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Ceratamines A and B, Antimitotic Heterocyclic Alkaloids Isolated from the Marine Sponge *Pseudoceratina* sp. Collected in Papua New Guinea

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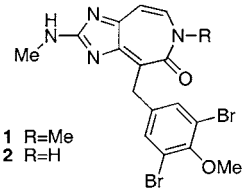
ABSTRACT



Two novel antimitotic heterocyclic alkaloids, ceratamines A (1) and B (2), have been isolated from the marine sponge *Pseudoceratina* sp., collected in Papua New Guinea. The structures of 1 and 2 were elucidated by analysis of spectroscopic data.

Naturally occurring antimitotic agents continue to attract significant attention as lead compounds for the development of new anticancer drugs.[1,2] More than two dozen antimitotic natural products or synthetic/semisynthetic analogues of antimitotic natural products are currently in preclinical evaluation or in various stages of clinical trials.[3] Many of the lead compounds for these development programs, including curacin, diazonamides, discodermolide, dolastatin 10, eleutherobin, halichondrin B, halimide, hemiasterlin, laulimalide, peloruside, spirastrellolides, spongistatins, and vitilevuamide, are marine natural products. As part of an ongoing program designed to discover new antimitotic natural product chemotypes,[4] a cell-based assay[5] has been used to screen crude extracts of marine invertebrates for their ability to arrest human breast cancer MCF-7 cells in mitosis. Extracts of the marine sponge *Pseudoceratina* sp. collected in Papua New Guinea showed promising activity in the assay. Bioassay guided fractionation of the extract resulted in the isolation of ceratamines A (**1**) and B (**2**), two novel antimitotic heterocyclic alkaloids. Details of the structure elucidation of **1** and **2** are described below.

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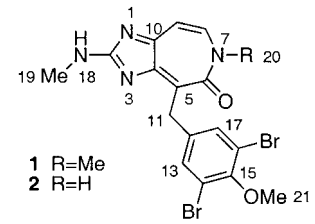

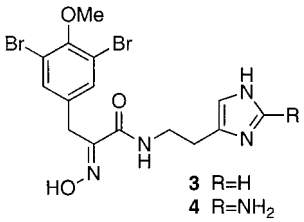

Specimens of *Pseudoceratina* sp.[6] were collected by hand with the use of SCUBA at −15 m on the outer reefs near Motupore Island, Papua New Guinea. Freshly collected sponge (190 g) was frozen on site and transferred to Vancouver over dry ice. Thawed sponge was extracted at room temperature with MeOH (3 × 0.5 L) and the combined MeOH extracts were evaporated in vacuo to give a gummy residue that was active in the antimitotic assay. The residue was partitioned between MeOH/H_2O (90:10) and hexanes, and then water was added to the MeOH phase (to reach 60:40 MeOH/H_2O) before extracting it with CH_2Cl_2. Fractionation of the antimitotic CH_2Cl_2 soluble materials via reversed-phase flash chromatography (Sep-pak 10 g) eluting with a step gradient from H_2O to MeOH gave activity in the MeOH/H_2O (8:2) wash. This material was further purified via reversed-phase HPLC eluting with MeOH/H_2O (8:2) to give ceratamines A (**1**) (8 mg) and B (**2**) (14 mg).

Ceratamine A (**1**) gave small yellow crystals from MeOH (mp 236 °C). The EIHRMS spectrum of **1** showed a molecular ion at *m*/*z* 467.9624 that corresponded to a molecular formula of $C_{17}H_{16}{}^{79}Br^{81}BrN_4O_2$ (calcd 467.9620) requiring 11 sites of unsaturation. Although the number of resonances in the ^{1}H NMR spectrum of **1** was quite small (Table 1), the spectrum showed additional complexity due to the presence of signals for two slowly interconverting forms. A 3,5-dibromo-4-methoxybenzyl fragment could be readily identified in **1** from the series of HMBC correlations shown in Figure 1A. The ^{13}C NMR chemical shifts assigned to this fragment in ceratamine A (**1**) (Table 1) were very similar to the shifts assigned to the identical fragment in 5-bromoverongamine (**3**) isolated from a Caribbean *Pseudoceratina* sp.[7]

Subtracting the atoms accounted for by the 3,5-dibromo-4-methoxybenzyl fragment ($C_8H_7Br_2O$) from the molecular formula of ceratamine A (**1**) indicated that the remaining portion of the molecule had to account for $C_9H_9N_4O$ and seven sites of unsaturation. The ^{1}H NMR resonances attributable to this undefined fragment of **1** included a two-proton spin system at δ 6.42 (d, J = 10 Hz; H-9) and 7.73 (d, J = 10 Hz; H-8) [assigned to a pair of vicinal cis olefinic hydrogens], a resonance at δ 3.07 (d, J = 3.7 Hz; H-19) [correlated in the COSY spectrum to an exchangeable proton resonance at δ 8.69 (H-18), and assigned to an *N*-methyl group on a secondary amine], and a methyl resonance at δ 3.56 (s; H-20) [correlated to a carbon at δ 43.7 in the HMQC

