Bioactive Brominated Metabolites from the Red Sea Sponge *Suberea mollis*

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Reinvestigation of the Red Sea sponge *Suberea mollis* afforded two new bromotyrosine-derived alkaloids, subereamollines A (**1**) and B (**2**), two new brominated phenolic compounds, subereaphenols B (**7**) and C (**9**), and the known compounds aerothionin (**3**), homoaerothionin (**4**), 11,19-dideoxyfistularin-3 (**5**), aeroplysinin-1 (**6**), and aeroplysinin-2 (**8**). The structure determination of the isolated compounds was assigned using one- and two-dimensional NMR spectra and HRFABMS data. The antimicrobial and antioxidant activities of the isolated compounds have been evaluated. Aeroplysinin-1 displayed significant antimicrobial activity against *S. aureus*, *P. aerugenosa*, and *K. pneumoniae*. The isolated compounds were examined for their antioxidant activity using a 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) solution-based chemical assay. Among the tested compounds, only subereaphenols B and C displayed a significant effect.

Marine sponges of the order Verongida are of much current biological and chemical interest. They are characterized by elaboration of typical brominated metabolites that are biogenetically related to tyrosine. These metabolites are considered as distinct markers for Verongid sponges. Diverse biological activities for these compounds have been reported including antifungal,[1] antibacterial,[1–4] cytotoxic,[4–9] and enzyme inhibitory effects.[10] In addition, an unprecedented spiro nonacyclic polyaromatic alkaloid with cytotoxic activity was isolated from a sponge of the genus *Suberea* collected at Lihou Reef in the Coral Sea.[11]

Previous study on the Red Sea sponge *Suberea mollis* (order Verongida, family Aplysinellidae) resulted in the isolation and identification of a number of cytotoxic and antimicrobial compounds,[12] which has prompted us to further explore this sponge. In the present work, chemical reinvestigation of the crude extract of *S. mollis* afforded four new compounds, subereamollines A (**1**) and B (**2**) and subereaphenols B (**7**) and C (**9**), together with the known compounds aerothionin (**3**),[1,13–15] homoaerothionin (**4**),[1] 11,19-dideoxyfistularin-3 (**5**),[1] aeroplysinin-1 (**6**),[16] and aeroplysinin-2 (**8**).[17] Optical rotations and detailed examination of the spectroscopic data, UV, NMR (1D and 2D), and HRFABMS secured the assignment of these compounds.

Subereamolline A (**1**) was purified as an optically active, white, amorphous powder. The FABMS spectrum of **1** showed three ion peaks at *m/z* 545.9, 547.9, and 549.9 in the ratio of 1:2:1, respectively, indicating the presence of two bromine atoms in the molecule. The HRFABMS allowed the assignment of the molecular formula as $C_{17}H_{23}Br_2N_3O_6$, requiring seven degrees of unsaturation. The ^{13}C NMR spectrum of **1** (Table 1) displayed signals for 17 carbons including two carbonyls, five quaternary carbons, two methines, six methylenes, and two methyls as assigned from an HSQC experiment. The ^1H NMR signals (Table 1) at δ 4.07 (1H, s, H-1), 6.42 (1H, s, H-5), 3.77 and 3.09 (each 1H, each d, J = 18.2 Hz, H-7a, H-7b), and 3.72 (3H, s, OCH_3), together with their corresponding carbons at δ 75.6 (C-1), 132.4 (C-5), 40.3 (C-7), and 60.5 (OCH_3), respectively, allowed the assignment of fragment A (Figure 1) as a 2,4-dibromo-1-hydroxy-3-methoxy-spirocyclohexadienylisoxazole moiety,[18] which was further confirmed from HMBC correlations (Table 1). In the $^1H-^1H$ COSY spectrum, the coupled signals at δ 3.30 (H_2-10) with the signal at δ 1.54 (H_2-11 and H_2-12), which was further coupled with the signal at δ 3.11 (H_2-13), allowed assignment of fragment B (Figure 1) as a butanediamine moiety. Similarly, the coupled signals at δ 4.04 (2H, q, J = 7.2 Hz, $COOCH_2$) and 1.22 (3H, t, J = 7.2 Hz, $COOCH_2CH_3$) secured the assignment of fragment C as an ethoxy moiety (Figure 1). The connectivity of fragments A−C through the signals at δ 161.7 (C=O, C-9) and 159.5 (C=O), which was supported from HMBC correlations of H_2-10/C-9, H_2-13/C=O, and OCH_2/C=O, allowed the assignment and secured the structure of subereamolline A.

