Amphimedonoic acid and psammaplysene E, novel brominated alkaloids from Amphimedon sp.

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ABSTRACT

Examination of the CH_2Cl_2-MeOH (1:1) extract from the Madagascan sponge *Amphimedon* sp. highlighted two new brominated alkaloids, amphimedonoic acid (**1**) and psammaplysene E (**2**), along with the known 3,5-dibromo-4-methoxybenzoic acid (**3**). Their structures were elucidated by 1D and 2D NMR spec-troscopy and HRESIMS data.

Marine sponges have been reported as a major source of bioactive secondary metabolites with a wide variety of unusual structures.[1] The genus *Amphimedon* has been known to produce various potent bioactive compounds, especially alkaloids with unique structures.[2–4] As part of our continued search for structurally unique metabolites from marine invertebrates,[5–7] the sponge *Amphimedon* sp., collected from the Mitsio Islands, Madagascar, was investigated. These investigations afforded two new brominated alkaloids, amphimedonoic acid (**1**) and psammaplysene E (**2**), along with the known 3,5-dibromo-4-methoxybenzoic acid (**3**).[8,9] Herein, the isolation and structure elucidation of **1**–**3** are described.

The sponge *Amphimedon* sp. (36.2 g, wet weight) collected off the Mitsio Islands, Madagascar, was extracted with CH_2Cl_2/MeOH (1:1). The crude extract (1.5 g) was subjected to MPLC over silica gel and separated into ten fractions (F1-F10) using a combination of isohexane, EtOAc and MeOH of increasing polarity. F9 (15 mg) was subjected to a subsequent reversed phase semi-preparative HPLC separation to yield pure compound **2** (0.7 mg). F10 (32 mg) was subjected to a reversed phase semi-preparative HPLC separation and led to the isolation of pure compounds **1** (2.8 mg), **2** (1.3 mg) and **3** (1.0 mg) (Fig. 1).

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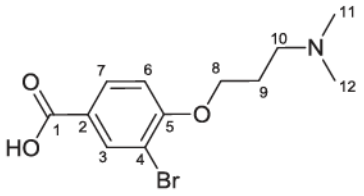
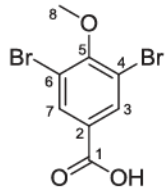
Amphimedonoic acid (**1**) was obtained as a colorless oil. The high resolution electrospray mass spectrum exhibited a molecular ion $[M+H]^+$ as a cluster of peaks *m*/*z* 302.0388/304.0388 in a 1:1 ratio, an isotope pattern characteristic of a brominated compound. Accordingly, based on HRESIMS, the molecular formula $C_{12}H_{16}BrNO_3$ (calcd for $C_{12}H_{17}{}^{79}BrNO_3^+$, 302.0386), with five degrees of unsaturation, was determined. The ^1H and ^{13}C NMR data displayed resonances and correlations for one carboxylic acid group, one 1,2,4-trisubstituted aromatic ring, three methylenes, one of which was oxygenated and two *N*-methyl groups (Table 1). The benzoic acid moiety was suggested by HMBC correlations from H-3 (δ_H 8.10) to C-1 (δ_C 172.6), C-4 (δ_C 111.7), C-5 (δ_C 157.6), C-7 (δ_C 131.1), from H-6 (δ_H 6.97) to C-2 (δ_C 132.2), C-4, C-5 and from H-7 (δ_H 7.83) to C-3 (δ_C 135.3) and C-5 (Fig. 2). The chemical shift (δ_C 111.7) of the quaternary carbon C-4 placed the bromine substituent at C-4. The substitution of C-5 was suggested by its chemical shift (δ_C 157.6) and also by the HMBC correlation from H-8 (δ_H 4.21) to C-5. Interpretation of the ^1H–^1H COSY correlations between H-8, H-9 and H10, revealed the propyl spin system C-8–C-9–C-10. The substitution of the amine moiety was determined by HMBC correlations from H-10 (δ_H 3.21) to C-11, C-12 (δ_C 43.9) and from H-11, H-12 (δ_H 2.81) to C-10 (δ_C 56.7), C-11 and C-12 (Fig. 2).