Table 1. NMR Data for Ceratamines A (**1**) and B (**2**) Recorded in DMSO-d_6 at 500 MHz for ^{1}H and 100 MHz for ^{13}C[a]

C/N no.	A (**1**) δ ^{1}H	A (**1**) δ ^{13}C	B (**2**) δ ^{1}H	B (**2**) δ ^{13}C
2		175.6		175.3
4		160.5		161.2
5		121.3		121.3
6		164.1		164.7
7			10.55, br s	
8	7.73, d (10.0)	142.9	7.14, t (9.5)	137.9
9	6.42, d (10.0)	100.4	6.42, d (9.5)	101.2
10		169.7		170.8
11	4.23, s	35.0	4.17, s	33.9
12		140.2		140.1
13	7.67, s	133.2	7.66, s	133.1
14		116.7		116.7
15		151.4		151.4
16		116.7		116.7
17	7.67, s	133.2	7.66, s	133.1
18	8.69	-	8.69	
19	3.07, d (3.7)	29.2	3.07, d (3.7)	28.9
20	3.56, s	43.7		
21	3.71, s	60.3	3.72, s	60.3

[a] The ^{13}C assignments are based on HMQC and HMBC data. Only the shifts for the major tautomers are listed.

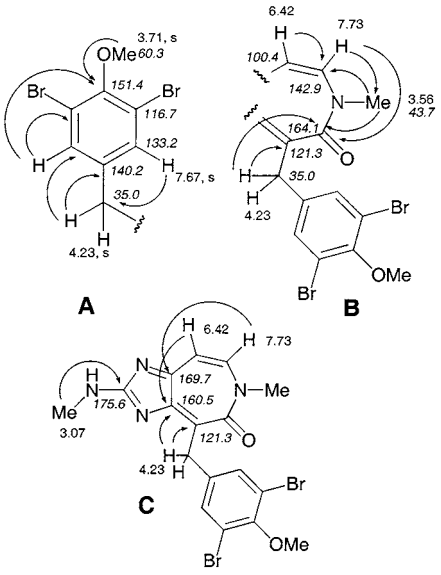

Figure 1. Correlations observed in the HMBC spectrum of ceratamine A (**1**).

(6) A voucher specimen (ZMAPOR 17512) has been deposited at the University of Amsterdam.

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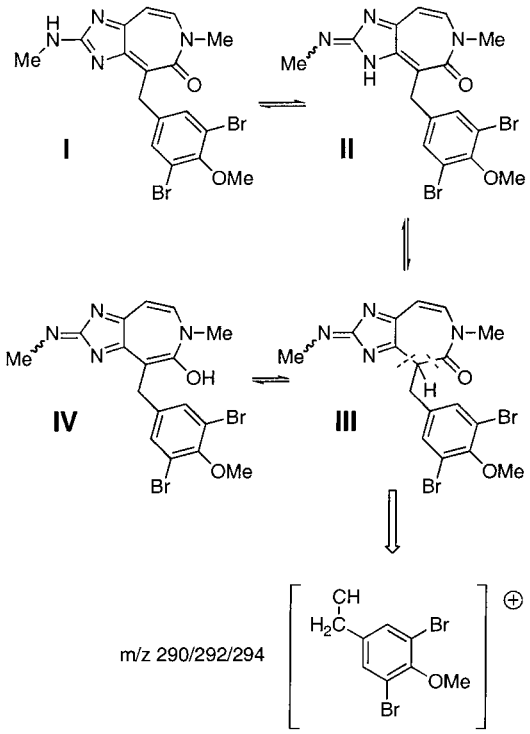
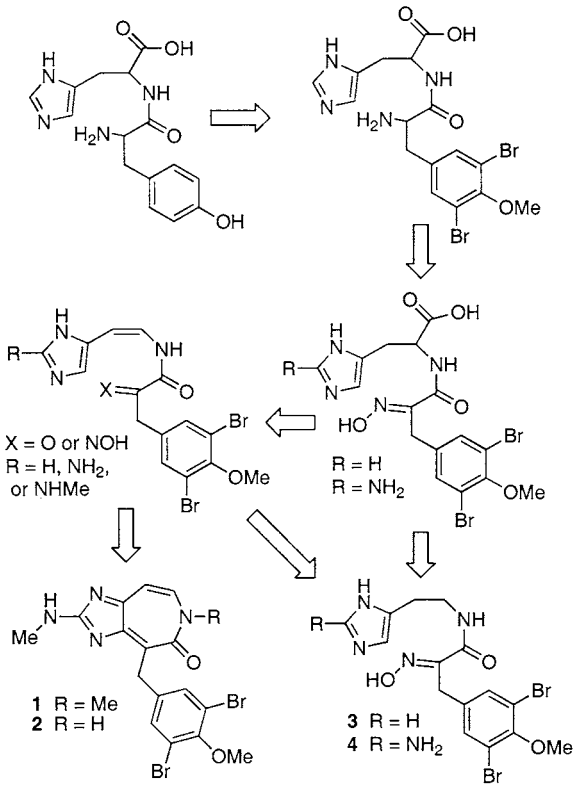


Figure 2. Structures of the possible tautomers of ceratamine A (**1**).

spectrum and assigned to a methyl group attached to a nonprotonated nitrogen atom].

