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New Bromotyrosine Metabolites from the Sponge *Aplysinella rhax*

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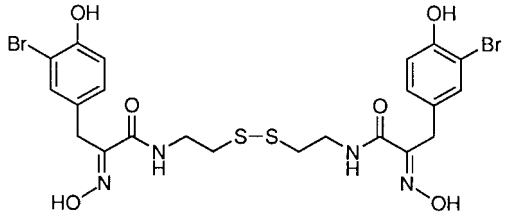
Abstract—Four new bromotyrosine metabolites (**3**–**6**) along with the previously described compounds psammaplin A (**1**) and bisaprasin (**2**) have been isolated from the sponge *Aplysinella rhax* collected from Guam, Palau, and Pohnpei. Based upon the results of extensive spectroscopic analysis and chemical reaction, the structures of psammaplins A_1 (**3**) and A_2 (**4**) have been determined to be a *N*,*N*-dimethylguanidium salt of psammaplin A sulfate and its bis-*N*,*N*-dimethylguanidium disulfate derivative, respectively. Aplysinellin A (**5**) possesses an additional bromotyrosine-derived C_9 unit connected directly to the carbon framework of psammaplin A by a biphenylic linkage while aplysinellin B (**6**) is the corresponding cyclic enol ether. These compounds exhibited moderate cytotoxicity and inhibitory activities against farnesyl protein transferase and leucine aminopeptidase. © 2000 Elsevier Science Ltd. All rights reserved.

Introduction

Bromotyrosine-derived secondary metabolites are frequently encountered in sponges of the order Verongida.[1,2] Of these sponge-derived bromotyrosines, psammaplins and related compounds comprise a small group of metabolites that appear to be biosynthesized by the linear connection of bromotyrosines and modified cysteines. Psammaplin A, the first metabolite of this series, is a symmetrical tetrapeptide composed of two units each of bromotyrosines and cysteines while bisaprasin is a biphenylic dimer of psammaplin A.[3–5] Other psammaplins are the linear dipeptides and tripeptides containing various sulfur functionalities at the chain originating from a modified cysteine.[6]

In the course of our continuing search for secondary metabolites possessing ecological and biomedical significance from tropical marine animals, we collected the sponge *Aplysinella rhax* from various locations of Micronesia. The crude extract of this animal showed significant cytotoxicity against a human leukemia cell K562 (LC_{50} 21 μg/mL). Bioactivity-directed fractionation of the crude extract led to the isolation of several bromotyrosine-derived metabolites. Herein we report the isolation and structure determination of six compounds of the psammaplin class including two previously reported metabolites, psammaplin A (**1**) and bisaprasin (**2**). The new compounds, psammaplin A_1 (**3**) and A_2 (**4**) are the structural analogs of psammaplin A containing



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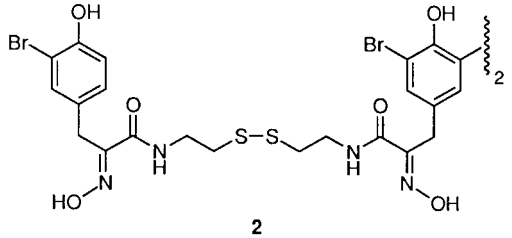

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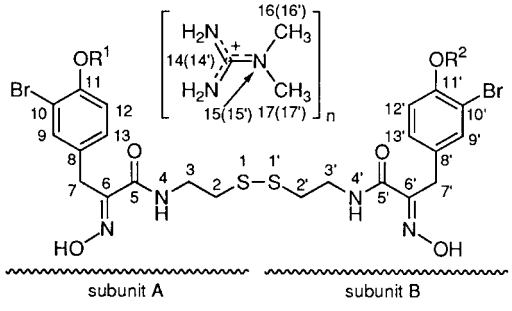

3 R^1 = H, R^2 = SO_3^-, n = 1

4 R^1 = R^2 = SO_3^-, n = 2

Keywords: bromotyrosines; psammaplins; aplysinellins; sponge; *Aplysinella rhax*.

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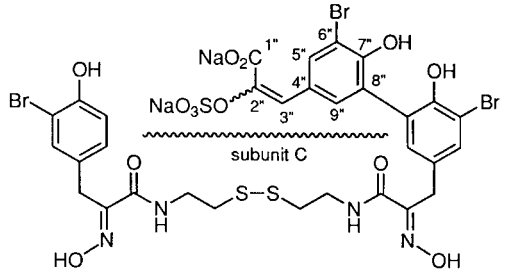
Table 1. NMR data (CD_3OD) for psammaplin A_1 (**3**)

