Bioactive Brominated Metabolites from the Red Sea Sponge *Pseudoceratina arabica*

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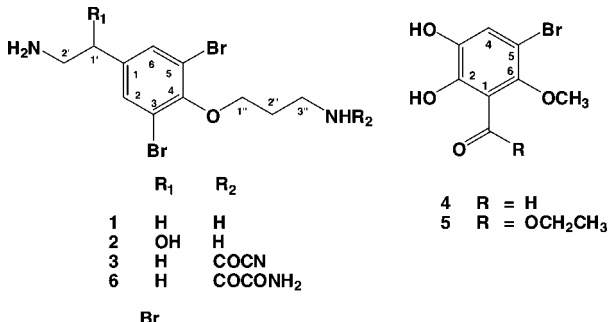
Chemical investigation of the Red Sea sponge *Pseudoceratina arabica* has led to the isolation and identification of seven brominated compounds including two new bromotyramine derivatives, hydroxymoloka'iamine (**2**) and moloka'iakitamide (**6**), and a new brominated phenolic compound, ceratinophenol A (**5**), together with the known compounds moloka'iamine (**1**), ceratinamine (**3**), 5-bromo-2,3-dihydroxy-6-methoxybenzaldehyde (**4**), and psammaplysin-A (**7**). Biological evaluation of these metabolites indicated that moloka'iamine and moloka'iakitamide possess significant parasympatholytic effects on isolated rabbit heart and jejunum. This finding has important implications for further biological investigation of this class of compounds. Moreover, these compounds showed weak antibacterial and antifungal activities.

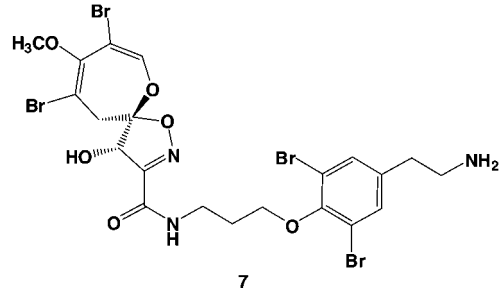
In view of the interest in expanding sources for new drug leads from the sea, and considering the enormous potential that the sea holds as a supply for new pharmaceuticals, we have focused our research in this direction. Several species of the genus *Pseudoceratina* (family Pseudoceratinidae, order Verongida) have been studied and attracted significant attention for their diverse biological activities. Among these activities are antibacterial,[1] cytotoxic,[2,3] enzyme inhibitoty,[4] antifouling,[5,6] antifungal,[7,8] and antiviral activity against HIV.[9]

The current study describes an investigation of the Red Sea sponge *Pseudoceratna arabica*, which led to the isolation and identification of three new compounds; hydroxymoloka'iamine (**2**), moloka'iakitamide (**6**), and ceratinophenol A (**5**), in addition to the four known metabolites moloka'iamine (**1**),[10] ceratinamine (**3**),[11] 5-bromo-2,3-dihydroxy-6-methoxybenzaldehyde (**4**),[10] and psammaplysin-A (**7**).[12]



	R_1	R_2
1	H	H
2	OH	H
3	H	COCN
6	H	$COCONH_2$

4 R = H
5 R = OCH_2CH_3



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The crude aqueous-methanol extract of the sponge was successively extracted with hexanes, CH_2Cl_2, and ethyl acetate. The ethyl acetate residue was fractionated on an open silica gel column using $CHCl_3$/MeOH. The product fractions were further purified on Sephadex LH-20 followed by a C-18 Sep-Pak cartridge (Waters) to yield compounds **1**−**3**. The CH_2Cl_2 portion was extracted by dilute acid to extract the basic alkaloidal compounds. The neutral nonalkaloidal portion was fractionated over a silica gel column using petroleum ether/CH_2Cl_2/MeOH gradient. The column fractions were then repeatedly purified using Sephadex LH-20 to give compounds **4** and **5**. The acidic solution was made alkaline and re-extracted with CH_2Cl_2 followed by purification on silica gel and Sephadex LH-20 to give compounds **6** and **7**.