HMBC correlations between the olefinic proton resonance at δ 7.73 (H-8) and the *N*-methyl carbon resonance at δ 43.7 (Me-20), and between the *N*-methyl resonance at δ 3.56 (Me-20) and the olefinic carbon at 142.9 (C-8) (Figure 1B), linked the disubsituted olefin to the methylated nitrogen atom. Additional HMBC correlations from both the olefinic resonance (δ 7.73; H-8) and the *N*-methyl resonance (δ 3.56; Me-20) to a carbon resonance at δ 164.1 indicated that the third substituent on the nitrogen was a carbonyl (C-6). The benzylic proton resonance at δ 4.23 (H-11) showed HMBC correlations to the carbonyl resonance (δ 164.1; C-6) and to two additional carbon resonances at δ 121.3 (C-5) and 160.5 (C-4), indicating that the 3,5-dibromo-4-methoxybenzyl fragment was two bonds removed from the carbonyl as shown in Figure 1B. An HMBC correlation between the olefinic resonance at δ 7.73 (H-8) and a carbon resonance at δ 169.7 identified an sp^2 hybridized carbon (C-10) as the second substituent on the cis olefin, and HMBC correlations between the carbon resonance at δ 160.5 (C-4) and both the olefinic resonance at δ 6.42 (H-9) and the benzylic proton resonance at 4.23 (H-11) required the seven-membered azepine ring as shown in Figure 1C. The remaining atoms ($C_2H_4N_3$) and three sites of unsaturation could be accommodated by fusing a *N*-methylaminoimidazole ring to the azepine ring as shown in Figure 1C. An HMBC correlation observed between the *N*-methyl resonance at δ 3.07 (Me-19) and the carbon resonance at δ 175.6 (C-2) was consistent with this assignment.

Scheme 1. Proposed Biogenesis of Ceratamines A (**1**) and B (**2**)



A number of possible tautomers exist for ceratamine A (**1**) as shown in Figure 2. Each of the constitutional isomers **II**, **III**, and **IV** can exist as the *E* and *Z* stereoisomers about the C-2/N-8 imine bond. Both the ^1H and ^{13}C NMR spectra showed evidence for two forms. This was particularly apparent in the resonances assigned to H-13/H-17 (δ 7.41 and 7.55; ratio of peak heights δ 4:1), H-9 (δ 6.42 and 6.56; ratio of peak heights δ 4:1), and H-8 (δ 7.73 and 7.87; ratio of peak heights δ 4:1). Scalar coupling observed between the Me-19 (δ 3.07) and NH-18 (δ 8.69) resonances provided evidence that the major tautomer observed in the ^1H NMR spectrum of **1** was **I**. A significant fragment peak cluster at *m*/*z* 290/292/294 (1:2:1) in the EIMS of ceratamine A (**1**) could formally arise from tautomer(s) **III** via an α cleavage next to the carbonyl accompanied by cleavage of the bond linking the substituted ethylbenzene fragment to the imidazole ring carbon as shown.

Ceratamine B (**2**) was obtained as small yellow crystals from MeOH (mp 242 °C) that gave a molecular ion in the HREIMS at *m*/*z* 453.9460 consistent with a molecular formula of $C_{16}H_{14}{}^{79}Br^{81}BrN_4O_2$ (calcd 453.9463) that differed form the molecular formula of **1** only by the loss of CH_2. The 1D and 2D ^1H and ^{13}C NMR data obtained for **2** showed a strong resemblance to the data for **1**, including evidence for the presence of multiple interconverting forms (Table 1). It was apparent from routine analysis of the NMR data for **2** that it differed from **1** simply by replacement of the methyl substituent (Me-20) on N-7 with a proton.

Support for the proposed structures **1** and **2** for ceratamines A and B comes from their obvious biogenetic relationship to 5-bromoverongamine (**3**), also isolated from a *Pseudoceratina* sp., and ianthelline (**4**), isolated from *Ianthella ardis* (Scheme 1).[7] The putative biogenetic precursors to these compounds are histidine and tyrosine as shown.

Ceratamines A (**1**) and B (**2**) both had IC_{50}s of 10 μg/mL in the cell-based antimitotic assay. They represent the first examples of a novel family of antimitotic heterocyclic alkaloids. The imidazo[4,5,*d*]azepine core heterocycle in the ceratamines appears to have no precedent at any oxidation level among known natural products or synthetic compounds.

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Supporting Information Available: 1D ^{1}H and ^{13}C NMR spectra of ceratamines A (**1**) and B (**2**). This material is available free of charge via the Internet at http://pubs.acs.org.

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