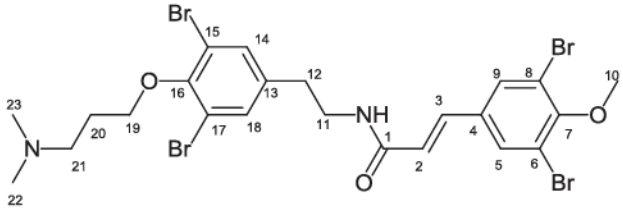
Psammaplysene E (**2**) was obtained as a colorless oil. The high resolution electrospray mass spectrum showed four isotopic peaks



amphimedonoic acid (**1**)



3,5-dibromo-4-methoxybenzoic acid (**3**)



psammaplysene E (**2**)

Fig. 1. Structures of isolated compounds **1**–**3**.

Table 1
^{1}H and ^{13}C NMR data for amphimedonoic acid (**1**), psammaplysene E (**2**) and 3,5-dibromo-4-methoxybenzoic acid (**3**) (^{1}H 500 MHz, ^{13}C 125 MHz, CD_3OD).

Position	**1**		**2**		**3**	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	172.6	–	167.6	–	171.0	–
2	132.2	–	123.6	6.52 (1H, d, 15.7)	–	–
3	135.3	8.10 (1H, d, 1.9)	138.1	7.37 (1H, d, 15.7)	134.7	8.12 (1H, s)
4	111.7	–	135.1	–	118.0	–
5	157.6	–	132.8	7.79 (1H, s)	156.6	–
6	112.9	6.97 (1H, d, 8.5)	119.3	–	118.0	–
7	131.1	7.83 (1H, dd, 8.6, 2.0)	156.1	–	134.7	8.12 (1H, s)
8	67.2	4.21 (2H, t, 5.7)	119.3	–	60.8	3.87 (3H, s)
9	25.9	2.23 (2H, m)	132.8	7.79 (1H, s)	–	–
10	56.7	3.21 (2H, t, 7.4)	60.9	3.88 (3H, s)	–	–
11	43.9	2.81 (3H, s)	41.5	3.51 (2H, t, 7.1)	–	–
12	43.9	2.81 (3H, s)	34.9	2.82 (2H, t, 7.0)	–	–
13	–	–	139.9	–	–	–
14	–	–	134.2	7.50 (1H, s)	–	–
15	–	–	118.7	–	–	–
16	–	–	152.3	–	–	–
17	–	–	118.7	–	–	–
18	–	–	134.2	7.50 (1H, s)	–	–
19	–	–	71.6	4.07 (2H, t, 5.8)	–	–
20	–	–	27.4	2.15 (2H, m)	–	–
21	–	–	57.1	3.07 (2H, t, 7.3)	–	–
22	–	–	44.3	2.63 (3H, s)	–	–
23	–	–	44.3	2.63 (3H, s)	–	–

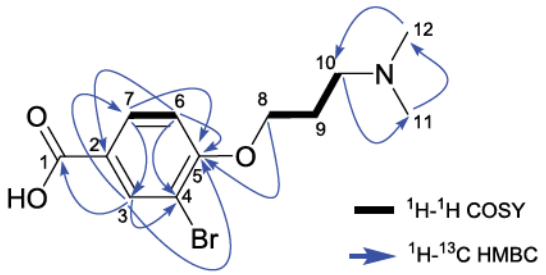


Fig. 2. Key ^{1}H–^{1}H COSY and ^{1}H–^{13}C HMBC correlations for **1**.