The FABMS spectrum of **1** showed three ion peaks at *m*/*z* 350.9/352.9/354.9 in the ratio 1:2:1, indicating the presence of two bromine atoms. The HRFABMS supported the molecular formula $C_{11}H_{16}Br_2N_2O$. The ^{1}H NMR and ^{13}C NMR data of **1** were in accordance with the known bromotyrosine metabolite moloka'iamine.[10]

The FABMS of **2** also showed three ion peaks at *m*/*z* 366.9/368.9/370.9 in the ratio 1:2:1, again indicating the presence of two bromine atoms in the molecule. The HRFABMS data of **2** displayed a molecular ion peak at *m*/*z* 366.9662 $[M + H]^+$, being larger than that of **1** by 16 mass units, suggesting the presence of an additional oxygen in the molecule. The ^{1}H NMR signal at δ 4.84 (dd, J = 9.3 and 3.5 Hz) together with a carbon signal at δ 69.6 indicated the oxygenation of one of the side-chain carbons. The COSY and HMBC data confirmed the location of a hydroxyl functionality on the ethylamine moiety through the cross-peaks of H-1′/C-2,6 and H_2-2′/C-1′. Moreover, a bathochromic shift in the UV spectrum upon addition of chromic acid gave further evidence for the hydroxyl group position. The generic name hydroxymoloka'iamine was given to compound **2**.

The data from different NMR experiments of compound **6** indicated its similarity with that of moloka'iamine. Accurate mass determination confirmed the pseudomolecular formula of $C_{13}H_{18}Br_2N_3O_3$ at *m*/*z* 421.9719, corresponding to $[M + H]^+$. These findings showed that compound **6** possessed 71 mass units more than that of moloka'iamine, suggesting the presence of an additional COCONH fragment. This postulation was confirmed from the ^{13}C NMR data, which revealed the presence of two carbonyl functionalities through the signals resonating at δ 160.4 (C-4″) and 162.5 (C-5″). The connectivity of the oxalamide moiety was supported from HMBC cross-peaks between the methylene signal at δ 3.60 (H_2-3″) and the carbonyl at δ 160.4 (C-4″). Finally, the chemical shift values of C-4″ and C-5″ were found to be in good agreement with the reported values for such carbons.[5]

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Table 1. NMR Data and HMBC Correlations for Hydroxymoloka'iamine (**2**) and Moloka'iakitamide (**6**) (CD_3OD)[a]

	2			**6**		
position	δ_H [mult., *J* (Hz)]	δ_C (mult.)	HMBC (H→C)	δ_H [mult., *J* (Hz)]	δ_C (mult.)	HMBC (H→C)
1		142.4 (C)	H-1′		135.7 (C)	H_2-1′, H_2-2′
2, 6	7.68 (2H, s)	131.7 (CH)	H-1′	7.57 (2H, s)	132.9 (CH)	H-3, H-5, H-1′
3, 5		119.3 (C)	H-2, H-6		118.1 (C)	H-2, H-6
4		153.6 (C)	H-2, H-6, H_2-1″		152.1 (C)	H_2-1″, H-2, H-6
1′	4.84 (1H, dd, 9.3, 3.5)	69.9 (CH)	H_2-2″, H-2, H-6	2.93 (2H, t, 7.5)	31.7 (CH_2)	H_2-2″, H-2, H-6
2′	2.93 (1H, dd,12.9, 9.3) 3.14 (1H, dd,12.9, 3.5)	47.2 (CH_2)	H-1′	3.19 (2H, t, 7.5)	40.0 (CH_2)	H_2-1′
1″	4.13 (2H, t, 6.5)	71.6 (CH_2)	H_2-2″, H_2-3″	4.10 (2H, t, 6.5)	70.7 (CH_2)	H_2-2″, H_2-3″
2″	2.21 (2H, quin, 6.5)	29.2 (CH_2)	H_2-1″, H_2-3″	2.14 (2H, quin, 6.5)	28.9 (CH_2)	H_2-1″, H_2-3″
3″	3.30 (2H, t, 6.5)	38.8 (CH_2)	H_2-1″, H_2-2″	3.60 (2H, t, 6.5)	36.5 (CH_2)	H_2-1″, H_2-2″
4″					160.4 (C)	H_2-3″
5″					162.5 (C)	

[a] NMR data were obtained at 400/100 MHz for $^1H/^{13}C$.