No.	δ_H (Hz)	δ_C	HMBC	No.	δ_H (Hz)	δ_C	HMBC
2	2.80, t (6.8)	38.6[a] (t)	3	2′	2.80, t (6.8)	38.5[a] (t)	3′
3	3.51, t (6.8)	39.7[b] (t)	2, 5	3′	3.51, t (6.8)	39.6[b] (t)	2′, 5′
5		165.8 (s)		5′		165.6 (s)	
6		153.0 (s)		6′		152.5 (s)	
7	3.78, s	28.7 (t)	5, 6, 9, 13	7′	3.86, s	29.0 (t)	5′, 6′, 8′, 9′, 13′
8		130.6 (s)		8′		136.0 (s)	
9	7.36, d (2.0)	134.4 (d)	7, 10, 11, 13	9′	7.49, d (2.0)	134.6 (d)	7′, 8′, 10′, 11′, 13′
10		110.4 (s)		10′		116.5 (s)	
11		153.7 (s)		11′		149.6 (s)	
12	6.75, d (8.3)	117.0 (d)	8, 10, 11	12′	7.47, d (8.8)	123.3 (d)	8′, 10′, 11′
13	7.06, dd (8.3, 2.0)	130.3 (d)	7, 9, 11	13′	7.21, dd (8.8, 2.0)	130.0 (d)	7′, 9′, 11′
				14′		158.6 (s)	
				16′	3.02, s	38.3 (q)	14′, 17′
				17′	3.02, s	38.3 (q)	14′, 16′

[a] Interchangeable signals.
[b] Interchangeable signals.

sulfate groups and *N*,*N*-dimethylguanidium as counter ions. Aplysinellin A (**5**) possesses an additional C_9 unit that appears to be biogenetically derived from bromotyrosine while aplysinellin B (**6**) is the corresponding cyclic enol ether.



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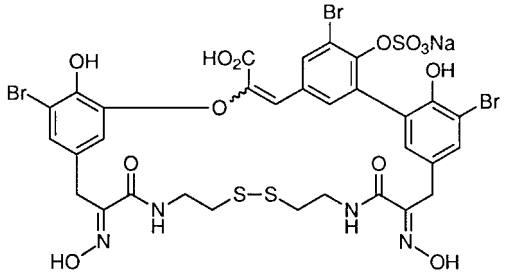

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Results and Discussion

The specimens were collected from the Pacific Islands of Guam, Palau, and Pohnpei. The lyophilized specimens were extracted with MeOH and CH_2Cl_2. The combined crude extracts were solvent-partitioned and the polar organic materials were separated by C_{18} reversed-phase flash chromatography and C_{18} HPLC to afford pure metabolites. The results of separation process showed no significant difference in distribution of metabolites among collections suggesting the chemical homogeneity of this sponge in diverse habitats (Extraction and isolation).

Compounds **1** and **2**, the least polar constituents, were obtained as white amorphous solids. Based on the results of combined spectroscopic analyses, the structures of these compounds were defined as psammaplin A (**1**) and bisaprasin (**2**). Psammaplin A was previously reported as a bioactive constituent of an unidentified sponge, *Psammaplysilla* sp., and *Psammaplysilla purpurea*.[3,4,6] This compound and bisaprasin were also isolated from the sponge *Thorectopsamma xana*.[5] The spectral data of these compounds were in good agreement with those reported previously.

Psammaplin A_1 (**3**) was isolated as a pale-yellow amorphous solid. The ^{13}C NMR spectra of this compound displayed signals of 25 carbons. However, the positive and negative HRFABMS data showed *m/z* clusters corresponding to $C_{25}H_{34}Br_2N_7O_6S_2$ (752.0381, Δ −2.3 mmu) and $C_{22}H_{23}Br_2N_4O_9S_3$ (742.8976, Δ −0.3 mmu), respectively. The negative LRESIMS data also showed a peak at *m/z* 743. The significant discrepancy in the mass data was indicative of the presence of a charged functionality in the molecule. The NMR data of this compound were highly comparable with those obtained for **1**. A combination of ^1H COSY, TOCSY, gradient HSQC, and gradient HMBC data revealed that **3** possessed the same bis-SCH_2CH_2NHCO-group as **1**. In addition, **3** had two 1,3,4-trisubstituted benzene moieties as found in **1**. The chemical shifts of signals of the aromatic carbons and protons of subunit A were almost identical to those of the 3-bromo-4-hydroxy-benzyl moiety in **1**. However, the NMR signals corresponding to the aromatic protons and carbons of subunit B were significantly shifted from those observed for **1** (Table 1). Since the mass data and chemical shift of C-10′ at δ 116.5 in the ^{13}C NMR spectrum indicated the attachment of a bromine at this position, another substituent clearly replaced the hydroxyl group of **1** at C-11′. Comparison of the partial molecular formula with the negative mass data containing the identical number of carbons revealed that the new substituent was a sulfate group. This interpretation was confirmed by a strong absorption band at 1250 cm^{-1} in the IR spectrum.

