1**) is described using the data obtained in DMSO-d_6.

Table 1. NMR spectral data for thorectandramine (**1**) recorded in DMSO-d_6 and MeOH-d_4 at 500 MHz

	DMSO-d_6				MeOH-d_4	
Pos.	δ ^{13}C	δ ^1H (mult., J=Hz)	COSY	HMBC	δ ^{13}C	δ ^1H (mult., J=Hz)
1	113.3	7.70 (d, 8.8)	H2	C3, C4a, C13a	114.1	7.67 (d, 8.2)
2	132.5	7.78 (t, 7.6)	H1, H3	C3†, C4, C13a	133.8	7.77 (t, 7.6)
3	122.1	7.47 (t, 7.3)	H2, H4	C1, C4a	123.6	7.47 (m)
4	123.9	8.55 (d, 7.8)	H3	C2, C13a	124.2	8.44 (d, 7.9)
4a	119.7	–			121.4	–
6	117.7	9.06 (d, 6.8)	H7	C4a†, C7, C12b	118.3	8.82 (d, 6.3)
7	123.8	9.63 (d, 6.4)	H6	C6, C7a, C7b, C12a	124.5	9.34 (d, 5.2)
7a	134.4	–			136.6	–
7b	141.2	–			142.8	–
8	114.1	8.47 (d, 7.8)	H9	C9/C10, C11a	114.8	8.27 (d, 7.1)
9	130.5	7.74 (t, 7.6)	H8, H10	C7b, C11†	131.8	7.70 (t, 7.7)
10	130.4	7.59 (t, 7.3)	H9, H11	C8, C11a	131.8	7.57 (m)
11	124.8	7.44 (d, 7.8)	H10	C7b	126.3	7.49 (m)
11a	138.3	–			139.7	–
12*	–	12.33 (bs), 12.28 (bs)			–	–
12a	144.5	–			146.4	–
12b	130.1	–			132.2	–
13	78.8	–			78.8	–
13a	144.8	–			146.6	–
14	132.3	–			133.5	–
14*	132.2	–			133.2	–
15	152.8	–			155.0	–
15*	152.7	–			155.0	–
16	115.2	6.46 (bt, 7.8)	H17	C14, C18	116.7	6.52 (m)
16*	115.0	6.46 (bt, 7.8)	H17	C14, C18	116.6	6.54 (m)
17	127.3	7.20 (bt, 10)	H16, H19	C15, C18†	129.2	7.29 (bd, 8.2)
17*	127.2	7.20 (bt, 10)	H16, H19	C15, C18†	128.9	7.29 (bd, 8.2)
18	123.8	–			125.6	–
18*	123.6	–			125.6	–
19	125.8	8.29 (bs)	H17	C13, C15	126.7	8.47 (bs)
19*	125.3	8.33 (bs)	H17	C13, C15	126.7	8.47 (bs)
20	68.3	5.00 (bs)			70.2	5.08 (m)
20*	68.1	4.99 (bs)			70.1	5.08 (m)
21	55.0	3.28 (2H, m)			56.8	3.36 (2H, m)
21*	54.8	3.19 (2H, m)			56.6	3.36 (2H, m)
22*	–	8.76 (bs), 8.69 (bs)			–	–
23	33.0	2.69 (3H, s)		C21	33.8	2.84 (3H, s)
23*	32.9	2.69 (3H, s)		C21	33.8	2.84 (3H, s)
24*	–	7.67 (bs), 7.66 (bs)		C13, C14	–	–
25*	–	9.34 (bs), 9.32 (bs)			–	–
26*	–	6.24 (bs), 6.14 (bs)			–	–

† Additional HMBC correlations only observed in MeOH-d_4.
* Doubled resonance.

A combination of the ^{1}H NMR of ring A (δ 7.44 d, $J=7.8$; 7.59 t, $J=7.3$; 7.74 t, $J=7.6$; 8.47 d, $J=7.8$) together with the data for the isolated AB spin system in ring C, (δ 9.06 d, $J=6.8$; 9.63 d, $J=6.4$) was indicative of a β-carbolinium subunit.[2] The presence of the β-carbolinium substructure was supported by the UV spectrum of **1** [(λ_{max} (log ε) 224 (4.16), 270 nm (3.97)]. The NMR assignments for the β-carbolinium substructure were all consistent with literature values.[2,3]

For the second ABCD aromatic spin system in ring E, which accounted for another four degrees of unsaturation, the HMBC of a well-resolved doublet at δ 7.70 (H1) correlated to two quaternary carbons at δ 144.8 (C13a) and δ 119.7 (C4a) and to a protonated carbon at δ 122.1 (C3). H2 at δ 7.78 correlated to a methine carbon at δ 123.9 (C4) and the quaternary carbon C13a. H3 at δ 7.47 was correlated in a COSY experiment to H2 and H4 (δ 8.55), and showed HMBC correlations to C1 and C4a. Similarly, H4 showed COSY correlations to H3 and HMBC correlations to C13a and C2, and thus completed the assignment of ring E. A key HMBC correlation, observed in MeOH-d_4, from H6 (δ 9.06) to C4a allowed the attachment of ring E to N5. The regiochemistry of ring E in relation to the β-carbolinium subunit was assigned from 1D-gNOESY[8] experiments. Strong NOE correlations between H4 and H6 established the arrangement shown in **1**. Also, NOE correlations between H7 and H8 established the orientation of ring A.