The FABMS of ceratinophenol A (**5**) showed two ion peaks at *m/z* 312.9 and 314.9 in the ratio 1:1, indicating the presence of one bromine atom, while its HRFABMS supported the pseudomolecular formula of $C_{10}H_{11}BrNaO_5$ at *m/z* 312.9673 corresponding to $[M + Na]^+$. The ^{13}C NMR spectrum of **5** displayed resonances for 10 carbons, of which six were detected in the region of δ 149.1−106.3, suggesting the presence of one aromatic ring in the molecule. Additionally, the signals at δ 144.4, 147.9, and 149.1 suggested these carbons were oxygenated as well. The nature of the substituents on this ring was secured as two ortho hydroxyls at C-2 and C-3, which was supported from the bathochromic shift noticed in the UV spectrum upon addition of $AlCl_3$, while the third substituent was shown to be a methoxyl functionality, which was detected at δ 3.75 (3H, s) and 62.4 in the NMR spectra. The $^{13}C/^1H$ NMR signals at δ 169.1 (C═O), 62.9/4.39 (q, CH_2), and 14.4/1.39 (t, CH_3) suggested the presence of the fragment ($COOCH_2CH_3$), which was secured from the HMBC cross-peaks of $OCH_2/C{=}O$ and OCH_2CH_3/OCH_2. Finally, interpretation of 1D and 2D NMR data proved the placement of all functionalities on the aromatic ring of **5**, which was assigned as ethyl (5-bromo-2,3-dihydroxy-6-methoxy)benzoate.

In conclusion, investigation of the Red Sea sponge *P. arabica* resulted in the isolation of six compounds including two new bromotyramine derivatives, hydroxymoloka'iamine and moloka'iakitamide, a new brominated phenolic compound, ceratinophenol A, and several known compounds. Biological evaluation of compounds **1**−**3**, **6**, and **7** showed that moloka'iamine and moloka'iakitamide possessed significant parasympatholytic effects on isolated rabbit heart and jejunum. In addition, compounds **4**, **5**, and **7** showed weak to moderate antibacterial activity, while compounds **5** and **6** were weakly antifungal. Moreover, these compounds were not toxic to human colon cells HCT-116 at 10 μg/mL. Such findings have important implications for further biological investigation of this class of compounds.

Experimental Section

General Experimental Procedures. Optical rotations were recorded on a JASCO DIP-730 digital polarimeter. Ultraviolet spectra were recorded on a Hitachi 300 spectrometer. NMR spectra were obtained in CD_3OD on Varian Mercury-400BB or Bruker Avance DRX 300 spectrometers at 400/300 MHz for ^1H NMR and 100/75 MHz for ^{13}C NMR. NMR chemical shifts are expressed in parts per million (ppm) referenced to CD_3OD solvent signals (δ 3.29 for ^1H and δ 49.0 for ^{13}C). Positive ion FAB mass spectral data were obtained with a Micromass Q-tof equipped with a lockspray mass spectrometer using leucine enkephalin at *m/z* 556.2771 $[M + H]^+$ as a reference mass. Precoated silica gel G-25 UV_{254} plates were used for thin-layer chromatography, and silica gel 60, 230−40 μm mesh (E. Merck), and Sephadex LH-20 (Pharmacia) were employed for column chromatography. Both acetylcholine and nicotine were purchased from Fluka (Fluka AG, Buchs, Switzerland).

Biological Material. The marine sponge *P. arabica* was collected from Sharm El-Sheikh at the Egyptian Red Sea coast at depths between 10 and 20 m. The sponge is massively encrusting with a conulose surface. The color of the living sponge underwater is yellow-green with a bright yellow interior. The preserved sample changed to a blackish-green color, and the alcohol became discolored to dark green as well. The surface conules are bluntly rounded, 2−5 mm apart with the consistency firmly compressible and rubbery. The voucher fragment is 10.0 × 4.0 × 1.0 cm. The skeleton consists of sparse irregular fibers consisting only of pith. The outline and branching is irregular, and thickness varies between 80 and 300 μm. The specimen conforms to the description of the type from the Eritrean Red Sea. The voucher is kept in the collections of the Zoological Museum of the University of Amsterdam under registration number 17951. Another voucher specimen was deposited in the Red Sea Invertebrates Collection of the Department of Pharmacognosy, Suez Canal University, under the code number DY-61.