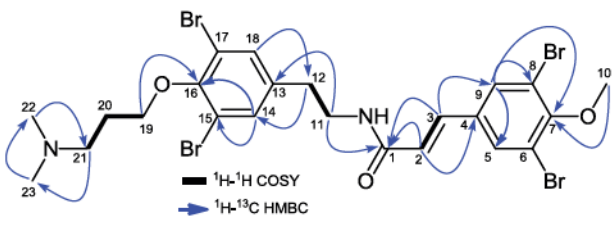


Fig. 3. Key ^{1}H–^{1}H COSY and ^{1}H–^{13}C HMBC correlations for **2**.

at m/z 694.8758, 696.8740, 698.8723, 700.8702, 702.8691 $[M+H]^+$ in a 1:4:6:4:1 ratio, respectively, indicating the presence of two bromine atoms in the molecule. The HRESIMS allowed assignment of the molecular formula as $C_{23}H_{27}Br_4N_2O_3^+$ (calcd for $C_{23}H_{27}{}^{79}Br_4N_2O_3^+$, 694.8750) requiring ten degrees of unsaturation. The ^{1}H and ^{13}C NMR data of **2** displayed the resonances of a *trans*-α,β-unsaturated carbonyl group, two symmetrical 1,2,4,6-tetrasubstituted aromatic rings, five methylenes, one of which was oxygenated, two *N*-methyl groups and one *O*-methyl group (Table 1).

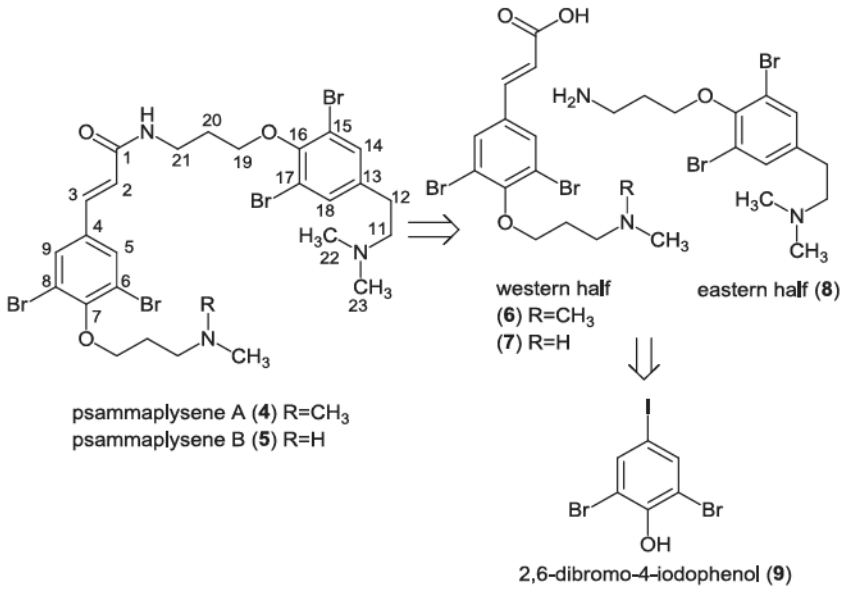


Fig. 4. Main retrosynthetic disconnection for psammaplysenes A (**4**) and B (**5**).

Interpretation of the ^{1}H–^{1}H COSY correlations between H-2 and H-3 reveal the connectivity of C-2 to C-3, between H-11 and H-12, reveal the connectivity of C-11 to C-12 and between H-19, H-20 and H-21, the propyl spin system C-19–C-20–C-21 (Fig. 3). The different partial structures were then linked together by interpretation of the correlations observed in the ^{1}H–^{13}C HMBC spectrum. The *N*-methyl H-22 and H-23 (δ_H 2.63) showed correlations to C-22, C23 (δ_C 44.3) and C-21 (δ_C 57.1). The correlation from H-19 (δ_H 2.63) to C-16 (δ_C 152.3) linked the *O*-methylene to the first 1,2,4,6-tetrasubstitued aromatic ring. The substitution of the aromatic moiety was suggested by the correlations from H-14, H-18 (δ_H 7.50) to C-12 (δ_C 34.9), C-15 (δ_C 118.7), C-16 (δ_C 152.3) and C-17 (δ_C 118.7), from H-12 (δ_H 2.82) to C-14 (δ_C 134.2) and from H-11 (δ_H 3.51) to C-13 (δ_C 139.9). The *N*-methylene C-11 was linked to the carbonyl moiety by the correlation from H-11 to C-1 (δ_C 171.0). The *trans*-α,β-unsaturation was demonstrated by the correlation from H-2 (δ_H 6.52) to C-1 and from H-3 (δ_H 7.37) to C-1. The substitution of the second 1,2,4,6-tetrasubstitued aromatic ring was explained by the correlations from H-2 to C-4 (δ_C 135.1), from H-3 to C-5, C-9 (δ_C 132.8), from H-5, H-9 (δ_H 7.79) to C-5, C-6 (δ_C 119.3), C-7 (δ_C 156.1), C-8 (δ_C 119.3), C-9 and from the *O*-methyl H-10 (δ_H 3.88) to C-7.