Ring F was defined in a similar manner. A resonance at δ 6.46 (H16) showed COSY correlations to H17 (δ 7.20) and H17 in turn showed long range COSY coupling to H19 (δ 8.29). H16 also showed HMBC correlations to C14 (δ 132.3) and to another quaternary carbon at δ 123.8 (C18), while H17 showed HMBC correlations to a quaternary carbon at δ 152.8 (C15). H19 showed HMBC correlations to C15 thus completing the assignment of the trisubstituted benzene ring F. The downfield shift of H19 (8.29 ppm) suggests it lies within a deshielding environment, perhaps from the β-carboline ring system.

COSY, TOCSY and HMBC data identified a C_3H_8NO substructure which was comprised of a methylene, an oxymethine, an NH and a *N*-methyl group. The oxymethine multiplet at δ 5.00 (H20) showed COSY correlations to a methylene multiplet centered at δ 3.19 (H21). The downfield ^{13}C chemical shift of C21 (δ 55.0) indicated that it was adjacent to a nitrogen, hence the *N*-methyl group was appropriately placed at position 22. This assignment was supported by HMBC correlations from the methyl singlet at δ 2.69 (H23) to C21.

The only remaining unassigned NMR resonances belonged to a quaternary carbon (δ 78.8) and a hydroxyl proton at δ 7.67. The molecular formula required 17.5 degrees of unsaturation. Since 16.5 degrees of unsaturation were already accounted for in the aforementioned substructures, the carbon bearing the hydroxyl had to be part of the sixth ring. Thus, the hydroxyl substituted carbon was placed at C13 on the basis of HMBC correlations from H19 (δ 8.29) to C13 (δ 78.8), and from the hydroxyl proton to C14 (δ 132.3).

The ^{13}C NMR spectrum of **1** recorded in both DMSO-d_6 and MeOH-d_4 revealed that nine carbon resonances were doubled (Table 1). Additionally, detailed analysis of the ^{1}H NMR indicated that proton resonances for ring F, the side chain at C18, the tertiary hydroxyl at position 24 and the secondary amine proton at N12 were also doubled. One explanation for the doubling of these resonances was that the compound underwent restricted rotation about the C13–C14 bond. In an attempt to verify this hypothesis, ^{1}H NMR spectra were recorded at variable temperatures (27, 40, 60 and 80°C) in DMSO-d_6. There were no significant spectral changes noted over this temperature range. However, when the ^{1}H NMR spectra were recorded in MeOH-d_4 at variable temperatures (27, 42 and 52°C) significant changes were noted in the doubled resonances. For example, H16 which appeared as a broad triplet at 27°C, collapsed into a clean doublet when the sample was heated to 52°C. These results thus confirmed that the doubling of resonances was indeed due to a dynamic molecular process such as restricted rotation around the C13–C14 bond. This does not appear to be due to the presence of strong hydrogen bonds, as all of the exchangeable protons appear as broad signals and variable temperature studies in DMSO-d_6 resulted in faster exchange rates for these protons since the signals first broadened and then disappeared into the baseline as the temperature increased.

The small sample size of **1** and the fact that it contains multiple reactive functional groups in close spatial proximity precluded the use of Mosher's ester methodology[9,10] to establish the absolute stereochemistry of **1**.

LC–MS analysis of compound **2**, isolated as bright red needles, showed an intense ion at m/z 271 [M$^+$] and was confirmed by HRFABMS to be $C_{18}H_{11}N_2O$. Mass spectral and NMR data established that compound **2** was the known compound fascaplysin.[2]

Both compounds **1** and **2** were tested for in vitro cytotoxicity against four human tumor cell lines, MALME-3M (melanoma), MCF-7 (breast), OVCAR-3 (ovarian) and A549 (non-small lung cell cancer).[11] Thorectandramine (**1**) was only weakly active in MCF-7, OVCAR-3 and A549 cell lines (EC_{50} 27.0–55.0 μg/mL), but fascaplysin (**2**) was potently cytotoxic to all the cell lines tested: MALME-3M (EC_{50} 0.03 μg/mL), MCF-7 (EC_{50} 0.14 μg/mL), OVCAR-3 (EC_{50} 0.16 μg/mL and A549 (EC_{50} 0.38 μg/mL).

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