Pergamon

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A New β-Carboline Alkaloid Isolated From the Marine Sponge *Hyrtios erecta*

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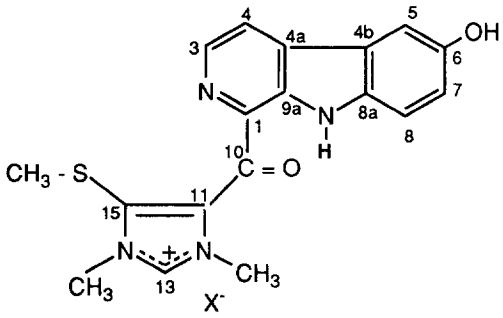
Abstract: A novel β-carboline alkaloid, called hyrtiomanzamine, was isolated from the marine sponge *Hyrtios erecta* collected in the Red Sea. Its structure elucidation was carried out by application of various one and two D NMR methods. Hyrtiomanzamine 1 is the first 6-OH-β-carboline ring associated to a betaine unit isolated from the marine sponge *Hyrtios erecta*. Hyrtiomanzamine 1 showed immunosuppressive activity in the B lymphocytes reaction assay. Copyright © 1996 Elsevier Science Ltd

The marine sponge *Hyrtios erecta* has proved to yield scalarane type sesterterpenoids[1]; heteronemin is the dominant metabolite with no restrictive geographic localization, since heteronemin was isolated from both Australian[2] and Red Sea[3] collections. However, previous chemical investigations from a New Caledonian sample led us to isolation, besides the known heteronemin, of 12-epi-heteronemin as the major compound[4]. In order to continue this study, we examined a sample collected in the Red Sea and compared it with the previously studied sample from New Caledonia. Intriguingly, heteronemin was not detected in the Red Sea sample, but a new β-carboline alkaloid associated to a betaine unit, called hyrtiomanzamine, was isolated from the methanolic extract of the Red Sea marine sponge. This paper deals with the isolation and structural characterization of hyrtiomanzamine deduced from spectroscopic analysis, as the first β-carboline alkaloid isolated from an *Hyrtios sp.*

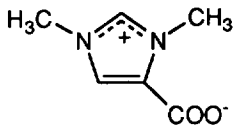
Fresh sponges (500g wet weight), collected in the Red Sea, were immersed in MeOH immediately after collection and subsequently extracted with a 1/1 MeOH/$CHCl_3$ mixture. The combined extracts were concentrated under reduced pressure and then extracted successively with $CHCl_3$ and MeOH. The MeOH extract (4.5g) was first chromatographed on a silicagel column eluted with $CHCl_3$ with increasing amounts of MeOH. The fraction eluted with $CHCl_3$ containing 30% MeOH gave an alkaloids containing fraction, detected with Dragendorff's reagent, further purified on Sephadex LH20 ($CHCl_3$/MeOH 2:8) and silicagel column (methylethylcetone/ethyl acetate/water/formic acid 5:3:0.5:0.5) to yield hyrtiomanzamine (2.10^{-6}% wet animal weight), as an orange glassy solid.

High resolution FABMS indicated the molecular formula $C_{18}H_{17}N_4SO_2$ (*m/z* 353.1053, M^+, Δmmu +1.8). The ^1H NMR spectrum of **1** measured in CD_3OD exhibited three aromatic protons at δ 7.20 (dd, 8.8 , 2.4 Hz), 7.60 (d, 9 Hz), 7.61 (d, 2.4 Hz) ppm engaged in a 1,2,4-trisubstituted benzene ring and two additional vicinal aromatic protons at δ 8.35 (d, 4.9 Hz) and 8.46 (d, 4.9 Hz) ppm, which coupling constants were typical of ortho protons on a pyrimidine ring. The remaining proton signals were assigned to a methyl singlet at δ 2.45 ppm and two N-methyl signals at δ 4.03 and 3.96 ppm, readily detected with the corresponding carbon signals at 34.8 and 36.9 ppm, respectively. Moreover, additional informations were furnished by the ^1H NMR spectrum of **1** recorded in DMSO-d_6. Three exchangeable proton signals appeared at δ 12.05, 9.54 and 7.60 ppm; however,

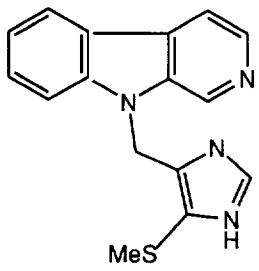
the two vicinal aromatic protons appeared overlapped at δ 8.46 ppm. The proton at δ 12.05 ppm was assigned to a NH group and gave long-range correlations with three quaternary carbons (120.8, 132.8 and 136.1 ppm).