Extraction and Isolation. The fresh sponge material (1.5 kg) was extracted exhaustively with MeOH (3 × 3000 mL) at room temperature to give 9.5 g of crude methanolic extract, which was successively extracted with *n*-hexane, CH_2Cl_2, and EtOAc, respectively. A portion of the crude EtOAc extract (3.33 g) was fractionated over an open silica gel column. Fractions were eluted with $CHCl_3$ and increasing polarity by MeOH and were further purified with Sephadex LH-20 (in MeOH) and C-18 Sep-Pak cartridge (Waters), eluted with H_2O/MeOH (1:1), to yield **1** (22 mg), **2** (10 mg), and **3** (50 mg). A portion of the crude CH_2Cl_2 extract (2.14 g) was made acidic with dilute HCl, then extracted several times with CH_2Cl_2. The organic solvent was distilled off to yield 0.9 g (nonalkaloid fraction). The mother liquor was then made alkaline with ammonia solution followed by extraction with CH_2Cl_2, which yielded 1.2 g (alkaloid fraction). About 0.8 g of the nonalkaloid fraction was fractionated over a silica gel column, eluted with a petroleum ether/CH_2Cl_2/MeOH gradient followed by running several times over Sephadex LH-20 (in CH_2Cl_2/MeOH, 1:1) to give **4** (12 mg) and **5** (3 mg). The alkaloid fraction (1.17 g) was chromatographed using silica gel and eluted with a $CHCl_3$/MeOH gradient, followed by purification on Sephadex LH-20 open columns several times using a mixture of CH_2Cl_2/MeOH (1:1) as eluent to afford two pure compounds, **6** (6 mg) and **7** (5.6 mg).

Determination of Inhibition Zone Using the Agar Diffusion Method. The antimicrobial activity of the isolated compounds was tested against the organisms *Staphylococcus aureus* (ATCC 6538P), *Pseudomonas aeruginosa* (ATCC 9027), *Klebsiella pneumoniae* (ATCC 10032), and *Candida albicans* (ATCC 2091) using the agar diffusion method. Accurately weighed 1 mg aliquots of each compound were dissolved in 1 mL of DMF, and 100 μL of each solution was inserted in the cups and then incubated at 37 °C for 24 h. Ceratinophenol A (**5**) displayed moderate activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* (7 and 4 mm, respectively). Ceratinophenol A (**5**), psammaplysin-A (**7**), and

Table 2. NMR Data and HMBC Correlations for Ceratinophenol A (**5**) (CD_3OD)[a]

position	δ_C (mult.)	δ_H [mult., *J* (Hz)]	HMBC (H→C)
1	115.0 (C)		
2	144.4 (C)		H-4
3	147.9 (C)		
4	121.9 (CH)	7.05 (1H, s)	
5	106.3 (C)		H-4
6	149.1 (C)		H-4, OC*H*$_3$
O=C	169.1 (C)		OC*H*$_2$
OCH_2	62.9 (CH_2)	4.39 (2H, q, 7.0)	C*H*$_3$
CH_3	14.4 (CH_3)	1.39 (3H, t, 7.0)	OC*H*$_2$
OCH_3	62.4 (CH_3)	3.75 (3H, s)	

[a] NMR data were obtained at 300/75 MHz for $^1H/^{13}C$.

compound **4** displayed a weak effect against *Klebsiella pneumoniae* (3 mm). Ceratinophenol A (**5**) and moloka'iakitamide (**6**) exerted a weak antifungal effect (2 and 3 mm, respectively).