The ^{13}C NMR data of **3** showed three additional carbons at δ 158.6 (C) and 38.3 (CH_3, two carbons). The latter carbons were assigned to bear the protons at δ 3.02 (6H, s) in the ^1H

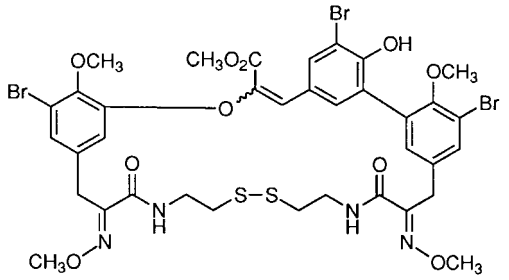
NMR spectrum on the basis of the gradient HSQC data. The gradient HMBC experiment showed 3-bond correlations between this proton and both carbons at δ 158.6 and 38.3. An *N*,*N*-dimethylguanidium group was in good agreement with the NMR data and positive HRFABMS data, interpreted as $[M-SO_3+C_3H_{10}N_3(N,N\text{-dimethylguanidium})+H]^+$. Alternatively the possibility of *N*,*N*′-dimethylguanidium group was less feasible on the basis of the downfield shift of the methyl carbons.[7] Thus, the structure of psammaplin A_1 (**3**) was determined to be the *N*,*N*-dimethylguanidium salt of psammaplin A 11′-sulfate.[8] The presence of the sulfate and guanidium group as counter ions in **3** was further supported by the structure elucidation of **4** as described below.

The negative HRFABMS data of psammaplin A_2 (**4**) gave an *m*/*z* cluster corresponding to $C_{25}H_{32}Br_2N_7O_{12}S_4$. The observation of signals of 14 carbons only in the ^{13}C NMR data revealed the molecular symmetry of this compound. The ^1H NMR spectra of this compound were also very simple and highly comparable with those of psammaplins A and A_1. Careful examination of the NMR data revealed that **4** had the same partial structure as subunit B of **3** plus *N*,*N*-dimethylguanidium group. This interpretation was confirmed by detailed interpretation of 2D NMR experiments. Therefore, the *m*/*z* cluster for this compound in the FABMS data corresponded to $[M+C_3H_{10}N_3(N,N\text{-dimethylguanidium})]^-$. The observation of a negatively charged mass cluster containing two sulfates and an *N*,*N*-dimethylguanidium group was evidence for the presence of both functionalities in the molecule. Thus, the structure of psammaplin A_2 (**4**) was determined to be the bis-*N*,*N*-dimethylguanidium salt of psammaplin A 11,11′-disulfate. A literature survey revealed that the isolation of organic sulfates with *N*,*N*-dimethylguanidium as counter ions is very rare among sponge metabolites.[1] To the best of our knowledge, only suvanine and sulfircin, both sesterterpenoid sulfates from the sponge *Ircinia* sp., have been described.[9,10]

In addition to the psammaplins, two minor constituents were also separated from the crude extract. The molecular formula of aplysinellin A (**5**) was established as $C_{31}H_{27}Br_3N_4O_{13}S_3$ by extensive mass analysis. The conspicuous *m*/*z* clusters in the positive FABMS data had three positive counter-ions, e.g. [M+3Na], [M+2Na+H], and $[M-SO_3+Na+2H]$ while those in the negative FABMS data had a positive counter-ion such as [M+2Na−H] and [M+Na]. This observation led to the conclusion that compound **5** was indeed a divalent anion. The ^1H and ^{13}C NMR data of this compound differed remarkably from those of other psammaplins. The most significant difference in the ^{13}C NMR spectra was the appearance of signals of nine additional carbons including a carbonyl carbon at δ 172.7. The downfield shift of all new carbon signals ($>\delta$ 115) and corresponding proton signals (δ 8.22, 7.65, and 6.93) in the NMR data suggested the addition of a new subunit (subunit C) composed of aromatic and/or olefinic moieties to the carbon framework of the psammaplins.

Careful examination of the NMR data showed that subunit A and the $-SCH_2CH_2NHCO-$ portion of subunit B of psammaplin A were intact in **5**. Starting from the conspicuous signal of the C-7′ methylene, however, the assignments of the NMR resonances on the basis of combined results of ^1H COSY, gradient HSQC, and gradient HMBC experiments revealed the presence of a 1,3,4,5-tetrasubstituted benzene ring instead of the 1,3,4-trisubstituted ring of subunit B in other psammaplins (Table 2). The attachment of a bromine and hydroxyl group at C-10′ and C-11′, respectively, was established by the chemical shifts of these and neighboring carbons as well as the comparison of the NMR data with those of **2**.