The molecular formula of subereamolline B (**2**) was assigned as $C_{18}H_{25}Br_2N_3O_6$ from the HRFABMS pseudomolecular ion peak at *m/z* 559.9990 $[M + Na]^+$, being 14 mass units larger than that of **1**, suggesting the presence of an additional methylene unit in the molecule. Comparison of the ^1H and ^{13}C NMR and HMBC data of **2** with those of **1** (Table 1) showed nearly identical signals. Moreover, the new NMR signals (Table 1) of the side chain in **2** at δ 40.3/3.30 (C-10/H_2-10), 30.1/1.53 (C-11/H_2-11), 25.2/1.36 (C-12/H_2-12), 30.7/1.53 (C-13/H_2-13), and 41.6/3.08 (C-14/H_2-14) confirmed the presence of an additional CH_2 in the side chain of **2**. Therefore the butanediamine moiety in **1** should be replaced by a pentanediamine moiety in **2**, which was secured from $^1H-^1H$ COSY data. In addition, the assignments of all protonated and quaternary carbons of **2** were secured from HSQC and HMBC experiments, respectively.

Accurate HRFABMS of **7** supported the molecular formula $C_9H_8Br_2O_4$. The ^{13}C NMR spectrum of **7** (Table 2) displayed nine signals including six aromatic carbons (δ 149.7−109.6), one carbonyl functionality at δ 171.5, and signals at δ 35.0 and 51.0. The ^1H NMR spectrum of **7** (Table 2) displayed a one-proton singlet at δ 7.01 (H-5) for a pentasubstituted aromatic ring. In addition, signals at δ 3.85 (2H, s) and 3.75 (3H, s, OCH_3) indicated the presence a methylene and a methoxy group. The HSQC data revealed the presence of six quaternary carbons, one methine, one methylene, and one methyl. The HMBC cross-peaks (Table 2) of H-5/C-1, H_2-7/C-1, H-5/C-3, H_2-7/C-6, H_2-7/C-8, and H_3CO/C-8 supported the placement of all functionalities on the aromatic moiety of **7**, which was assigned as methyl-6-(2,4-dibromo-1,3-dihydroxy)-phenacetate (subereaphenol B).

The molecular formula of subereaphenol C (**9**) was deduced as $C_{10}H_{10}Br_4O_4$ from its HRFABMS and thus was a methylene homologue of compound **7**. Comparison of the ^{13}C and ^1H NMR data of compound **9** (Table 2) with those of **7** showed nearly identical signals for both compounds. The only difference was the replacement of the methoxy moiety (δ 171.5, 51.0/3.75) in **7** with signals (δ 172.7, 62.1/4.14, 14.6/1.23) for an ethoxy fragment in **9** (Table 2). Again, the assignment of the protonated carbons as well as the quaternary carbons and the placement of all moieties on the

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Chart 1

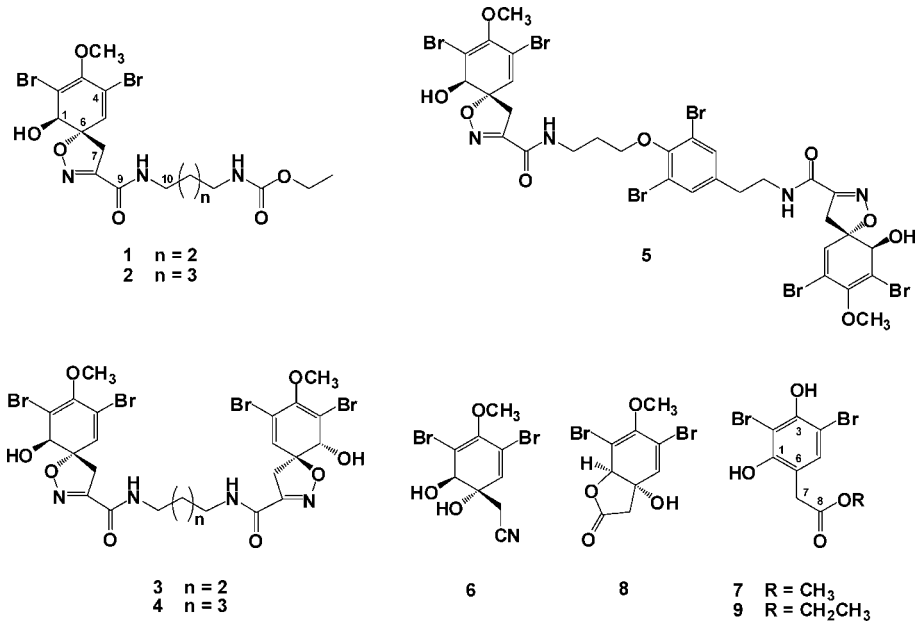

Table 1. NMR Data and HMBC Correlations of Subereamollines A (**1**) and B (**2**) (CD_3OD)