Psammaplysenes previously isolated from two sponges, *Psammaplysilla* sp.[10] and *Psammoclemma* sp.,[11] are known to possess interesting biological activities. To avoid the limited supply of material from sponge collections, Georgiades and Clardy have developed an efficient synthesis of psammaplysenes A (**4**) and B (**5**) as well as other derivatives.[12,13] They first considered a retrosynthetic disconnection at the amide bond (Fig. 4) yielding two fragments **6** (or **7**) and **8**. Both of these fragments could be synthetized from the 2,6-dibromo-4-iodophenol (**9**). Due to the great similarities between **2**, **4** and **5** (see comparison of the ^{1}H and ^{13}C NMR data for psammaplysenes A and E, Table 2) and in the light of this work, a similar retrosynthetic pathway with a disconnection at the amide bond could also be envisaged for psammaplysene E (**2**) (Fig. 5). Although similar to the fragments **6** and **8**, the two synthons **10** and **11** for psammaplysene E (**2**) show some differences. Compound **10** differs from **6** by a methoxy group instead of a *N*,*N*-dimethylamine-3-propoxy group. Compound **11** differs from **8** by the substitutions of the amines, the primary amine of **8** is replaced by a tertiary amine for **11** and the tertiary amine of **8** is replaced by a primary amine for **11**. Thus, as the primary amine is not located on the same side-chain, the skeleton obtained after the final coupling step between the primary amine of the "eastern half" and the carboxylic acid of the "western half", will be different

Table 2
Comparison of ^{1}H and ^{13}C NMR data for psammaplysene A (**4**)[10] and psammaplysene E (**2**) (CD_3OD).

Position	δ_C		δ_H (*J* in Hz)	
	2	**4**	**2**	**4**
1	167.6	167.5	–	–
2	123.6	124.1	6.52 (1H, d, 15.7)	6.64 (1H, d, 15.7)
3	138.1	137.6	7.37 (1H, d, 15.7)	7.39 (1H, d, 15.7)
4	135.1	135.8	–	–
5	132.8	132.8	7.79 (1H, s)	7.82 (1H, s)
6	119.3	119.3	–	–
7	156.1	154.2	–	–
8	119.3	119.3	–	–
9	132.8	132.8	7.79 (1H, s)	7.82 (1H, s)
10	60.9	–	3.88 (3H, s)	–
11	41.5	59.0	3.51 (2H, t, 7.1)	3.27 (2H, t, 7.0)
12	34.9	30.3	2.82 (2H, t, 7.0)	3.00 (2H, t, 7.0)
13	139.9	136.8	–	–
14	134.2	134.1	7.50 (1H, s)	7.59 (1H, s)
15	118.7	119.1	–	–
16	152.3	153.2	–	–
17	118.7	119.1	–	–
18	134.2	134.1	7.50 (1H, s)	7.59 (1H, s)
19	71.6	71.7	4.07 (2H, t, 5.8)	4.07 (2H, t, 7.0)
20	27.4	30.5	2.15 (2H, m)	2.13 (2H, q, 7.0)
21	57.1	36.6	3.07 (2H, t, 7.3)	3.60 (2H, t, 7.0)
22	44.3	43.3	2.63 (3H, s)	2.87 (3H, s)
23	44.3	43.3	2.63 (3H, s)	2.87 (3H, s)