Consideration of the molecular formula revealed that the remaining part had a formular of $C_9H_4BrO_7S$. The combined 2D NMR experiment showed the presence of a 1,3,4,5-tetrasubstituted ring. The placement of a bromine and a lone-pair bearing heteroatom at C-6″ and C-7″, respectively, was established by the chemical shifts of these and neighboring carbons in the ^{13}C NMR data (Table 2). The chemical shifts of three additional carbons at δ 172.7 (C), 142.5 (C), and 123.8 (CH) suggested the presence of an α,β-unsaturated carboxyl group attached by an electron-withdrawing group at its α-position. This interpretation and the attachment of the partial structure at C-4″ of the aromatic ring was confirmed by the mutual HMBC correlations between protons and carbons at the β-carboxylic methine and C-9″. The hydroxyl and sulfate group, deduced from the molecular formula and a strong absorption band at 1270 cm^{-1} in the IR data, could be located at either of C-2″ and C-7″ of this C_9 unit. The enolized nature of the $\Delta^{2''}$ double bond required a charged functionality, that is the sulfate group at C-2″. Alternatively the attachment of the hydroxyl group at this position would transform the enol to the corresponding keto group, which was denied by the NMR data. Thus, the structure of subunit C was elucidated as a highly functionalized C_9 unit of cinnamic acid type which appeared to be derived biogenetically from bromotyrosine. The subunit C of **5** had an asymmetric double bond at $\Delta^{2''}$. Due to the lack of proton signals at this center, however, the geometry at this bond was not determined directly by NMR methods. The geometry was tentatively assigned as *E* on the basis of comparison of the spectral data with **7**, a derivatized product as described later.



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The connectivity between subunits B and C by a biphenylic linkage at C-12′ and C-8″ was determined by the HMBC correlations of H-13′ with C-8″ and H-9″ with C-12′. Further supporting evidence for this interpretation and the substitution pattern at the aromatic rings of subunits B and C was provided by the ROESY experiments. The benzylic H-7′ showed cross peaks with H-9′ and H-13′. The latter proton, in turn, showed a cross peak with H-9″. In addition,

Table 2. NMR data (CD_3OD) for aplysinellins A (**5**) and B (**6**)

No.	**5**			**6**		
	δ_H (Hz)	δ_C	HMBC	δ_H (Hz)	δ_C	HMBC
2	2.78, t (6.8)[a]	38.6 (t)[b]	3	2.87, m[d]	41.1 (t)	
3	3.49, t (6.8)	39.7 (t)[c]	2, 5	3.51, m[d]	40.0 (t)	
5		165.9 (s)			166.2 (s)	
6		153.0 (s)			153.1 (s)	
7	3.77, s	28.7 (t)	5, 6, 9, 13	3.72, s	28.3 (t)	5, 6, 8, 9, 13
8		130.0 (s)			129.6 (s)	
9	7.34, d (2.0)	134.4 (d)	10, 11, 13	6.81, d (2.0)	126.3 (d)	7, 10, 11, 13
10		110.8 (s)			110.5 (s)	
11		154.3 (s)			143.7 (s)	
12	6.73, d (8.3)	117.3 (d)	8, 10, 11		147.5 (s)	
13	7.03, dd (8.3, 2.0)	130.3 (d)	7, 9, 11	6.18, d (2.0)	115.0 (d)	7, 9, 11, 12
2′	2.77, t (6.8)[a]	38.5 (t)[b]	3′	2.87, t (6.8)	38.4 (t)	3′
3′	3.51, t (6.8)	39.6 (t)[c]	2′	3.43, t (6.8)	39.6 (t)	2′, 5′
5′		166.1 (s)			165.4 (s)	
6		153.8 (s)			152.9 (s)	
7′	3.86, s	29.0 (t)	5′, 6′, 8′, 9′, 13′	3.68, br s[d]	28.7 (t)	5′
8′		127.9 (s)			130.3 (s)	
9′	7.31, d (2.0)	132.5 (d)	7′, 10′, 11′, 13′	7.32, d (2.0)	134.6 (d)	7′, 10′, 11′, 13′
10′		114.6 (s)			113.1 (s)	
11′		155.0 (s)			151.2 (s)	
12′		131.7 (s)			128.4 (s)	
13′	7.35, d (2.0)	132.2 (d)	7′, 9′, 11′, 8″	6.78, d (2.0)	131.3 (d)	7′, 9′, 11′, 8″
1″		172.7 (s)			171.4 (s)	
2″		142.5 (s)			146.8 (s)	
3″	6.93, s	123.8 (d)	1″, 2″, 5″, 9″	6.96, s	120.9 (d)	1″, 2″, 5″, 9″
4″		124.1 (s)			134.5 (s)	
5″	8.22, d (2.2)	134.3 (d)	4″, 6″, 7″, 9″	8.42, d (2.0)	135.7 (d)	3″, 6″, 7″, 9″
6″		116.4 (s)			118.2 (s)	
7″		160.2 (s)			150.4 (s)	
8″		130.8 (s)			135.5 (s)	
9″	7.65, d (2.2)	134.2 (d)	12′, 3″, 5″, 7″	7.59, d (2.0)	134.1 (d)	12′, 3″, 5″, 7″

[a] Interchangeable signals.
[b] Interchangeable signals.
[d] The shape of signal was changed in temperature-variable experiment in DMSO-d6.
[c] Interchangeable signals.