	1			2		
position	δ_H [mult., *J* (Hz)]	δ_C (mult.)	HMBC (H→C)	δ_H [mult., *J* (Hz)]	δ_C (mult.)	HMBC (H→C)
1	4.07 (1H, s)	75.6 (CH)	H-5, H_2-7	4.06 (1H, s)	75.6 (CH)	H-5, H_2-7
2		122.9 (qC)	H-5		122.9 (qC)	H-5
3		149.4 (qC)	H-1, H-5, OCH_3		149.4 (qC)	H-1, H-5, OCH_3
4		114.3 (qC)	H-1, H-5		114.3 (qC)	H-1, H-5
5	6.42 (1H, s)	132.4 (CH)	H-1, H_2-7	6.40 (1H, s)	132.4 (CH)	H-1, H_2-7
6		92.4 (qC)	H-1, H-5, H_2-7		92.4 (qC)	H-1, H_2-7
7	3.77 (1H, d, 18.2), 3.09 (1H, d, 18.2)	40.3 (CH_2)	H-1, H-5	3.77 (1H,d, 18.3), 3.09 (1H, d, 18.3)	40.4 (CH_2)	H-1, H-5
8		155.4 (qC)	H_2-7		155.4 (qC)	H_2-7
9		161.6 (qC)	H_2-10		161.6 (qC)	H_2-10
10	3.30 (2H, m)[a]	40.2 (CH_2)	H_2-11, H_2-12	3.30 (2H, m)[a]	40.3 (CH_2)	H_2-11, H_2-12
11	1.54 (2H, m)[b]	27.7 (CH_2)	H_2-10, H_2-12, H_2-13	1.53 (2H, m)[b]	30.1 (CH_2)	H_2-10
12	1.54 (2H, m)[b]	28.4 (CH_2)	H_2-10, H_2-13	1.36 (2H, m)	25.2 (CH_2)	H_2-10, H_2-13, H_2-14
13	3.11 (2H, m)	41.3 (CH_2)		1.53 (2H, m)[b]	30.7 (CH_2)	H_2-10, H_2-14
14				3.08 (2H, m)	41.6 (CH_2)	
CO		160.0 (qC)	H_2-13, OCH_2		159.0 (qC)	H_2-14
OC_2H_5	4.04 (2H, q, 7.2)	61.7 (CH_2)	CH_3	4.03 (2H, q, 7.0)	61.7 (CH_2)	CH_3
	1.22 (3H, t, 7.2)	15.1 (CH_3)	CH_2	1.22 (3H, t, 7.0)	15.1 (CH_3)	
OCH_3	3.72 (3H, s)	60.5 (CH_3)		3.72 (3H, s)	60.5 (CH_3)	

[a] Overlapped with solvent signal. [b] In each column overlapped signals.

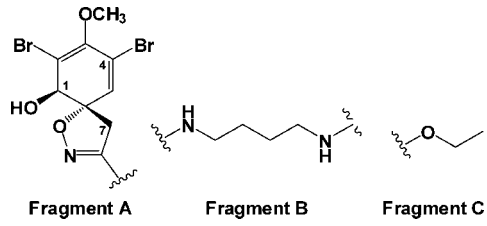

Figure 1. Partial structural fragments of subereamolline A (**1**).

skeleton of **9** were secured from HSQC and HMBC experiments, respectively. Therefore compound **9** was assigned as ethyl-6-(2,4-dibromo-1,3-dihydroxy)phenacetate.