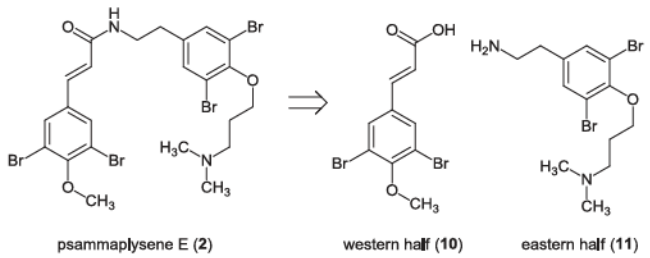


Fig. 5. Hypothetic main retrosynthetic disconnection for psammaplysene E (**2**).

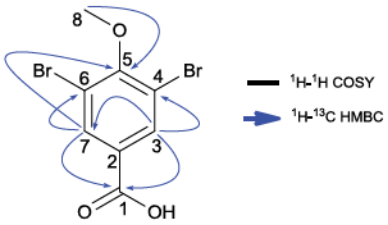


Fig. 6. Key ^{1}H–^{13}C HMBC correlations for **3**.

between psammaplysene E (**2**) and psammaplysenes A (**4**) and B (**5**) (Fig. 6).

3,5-Dibromo-4-methoxybenzoic acid (**3**) was obtained as a colorless oil. Although known, no spectral data has been reported in the literature for this compound. Therefore, as it was not possible to compare our data with those previously published, the structure was completely elucidated. The HRESIMS showed a cluster of isotopic $[M-H]^-$ peaks at *m/z* 306.8618, 308.8576 and 310.8582 in a 1:2:1 ratio, respectively. The molecular formula was deduced to be $C_8H_6Br_2O_3$ (calcd for $C_8H_5{}^{79}Br_2O_3^-$, 306.8611) indicating five degrees of unsaturation. The ^{1}H and ^{13}C NMR data of **3** displayed the resonances of one carboxylic acid group, one symmetrical 1,2,4,6-tetrasubstituted aromatic ring and one *O*-methyl group (Table 1). There was no ^{1}H–^{1}H COSY correlation. The substitution of the 1,2,4,6-tetrasubstituted aromatic ring was established by ^{1}H–^{13}C HMBC correlations from H-3, H-7 (δ_H 8.12) to C-1 (δ_C 171.0), C-3 (δ_C 134.7), C-4 (δ_C 118.0), C-5 (δ_C 156.6), C-6 (δ_C 118.0) and C-7 (δ_C 1134.7) and from H-8 (δ_H 3.87) to C-5.

Amphimedonoic acid (**1**), psammaplysene E (**2**) are both bromotyrosine-derived alkaloids. The genus *Amphimedon* has proved to be a powerful producer of various alkaloids with diverse structures, such as manzanine analogs or 3-alkylpyridine alkaloids,[3,4,14,15] but this is the first report of bromotyrosine-derived metabolites from *Amphimedon* sponges. Some of these metabolites have also been isolated from an *Oceanapia* sp. sponge and a *Psammoclemma* sp. sponge,[11,16] except that they have been limited exclusively to sponges of the order Verongida and were considered as one of the most solid chemotaxonomic groupings among the Porifera until recent years.[17]

Amphimedonoic acid (**1**), psammaplysene E (**2**) and 3,5-dibromo-4-methoxybenzoic acid (**3**) did not show *in vitro* cytotoxicity against human epidermoid carcinoma KB cells (IC_{50} > 10 μg/mL).

Acknowledgments

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A. Supplementary data

Supplementary data associated (experimental section, compound characterization, UV spectrum, ^{1}H, HSQC, HMBC, COSY and HRESIMS spectra of compounds **1**, **2** and **3**) with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2017.08.072.

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