spacial proximity was found between H-5″ and H-3″. Thus, the structure of aplysinellin A (**5**) was determined as a bromotyrosine metabolite of the psammaplin class. A literature survey revealed that the attachment of a bromotyrosine-derived C_9 unit of cinnamic acid type to another bromotyrosine moiety by a biphenylic linkage, as observed for this compound, is uncommon among sponge metabolites.[1] To the best of our knowledge, the biphenylic linkage between bromotyrosine units of aplysinellin A is precedented only by those in bastadin-3 and its 10-sulfated analog.[11,12]

The molecular formula of aplysinellin B (**6**) was deduced as $C_{31}H_{26}Br_3N_4O_{13}S_3$, less than **5** by a hydrogen, on the basis of extensive mass analysis. The NMR data for this compound were also highly comparable with those obtained for **5**. However, the ^{13}C NMR data revealed the replacement of a signal of an aromatic proton-bearing carbon by that of a quaternary carbon. In addition, several carbon signals in subunits A and C were considerably shifted. The corresponding difference was also found in the ^1H NMR and ^1H COSY data in which the AMX coupling pattern of protons at the aromatic ring of subunit A of **5** was replaced by an AB pattern (Table 2). A small ^1H–^1H coupling constant (J=2.0 Hz), combined with the results of gradient HSQC and HMBC experiments showed that a new substituent was attached at C-12 of subunit A. The downfield shift of this carbon (δ 147.5) as well as the upfield shifts of the neighboring carbon (δ 115.0) and proton (δ 6.18) in the NMR data suggested the attachment of an electron-withdrawing group at this position.

The detailed 2D NMR interpretation revealed the almost identical ^1H–^1H and ^1H–^{13}C correlations for subunits B and C between **5** and **6**. Consequently the placement of a new substituent at C-12, combined with the loss of a hydrogen seen in the mass data of **6**, was explained by a connection between subunits A and C. The supporting data for this interpretation was provided by the ROESY experiment in which spatial proximity was observed among H-13, H-13′, and H-9″. In addition, the splitting pattern (multiplet) of H-2 and H-3 in the ^1H NMR data were indicative of the unequivalence of the methylene protons due to the hindrance of free rotation by cyclization of the molecule (Table 2). The attachment of subunit C at C-12 of the subunit A could occur by either the C-1″ carboxylic or C-2″ sulfate (or hydroxyl) group. That is, **6** could be either a lactone or a cyclic enol ether.

This problem was solved by a chemical transformation. Treatment of **6** with $(CH_3)_2SO_4$ afforded **7** as the major product. The structure of this compound was unambiguously defined as a pentamethylated derivative of **6** by combined 2D NMR experiments. The ROESY data

showed cross peaks H-3/H-13′, H-3/H-9″, H-13/H-13′, and H-13/H-3″ confirming the cyclized nature of this compound. The gradient HMBC data showed a long-range correlation of the C-1″ carbonyl carbon (δ 166.2) with a methoxy proton (δ 3.92). Accordingly the connection between subunits A and C was assigned to occur with formation of an ether bridge between C-12 and C-2″.

The molecular formula and IR data (1270 cm^{-1}) of **6** indicated the presence of a sulfate group as found for **5**. However, the presence of an ether functionality at C-2″ of **6** suggested different location for this group between **5** and **6**. Of the three possible positions at C-11, C-11′, and C-7″, the observation of a free hydroxyl group at C-7″ of **7** showed the attachment of the sulfate functionality at this position in **6**.[13] This interpretation was supported by the ^{13}C NMR data in which substantial difference was observed for C-7″, its *ortho* and *para* carbons between **5** and **6** (Table 2). Although it was inconclusive, the *E* configuration was suggested for $\Delta^{2''}$ on the basis of the absence of a ROESY correlation between the methoxy protons at C-1″ and H-3″. Thus, the structure of aplysinellin B (**6**) was defined as a derivative of aplysinellin A (**5**) possessing a cyclic enol ether functionality.

Psammaplins and related bromotyrosine metabolites exhibit diverse bioactivities. For example, psammaplin A was significantly cytotoxic against the leukemia cell-line P388.[4] This compound and bisaprasin were also reported to possess antimicrobial activity.[5] Psammaplin A and the recently isolated sodium salt of psammaplin A 11′-sulfate inhibited ligand binding to rat-brain adenosine A_1 receptors.[8] Psammaplin D, a truncated derivative of psammaplin A, exhibited antimicrobial and tyrosine kinase D inhibition activity.[6] In our studies, compounds **1**–**6** were also active against the K562 cell-line with LC_{50} of 0.4, 0.7, 1.9, 4.2, 10.7, and 7.1 mM, respectively. These metabolites also exhibited inhibitory activity against farnesyl protein transferase with IC_{50} of 7.0, 4.2, 3.0, 4.4, 85.2, and 25.1 mM for **1**–**6**, respectively. In addition, compounds **1**, **2**, and **5** exhibited inhibitory activity against leucine aminopeptidase (AP-N) with IC_{50} of 70.9, 30.2, and 2.4 mM while others were inactive (IC_{50}>100 mM). Despite the potent and diverse bioactivies of these compounds in biomedical studies, however, the ecological roles of psammaplins remain to be unveiled. In field feeding assays, psammaplin A (**1**) and A_1 (**3**), the major constituents of this sponge, failed to deter feeding by carnivorous and omnivorous coral reef fishes.