In conclusion, reinvestigation of the Red Sea sponge *S. mollis* resulted in the isolation of eight compounds including two new bromotyrosine-derived alkaloids, subereamollines A (**1**) and B (**2**), two new brominated phenolic compounds, subereaphenols B (**7**) and C (**9**), and several known compounds. Biological evaluation of the isolated compounds showed that aeroplysinin-1 possesses significant antimicrobial activity against *S. aureus*, *P. aerugenosa*, and *K. pneumoniae*. In addition, subereaphenols B (**7**) and C (**9**) showed significant antioxidant activity. The high antioxidant activity of subereaphenols B and C could be attributed to the phenolic nature of these compounds. This finding has 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. UV spectra were recorded on a Hitachi 300 spectrometer. NMR spectra were obtained in CD_3OD on a Bruker Avance DRX300 spectrometer at 300 MHz for ^1H NMR and 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 enkaphalin at *m/z* 556.2771 $[M + H]^+$ as a reference mass. A semipreparative HPLC system (Bischoff) was equipped with two HPLC compact pumps, a central processor, a Rheodyne injector, and a variable-wavelength UV detector. The HPLC separation and quantitation were made on a RP18, 250 × 10 mm, 5 μm Phenomenex Luna column. The mobile phase was 40:60 (v/v) CH_3CN and NaH_2PO_4 (pH 3.5) at 220 nm and at a flow rate of 2.0 mL/min. Precoated silica gel G-25 UV_{254} plates were used for thin-layer chromatography, and

Table 2. NMR Data and HMBC Correlations for Subereaphenols B (**7**) and C (**9**) (CD_3OD)

	7			**9**		
position	δ_H [mult., *J* (Hz)]	δ_C (mult.)	HMBC (H→C)	δ_H [mult., *J* (Hz)]	δ_C (mult.)	HMBC (H→C)
1		149.7 (qC)	H-5, H_2-7		151.4 (qC)	H-5, H_2-7
2		115.6 (qC)	H_2-7		117.2 (qC)	H_2-7
3		143.7 (qC)	H-5		143.0 (qC)[a]	H-5
4		109.6 (qC)	H-5		111.2 (qC)	H-5
5	7.01 (1H, s)	117.2 (CH)		6.98 (1H, s)	118.8 (CH)	H_2-7
6		122.2 (qC)	H-5, H_2-7		123.9 (qC)	H-5, H_2-7
7	3.85 (2H, s)	35.0 (CH_2)		3.81 (2H, s)	36.8 (CH_2)	H-5
8		171.5 (qC)	H_2-7, OCH_3		172.7 (qC)	H_2-7, CH_2
OC_2H_5				4.14 (2H, q, 7.1)	62.1 (CH_2)	CH_3
				1.23 (3H, t, 7.1)	14.6 (CH_3)	CH_2
OCH_3	3.75 (3H, s)	51.0 (CH_3)				

[a] Weak signal detected from HMBC.

silica gel 60, 230−40 μm mesh (E. Merck), and Sephadex LH-20 (Pharmacia) were used for column chromatography.

Animal Material. The marine sponge *Suberea mollis* was collected from Hurghada at the Egyptian Red Sea coast at depths between 10 and 20 m during the summer of 2004. A detailed description of the sponge was previously reported elsewhere.[12] The voucher specimen, measuring 3.5 cm, is incorporated in the collections of the Zoological Museum of the University of Amsterdam under registration number 16621. Another voucher specimen was deposited in the Red Sea Invertebrates Collection of the Department of Pharmacognosy, Suez Canal University, under the code number DY-8.

Extraction and Isolation. The fresh sponge material (1.5 kg) was chopped into small pieces and extracted with MeOH. The extract was defatted with hexanes. The mother liquor was concentrated under reduced pressure to yield a viscous, brown residue (4.5 g). The residue was fractionated by a silica gel column, eluted with a hexanes/CH_2Cl_2/MeOH gradient, to yield two fractions, A (1.475 g) and B (0.823 g). Fraction A (1.475 g) was further purified on a Sephadex LH-20 column using MeOH/CH_2Cl_2 (1:1) to produce major fraction C, while fraction B was treated similarly to give fraction D. A part of fraction C (100 mg) was purified on a RP C_{18} semipreparative HPLC column using 60% MeCN in H_2O at a flow rate of 2 mL/min using a UV detector at 254 nm to afford compounds **1** (4.9 mg, t_R = 8.29 min), **2** (2.0 mg, t_R = 11.54 min), **3** (10.7 mg, t_R = 12.0 min), **4** (4.2 mg, t_R = 13.95 min), and **5** (16.1 mg, t_R = 29.27 min). A part of fraction D (100 mg) was processed in the same manner to yield compounds **6** (8.6 mg, t_R = 6.23 min), **7** (10.5 mg, t_R = 9.56 min), **8** (8.0 mg, t_R = 12.92 min), and **9** (7.5 mg, t_R = 33.82 min).