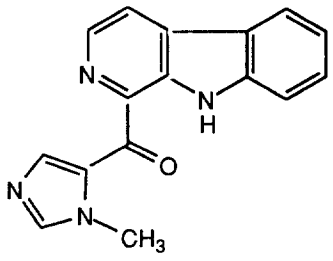
Hyrtiomanzamine **1**



norzooanemonin **2**



Didemnoline A **3**



Xestomanzamine A **4**

Long-range correlations were observed from the aromatic protons at δ 7.62 and 7.17 ppm to the quaternary carbon at 136.1 ppm and from one of the overlapped aromatic protons at δ 8.46 ppm to the quaternary carbons at 132.8 and 134.1ppm, establishing the presence of a 1,6-disubstituted β-carboline moiety. A long-range correlation was found from the proton signal at δ 7.63 ppm to the carbon resonating at δ 152.1 ppm, which positioned the hydroxyl group at position C-6 on the β-carboline ring. All these informations were supported by the IR spectrum, which displayed a NH band at 3408 cm^{-1}, aromatic absorptions at 1604 cm^{-1}, a phenol function band at 1356 cm^{-1} and the presence of a carbonyl band at 1720 cm^{-1}.

Moreover, an additional chromophore conjugated with the β-carboline moiety was revealed by the UV absorption at λ_{max} 394 nm[5]. The exchangeable proton at δ 9.54 ppm gave a direct correlation in the HMBC spectrum of **1** (in DMSO-d_6) and appeared correlated to a carbon, resonating at 140.2 ppm. Long-range correlations were also seen from this proton at δ 9.54 ppm to the N-methyl group carbons (33.9 and 37.8 ppm) as well as to carbons at position C-11 and C-15, constituing the N,N-dimethyl imidazole ring (see Fig.1 and 2). These data are very similar to those reported for norzooanemonin **2**[6]. This marine betaine was isolated from the caribbean gorgonian *Pseudopterogorgia americana*, for which a proton exchange at the 2-position of the dimethyl imidazolium nucleus was also observed.

A thiomethyl group was proposed to account for the deshielded methyl proton signal at δ 2.41 ppm (in DMSO-d_6), which correlated with the field carbon signal at 18.6 ppm. Additional evidence came from the one bond carbon-proton coupling constant ($^1J_{C-H}$= 141.7± 1.7 Hz), which is in accordance with that reported for

SCH_3 in didemnoline A **3** ($^1J_{C-H}$= 142 Hz) rather than a carbon-proton coupling constant of 127 Hz, found for example for the CH_3 of 3-methylimidazole[7]. The position of the thiomethyl group was confirmed by a ROESY correlation (in CD_3OD in order to avoid overlapping signals) between the methyl singlet at δ 2.45 ppm and the N-methyl group at δ 4.03 ppm. This experience also revealed a ROESY correlation between the proton H_4 at δ 8.35 ppm and the proton H_5 at δ 7.61 ppm. Therefore, construction of the betaine ring was accomplished through complete interpretation of 2D NMR data. Consequently, the structure of hyrtiomanzamine was established as **1**. All proton and carbon signals have been assigned (see Table 1).

Table 1: ^{13}C (75.45 MHz, δ in ppm) and ^1H NMR (300.13 MHz, δ in ppm, (mult.), *J* Hz) chemical shift assignments of **1**.

rf[a]	^{13}C DMSO-d_6	^1H DMSO-d_6	^{13}C CD_3OD	^1H CD_3OD
1	136.5			
3	137.3	8.46 (s)	139.1	8.46 (d) 4.9
4	121.0	8.46 (s)	121.8	8.35 (d) 4.9
4a	132.8			
4b	120.8			
5	106.1	7.62 (d) 2.3	107.5	7.61 (d) 2.4
6	152.1			
7	119.4	7.17 (dd) 2.3 ; 9	120.9	7.20 (dd) 2.4 ; 8.8
8	113.7	7.63 (d) 8.3	114.8	7.60 (d) 9
8a	136.1			
9a	134.1			
10	184.2			
11	134.4			
13	140.2	9.54 (s)		
15	131.6			
S-CH_3	18.6	2.41 (s)	19.6	2.45 (s)
N-CH_3	37.8	3.90 (s)	36.9	3.96 (s)
N-CH_3	33.9	3.95 (s)	34.8	4.03 (s)
NH		12.05 (s)		
0H		7.60 (br s)		

Hyrtiomanzamine displayed immunosuppressive activity with an EC_{50} of 2 μg/ml in the B lymphocytes reaction assay and no cytotoxic activity on KB cells. This indicates that the immunosuppressive activity is specific and not due to a general cytotoxic effect.