Experimental

General experimental procedures

Melting points were measured on a Fisher–Johns apparatus and are uncorrected. IR spectra were recorded on a Mattson GALAXY spectrophotometer. UV spectra were obtained in methanol using a Milton-Roy spectrophotometer. NMR spectra were recorded in CD_3OD solutions containing Me_4Si as internal standard on a Varian Unity 500 spectrometer. Proton and carbon NMR spectra were measured at 500 and 125 MHz, respectively. Mass spectra were obtained by using a VG ZAB-2FHF and a Jeol JMS-HX 110 high-resolution mass spectrometer and were provided by the Mass Spectrometry Facility, Department of Chemistry, University of California, Riverside (**1**, negative HRFABMS data of **3**–**6**) and Korea Basic Science Institute, Taejeon, Korea (**2**, positive FABMS, LRFABMS, and ESIMS data of **3**–**7**), respectively. All solvents used were spectral grade or were distilled from glass prior to use.

Animal material

The specimens of *Aplysinella rhax* (family Aplysinellidae) (BMNH 1995.6.22.30) were collected by hand using SCUBA at 6–10 m depth on 4 April 1996 at Mant Channel, Pohnpei. The same sponge was also collected at Wonder Channel and Omodes in Palau in April 1999. Specimens of *A. rhax* were also collected from Apra Harbor, Guam in September 1999.

Extraction and isolation

The freshly collected specimens were immediately frozen and kept at −20°C until investigated chemically. The specimens from Pohnpei were lyophilized (dry wt 503.5 g), macerated, and repeatedly extracted with MeOH (3 L×5) and CH_2Cl_2 (3 L×2). The combined crude extracts were evaporated in vacuo (114.25 g) and partitioned between *n*-BuOH and H_2O. A portion (26.30 g) of the *n*-BuOH layer (34.16 g) was re-partitioned between 15% aqueous MeOH and *n*-hexane. The aqueous MeOH layer was dried and the residue (17.67 g) subjected to C_{18} reversed-phase vacuum flash chromatography using gradient mixtures of MeOH and H_2O (elution order: 50, 30, 20, 10% aqueous MeOH, 100% MeOH), acetone, and EtOAc as eluents. The fraction eluted with 50% aqueous MeOH was dried and the residue (3.82 g) separated by semi-preparative reversed phase HPLC (YMC ODS-A column, 1 cm×25 cm, 60% aqueous MeOH) to yield, in the order of elution, a mixture of psammaplin A_2 (**4**) and aplysinellin A (**5**), aplysinellin B (**6**), and psammaplin A_1 (**3**), respectively. Separation by reversed-phase HPLC (90% aqueous MeCN) gave 26.2 and 12.4 mg of **4** and **5**, respectively. Purification of the other compounds was also accomplished by reversed-phase HPLC (YMC ODS-H80 column, 80% aqueous MeCN for **6**, and 60% aqueous MeCN for **3**) to afford 1026 and 29.2 mg of **3** and **6**, respectively.

A portion (460 mg) of the fraction eluted with 30% aqueous MeOH (4.63 g) in the flash chromatography was separated by reversed phase HPLC (YMC ODS-A column, 40% aqueous MeOH) to yield 120.6 and 138.5 mg of psammaplins A (**1**) and A_1 (**3**) as yellow amorphous solids, respectively. The 20% aqueous MeOH fraction (0.73 g) was also chromatogramed on HPLC (YMC-ODS column, 35% aqueous MeOH) to afford 147.3 and 87.6 mg of psammaplin A (**1**) and bisaprasin (**2**), respectively.

Metabolites from the Palau specimens were also isolated following the same procedure as described above for the Pohnpei collection. From 590.2 g of the lyophilized animals, 2,450, 960, 3,200, 18.1, 22.3, and 54.1 mg of pure **1**–**6** were obtained, respectively. The concentration of each compound was 0.42, 0.16, 0.54, 3.1×10^{-3},

3.8×10^{-3}, and 9.1×10^{-3}% of dry weight of the animal for **1**–**6**, respectively.

The C_{18} vacuum flash chromatographic fractions of the extracts from the Guam collection also contained psammaplins A (**1**), A_1 (**3**), and bisaprasin (**2**) as the major constituents based on ^{1}H NMR analysis. Further separation has not been carried out for the individual metabolites.

Psammaplin A (1). A white solid; mp 174–176°C, [Lit. 172–174°C];[2] UV (MeOH) λ_{max} (log ϵ) 205 (4.75), 282 (3.88) nm, [Lit. 220, 280 nm];[3] IR (KBr) ν_{max} 3350 (br), 2930, 1660, 1535, 1420, 1285, 1210, 1015 cm^{-1}; (+)-HRFABMS $[M+H]^+$ *m/z* 664.9566 (calcd for $C_{22}H_{25}{}^{79}Br^{81}BrN_4O_6S_2$ 664.9562, Δ −0.4 mmu).