Determination of the Antimicrobial Activity Using the Agar Diffusion Method. The antimicrobial activities of the isolated compounds were evaluated against *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 quantities of each compound were dissolved in 1 mL of DMF, and 100 μL of each solution was inserted in a small hole previously made in the agar followed by incubation at 37 °C for 24 h. The inhibition zones were measured and compared with those produced by reference erythromycin (15 μg/disk), ciprofloxacin (5 μg/disk), chloramphenicol (30 μg/disk), and clotrimazole (10 mg/mL, 100 μL). Most of the examined compounds showed activity against *S. aureus* to variable degrees. The inhibition zones of the tested compounds are presented in Table 3. None of the tested compounds exhibited an antifungal effect.

Evaluation of the Antioxidant Activity. Compounds **1**−**9** were examined for their antioxidant activity using a 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) solution-based chemical assay.[19] The compounds were dissolved in MeOH at a concentration of 1 mg/mL, and vitamin E was prepared at a similar concentration and used as a positive control. Six micrograms of each compound were applied in the form of a spot of 4 mm in diameter. The radical-scavenging effects were detected on a TLC plate, using a spray reagent composed of a 0.2% (w/v) solution of 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) in MeOH. The plate was observed 30 min after spraying. Aeroplysinin-1 (**6**) showed a yellow color almost comparable to that of vitamin E, while subereaphenols B (**7**) and C (**9**) showed a very strong bright yellow color, suggesting their powerful antioxidant activities.

Table 3. Antimicrobial Activity of Compounds **1**−**9** against Different Microorganisms.

	inhibition zone (mm)		
compound	*S. aureus*	*P. aeruginosa*	*K. pneumoniae*
1	3	NI[b]	NI
2	−[a]	−	−
3	5	NI	3
4	3	NI	NI
5	NI	NI	NI
6	9	11	7
7	5	NI	NI
8	5	NI	NI
9	−	−	−
chloramphenicol	9	7	10
erythromycin	8	NI	NI
ciprofloxacin	NI	2	8
clotrimazole	−	−	−

[a] − = not tested. [b] NI = no inhibition.

Determination of the Cytotoxic Activity of the Compounds. The cytotoxic activities of the isolated compounds were determined against human colon tumor cells (HCT-116) using the MTT assay.[20] Makulavamine C was used as a positive cytotoxic control. None of the tested compounds showed any significant cytotoxicity against human colon carcinoma at a concentration of 10 μg/mL.

Subereamolline A (1): white, amorphous powder; $[\alpha]_D$ +156.5 (*c* 0.55, MeOH); UV (MeOH) λ_{max} nm (log ε) 280 (3.63), 228 (3.72), 205 (3.72); ^1H and ^{13}C NMR data, see Table 1; positive HRFABMS *m/z* 547.9846 (calcd for $C_{17}H_{23}{}^{79}Br^{81}BrN_3NaO_6$, $[M + Na]^+$, 547.9831).

Subereamolline B (2): white, amorphous powder; $[\alpha]_D$ +22.9 (*c* 6.25, CH_2Cl_2); UV (MeOH) λ_{max} nm (log ε) 281 (3.65), 236 (3.86), 225 (3.86); ^1H and ^{13}C NMR data, see Table 1; positive HRFABMS *m/z* 559.9990 (calcd for $C_{18}H_{25}{}^{79}Br_2N_3NaO_6$, $[M + Na]^+$, 560.0005).

Subereaphenol B (7): dark brown residue; UV (MeOH) λ_{max} nm (log ε) 301 (2.83), 206 (3.62); ^1H and ^{13}C NMR data, see Table 2; positive HRFABMS *m/z* 360.8690 (calcd for $C_9H_8{}^{79}Br_2NaO_4$, $[M + Na]^+$, 360.8687).

Subereaphenol C (9): dark brown residue; UV (MeOH) λ_{max} nm (log ε) 301 (3.07), 211 (3.60); ^1H and ^{13}C NMR data, see Table 2; positive HRFABMS *m/z* 376.8822 (calcd for $C_{10}H_{10}{}^{79}Br^{81}BrNaO_4$, $[M + Na]^+$, 376.8823).

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Supporting Information Available: UV, HRFABMS, ^1H and ^{13}C NMR, COSY, HSQC, and HMBC spectra of compounds **1**−**9**. This material is available free of charge via the Internet at http://pubs.acs.org.

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