Determination of the Cytotoxic Activity of the Compounds.[13] The cytotoxic activities of the isolated compounds were tested against human colon tumor cells (HCT-116) using the MTT assay.[13] Makulavamine C was used as a positive cytotoxic control. None of the tested compounds showed significant cytotoxicity against human colon carcinoma at a test dose of 10 μg/mL.

Study of the Effect of Moloka'iamine and Its Derivatives on Rabbit Intestine and Heart. The effect of moloka'iamine (**1**) and its derivatives hydroxymoloka'iamine (**2**), ceratinamine (**3**), moloka'iakitamide (**6**), and psammaplysin-A (**7**) on rabbit intestine and heart was studied.

Isolated Rabbit Jujunum. [14] The pure compounds were dissolved in distilled H_2O (1.0 mg/mL stock solution). Two dilutions (1/1000 and 1/100) were prepared and used in this investigation. The normal intestinal contractions were initially recorded, and then the nicotinic, muscarinic, histaminergic, and adrenergic receptors were tested. The available pure isolated compounds, at a concentration of 1/1000, produced no change in the intestinal contractions (tone and motility). Only moloka'iamine (**1**) and moloka'iakitamide (**6**) slightly decreased the intestinal contractions at a concentration of 1/100; the other compounds did not produce any significant activity. Application of histamine after the test compounds produced stimulation of the intestinal contractions. Thus, the tested compounds are not histaminergic receptor blockers. Addition of 0.1 mL of nicotine small dose (0.1 mL of 1/1000 dilution of the original solution) or 10 gamma (0.1 mL of 1/10 000 dilution of the original solution)[14] of acetylcholine after application of compounds **1** and **6** produced no effect. Accordingly, these two compounds are most probably parasympatholytic, as they blocked the stimulating effect of both nicotine and acetylcholine on the isolated intestine.

Isolated Perfused Rabbit Heart (modified Langendroff's method). [14] After recording normal contractions and testing the nicotinic, muscarinic, histaminergic, and adrenergic receptors, the tested pure compounds (**1** and **6**) were added in a concentration of 1/100. Addition of 0.1 mL nicotine small dose (0.1 mL of the 1/1000 dilution of the original solution, 1 mL nicotine/100 mL H_2O) and 10 gamma acetylcholine (0.1 mL of the 1/10 000 dilution of the original solution, 1 g acetylcholine/100 mL H_2O)[14] after application of the test compounds produced no effect on the heart. Therefore, the compounds are most probably parasympatholytic, as they blocked the inhibitory effect of both nicotine and acetylcholine on the isolated mammalian heart.

Hydroxymoloka'iamine (2): white, amorphous powder; $[\alpha]_D = -16.1$ (*c* 0.47, MeOH); UV (MeOH) λ_{max} nm (log ε) 275 (2.19), 224 (3.66), 217 (3.67); UV (MeOH + chromic acid) λ_{max} nm (log ε) 275 (2.46), 231 (3.69), 225 (3.69); ^1H and ^{13}C NMR data, see Table 1; positive HRFABMS *m/z* 366.9662 (calculated for $C_{11}H_{17}Br_2N_2O_2$, $[M + H]^+$, 366.9657).

Ceratinophenol A (5): grayish powder; UV (MeOH) λ_{max} nm (log ε) 338 (3.42), 298 (3.70); UV (MeOH + $AlCl_3$) λ_{max} nm (log ε) 385nm (3.29), 312 (3.85); ^1H and ^{13}C NMR data, see Table 2; positive HRFABMS *m/z* 312.9673 (calculated for $C_{10}H_{11}BrNaO_5$, $[M + Na]^+$, 312.9688).

Moloka'iakitamide (6): amorphous, white powder; UV (MeOH) λ_{max} nm (log ε) 282 (2.26), 274 (2.32), 208 (3.71); ^1H and ^{13}C NMR data, see Table 1; positive HRFABMS *m/z* 421.9719 (calculated for $C_{13}H_{18}Br_2N_3O_3$, $[M + H]^+$, 421.9715).

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Supporting Information Available: UV, HRFABMS, ^1H and ^{13}C NMR, COSY, HSQC, and HMBC spectra of compounds **1**−**7**. This material is available free of charge via the Internet at http://pubs.acs.org.

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