Bisaprasin (2). A white solid; mp 101–104°C; UV (MeOH) λ_{max} (log ϵ) 204 (4.95), 284 (4.15) nm; IR (KBr) ν_{max} 3400 (br), 1655, 1530, 1420, 1365, 1215, 1010 cm^{-1}; (+)-HRFABMS $[M+H]^+$ *m/z* 1326.8894 (calcd for $C_{44}H_{47}{}^{79}Br_2^{81}Br_2N_8O_{12}S_4$ 1326.8892, Δ 0.2 mmu).

Psammaplin A_1 (3). A pale-yellow amorphous solid; mp 76–80°C; UV (MeOH) λ_{max} (log ϵ) 204 (4.73), 280 (3.88) nm; IR (KBr) ν_{max} 3400 (br), 2925, 1655, 1530, 1485, 1250, 1230, 1040 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; (+)-HRFABMS $[M-SO_3+C_3H_{10}N_3(N,N\text{-dimethylguanidium})+H]^+$ *m/z* 752.0381 (calcd for $C_{25}H_{34}{}^{79}Br^{81}BrN_7O_6S_2$ 752.0358, Δ −2.3 mmu); (−)-HRFABMS $[M]^-$ *m/z* 742.8976 (calcd for $C_{22}H_{23}{}^{79}Br^{81}BrN_4O_9S_3$ 742.8973, Δ −0.3 mmu); (−)-ESIMS *m/z* 743.

Psammaplin A_2 (4). A pale-yellow amorphous solid; mp 155–160°C; UV (MeOH) λ_{max} (log ϵ) 204 (4.60), 281 (3.98) nm; IR (KBr) ν_{max} 3400 (br), 2930, 1660, 1530, 1485, 1230, 1040 cm^{-1}; ^{1}H NMR (CD_3OD) δ 7.49 (2H, d, *J*=2.0 Hz, H-9, H-9′), 7.47 (2H, d, *J*=8.3 Hz, H-12, H-12′), 7.21 (2H, dd, *J*=8.3, 2.0 Hz, H-13), 3.86 (4H, s, H-7, H-7′), 3.52 (4H, t, *J*=6.8 Hz, H-3, H-3′), 3.01 (12H, s, H-16, H-17, H-16′, H-17′), 2.80 (4H, t, *J*=6.8 Hz, H-2, H-2′); ^{13}C NMR (CD_3OD) δ 165.6 (C, C-5, C-5′), 158.6 (C, C-14, C-14′), 152.5 (C, C-6, C-6′), 149.5 (C, C-11, C-11′), 136.1 (C, C-8, C-8′), 134.6 (CH, C-9, C-9′), 130.0 (CH, C-13, C-13′), 123.3 (CH, C-12, C-12′), 116.5 (C, C-10, C-10′), 39.6 (CH_2, C-3, C-3′), 38.5 (CH_2, C-2, C-2′), 38.3 (CH_3, C-16, C-17, C-16′, C-17′), 29.0 (CH_2, C-7, C-7′); (−)-HRFABMS $[M+C_3H_{10}N_3(N,N\text{-dimethylguanidium})]^-$ *m/z* 909.9285 (calcd for $C_{25}H_{32}{}^{79}Br^{81}BrN_7O_{12}S_4$ 909.9338, Δ 5.3 mmu).

Aplysinellin A (5). A yellow amorphous solid; mp 191–194°C (decomposed); UV (MeOH) λ_{max} (log ϵ) 204 (4.83), 291 (4.11) nm; IR (KBr) ν_{max} 3400 (br), 2925, 1660, 1565, 1270, 1230, 1040 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 2; (+)-HRFABMS $[M+3Na]^+$ *m/z* 1068.7994 (calcd for $C_{31}H_{27}{}^{79}Br^{81}Br_2N_4O_{13}S_3Na_3$ 1068.7943, Δ 5.5 mmu), $[M+2Na+H]^+$ *m/z* 1046.8165 (calcd for $C_{31}H_{28}{}^{79}Br^{81}Br_2N_4O_{13}S_3Na_2$ 1046.8124, Δ 4.5 mmu); (+)-LRFABMS *m/z* 1068.8/1066.8, 1046.8/1044.8, $[M-SO_3+Na+2H]^+$ *m/z* 944.8/942.8; (−)-LRFABMS $[M+2Na-H]^-$ *m/z* 1044.9/1042.9, $[M+Na]^-$ *m/z* 1022.9/1020.9.