Recently, xestomanzamine A **4**, a cytotoxic β-carboline alkaloid closely related to hyrtiomanzamine was isolated from an Okinawan marine sponge *Xestospongia sp*[8].

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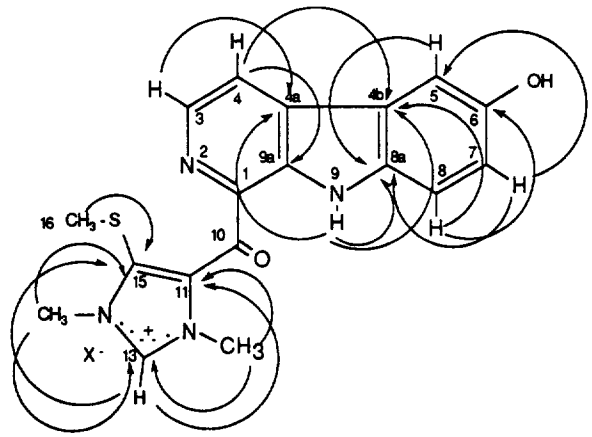

Fig. 1: Long-range ^{1}H-^{13}C correlations (HMBC observed in DMSO-d_6)

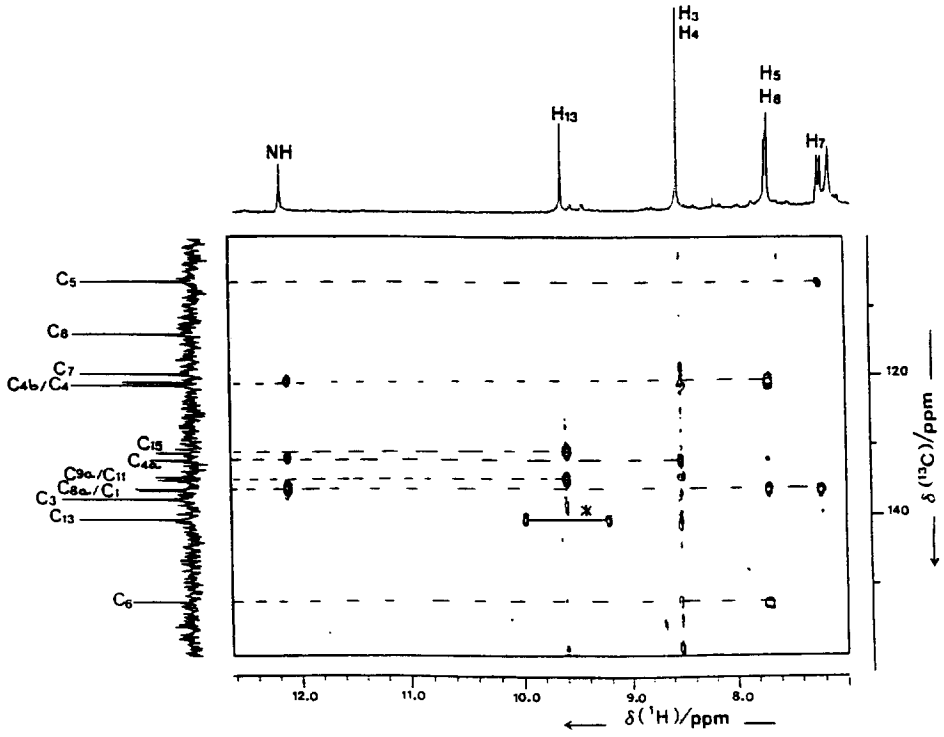

Fig.2: Part of the HMBC spectrum (290°K) of hyrtiomanzamine **1** in DMSO-d_6

* In this experiment the low pass *J*-filter used to suppress one-bond correlations cannot be effective for the whole range of ^{1}JH-^{13}C (from 140 to 220 Hz). Here we observe some residual one-bond H13-C13 coupling (220 Hz).

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