Aplysinellin B (6). A yellow amorphous solid; mp 236–239°C (decomposed); UV (MeOH) λ_{max} (log ϵ) 205 (4.88), 280 (3.93) nm; IR (KBr) ν_{max} 3400 (br), 2925, 1655, 1540, 1385, 1270, 1235, 1045 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 2; (+)-HRFABMS $[M+2Na]^+$ *m/z* 1044.8008 (calcd for $C_{31}H_{26}{}^{79}Br^{81}Br_2N_4O_{13}S_3Na_2$ 1044.7967, Δ 4.5 mmu); (+)-LRFABMS *m/z* 1044.7/1042.7, $[M+Na+H]^+$ *m/z* 1022.6/1020.6, $[M-SO_3+Na+H]^+$ *m/z* 942.8/940.8; (−)-LRFABMS $[M]^-$ *m/z* 998.7/996.8; (−)-ESIMS $[M]^-$ *m/z* 998.7/996.8.

Methylation of aplysinellin B. To a stirred solution of **6** (15.1 mg, 14.7×10^{-6} mol) in dry MeOH (5 mL) were added $(CH_3)_2SO_4$ (17 mg, 135×10^{-3} mol) and K_2CO_3 (12 mg). The suspension was stirred at 40°C under N_2 for 8 h. After removing the solvent and excess $(CH_3)_2SO_4$ under reduced pressure, the residue was dissolved in CH_2Cl_2 (5 mL) and washed with H_2O. After drying the mixture, the residue was separated by C_{18} HPLC (YMC-ODS-A column, 10% aqueous MeOH) to afford **7** as the major product (5.3 mg, 37% yield). Compound **7**, a white solid (mp 136–138°C), had the following spectral features: ^{1}H NMR data ($CDCl_3$) δ 8.12 (1H, d, *J*=1.8 Hz, H-5″), 7.71 (1H, br s, H-4), 7.67 (1H, d, *J*=1.8 Hz, H-9″), 7.43 (1H, d, *J*=1.8 Hz, H-9′), 7.23 (1H, t, *J*=6.3 Hz, H-4′), 6.95 (1H, d, *J*=1.4 Hz, H-9), 6.65 (1H, d, *J*=1.8 Hz, H-13′), 6.50 (1H, s, H-3″), 6.17 (1H, d, *J*=1.4 Hz, H-13), 3.92 (3H, s, 1″-OMe), 3.91 (3H, s, 6/6′-OMe), 3.85 (3H, s, 6/6′-OMe), 3.71 (2H, s, H-7), 3.63 (2H, s, H-7′), 3.61 (3H, s, 11-OMe), 3.58 (3H, s, 11′-OMe), 3.54 (2H, t, *J*=6.1 Hz, H-3′), 3.48 (2H, br s, H-3), 2.85 (4H, br s, H-2, H-2′); ^{13}C NMR data ($CDCl_3$) δ 166.2 (C, C-1″), 163.1 (C, C-5), 162.7 (C, C-5′), 153.5 (C, C-11′), 151.7 (C, C-6), 151.2 (C, C-12), 151.1 (C, C-6′), 148.1 (C, C-7″), 144.3 (C, C-11), 140.0 (C, C-2″), 134.74 (CH, C-9′), 134.69 (CH, C-5″), 133.8 (C, C-8″), 133.1 (C, C-8), 132.9 (C, C-8′/C-4″), 132.6 (C, C-8′/C-4″), 132.1 (CH, C-9″), 132.0 (C, C-12′), 129.6 (CH, C-13′), 126.0 (CH, C-9), 117.9 (C, C-6″), 117.6 (C, C-10), 117.1 (C, C-10′), 114.3 (CH, C-13), 108.5 (CH, C-3″), 63.0 (CH_3, 6′-OMe), 62.9 (CH_3, 6-OMe), 61.1 (CH_3, 11′-OMe), 60.5 (CH_3, 11-OMe), 53.5 (CH_3, 1″-OMe), 39.0 (CH_2, C-3′), 38.8 (CH_2×2, C-2, C-2′), 38.7 (CH_2, C-3), 28.6 (CH_2×2, C-7, C-7′); HMBC correlations H-7/C-5, C-6, C-8, C-9, C-13; H-9/C-7, C-10, C-11, C-13; H-13/C-7, C-9, C-11, C-12; H-4′/C-5′; H-9′/C-7′, C-10′, C-11′, C-13′; H-13′/C-7′, C-9′, C-11′, C-8″; H-3″/C-1″, C-2″, C-5″, C-9″; H-5″/C-3″, C-6″, C-7″, C-9″; H-9″/C-12′, C-3″, C-5″, C-7″; 11-OMe/C-11; 11′-OMe/C-11′; 1″-OMe/C-1″; ROESY correlations H-3/H-13′, H-3/H-9″, H-7/H-9, H-7/H-13, H-9/H-3″, H-13/H-13′, H-13/H-3″, H-7′/H-9′, H-7′/H-13′, H-13′/H-9″, H-3″/H-5″, H-3″/H-9″, H-3″/1″-OMe, H-9″/11′-OMe; (+)-HRFABMS $[M+H]^+$ *m/z* 990.9540 (calcd for $C_{36}H_{38}{}^{79}Br^{81}Br_2N_4O_{10}S_2$ 990.9545, Δ 0.2 mmu).

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