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Kororamide A, a new tribrominated indole alkaloid from the Australian bryozoan *Amathia tortuosa*

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ABSTRACT

A new tribrominated indole alkaloid, kororamide A together with the known alkaloid convolutamine F, were isolated through the application of mass directed purification from the bryozoan, *Amathia tortuosa* collected from northern New South Wales, Australia. The structure of kororamide A was deduced from the analysis of 1D/2D NMR and MS data. Kororamide A exists in solution as a mixture of interconverting *cis–trans* amide regioisomers in a ratio of 4:5. Bioactivity testing demonstrated that kororamide A was marginally active against chloroquine-sensitive and resistant strains of the malarial parasite *Plasmodium falciparum*, and was inactive against normal human cell lines.

The natural products chemistry of southern Australian bryozoans was studied extensively between the mid 1980s and late 1990s by Blackman's group at the University of Tasmania.[1] A parallel ongoing study of the chemistry of New Zealand bryozoans has been pursued by Princep's group at the Waikato University in New Zealand, since the mid 1990s.[2] Both groups have shown that south western Pacific Foliose bryozoans produce a range of alkaloids possessing novel structures and interesting biological activities.[3] We have recently started a programme to investigate the chemistry and biological activity of temperate and subtropical eastern Australian bryozoans. This has so far resulted in the structures and *anti*-plasmodial activity of two novel alkaloids, wilsoniamine A and B, together with an additional amathamide alkaloid, amathamide H from a Tasmanian collection of *Amathia wilsoni* being reported.[4] Although, our initial study reported on the chemistry of a Tasmanian bryozoan, our collection has mainly focused on bryozoans inhabiting sub-tidal reefs off the northern New South Wales (NSW) coast. Mass spectrometric, NMR spectroscopic and HPLC analysis have been used as tools to identify specimens having unique chemistry and this highlighted *Amathia tortuosa* (ACENV0 0007) collected from storm debris on Korora beach near Coffs Harbour, NSW in 2009 for further analysis.[5] Herein, we report on the isolation, structure determination and biological activity of a new alkaloid, kororamide A and the known alkaloid, convolutamine F (Fig. 1) that we isolated from this species.

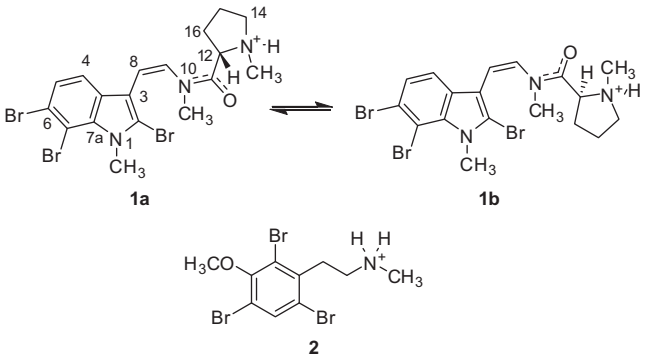

Figure 1. Structures of kororamide A (**1a** and **1b**) and convolutamine F (**2**).

The freeze-dried bryozoan (2.39 g) was extracted by repeated sonication in MeOH (4 × 200 mL). The MeOH extracts were combined, evaporated and the residue (668 mg) adsorbed onto C_{18} silica gel. The extract impregnated gel was placed in a HPLC pre-column cartridge (10 × 20 mm), connected in series to a C_{18}-bonded silica HPLC column (21 × 150 mm) and eluted with a gradient from 1% aqueous TFA to MeOH containing 1% TFA. Seventy fractions were collected and aliquots from these fractions were further analysed by (+)-LRESIMS. Only two groups of fractions

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(fraction 33 and fractions 43–45) showed prominent ion clusters by MS. Fraction 33 had a pseudomolecular ion cluster at *m/z* 400/402/404/406 and fractions 43–45 each had a pseudomolecular ion cluster at *m/z* 532/534/536/538. These ion clusters are characteristic of compounds each containing three bromine atoms and an odd number of nitrogen atoms. These fractions were evaporated and analysed by ^{1}H NMR spectroscopy. Fractions 43–45, possessing identical NMR spectra, were combined for detailed NMR analysis and shown to be a new compound that we have named kororamide A (**1**, 5.1 mg, 0.21% dry weight). Fraction 33 (8.0 mg, 0.33% dry weight) was pure and shown to be the known compound, convolutamine F (**2**), from interpretation of 2D NMR data and comparison with the literature data.[6]

Kororamide A (**1**) was isolated as a yellow optically active gum $[\alpha]_D$ −3.1 (0.24, MeOH).[7] Positive HRESIMS measurement of the pseudomolecular ion cluster peak (MH^+) at *m/z* 531.8237 (Δ 1.5 ppm) established a molecular formula of $C_{18}H_{21}Br_3N_3O$ for **1**. The IR spectrum of **1** had absorption bands at 2959, 1683, 1672 and 1652 cm^{-1} suggesting that it contained an amide group as well as aromatic or double bond systems. The UV spectrum had an absorption maximum at 282 nm indicating that **1** contained an aromatic moiety.

A prominent feature of the ^{1}H NMR spectrum of **1** (Table 1) was the presence of multiple pairs of signals each possessing the same splitting pattern. This suggested that the sample was a mixture of two compounds. Chemical exchange correlations observed between these paired signals in a ROESY spectrum however, indicated that the molecule was a single compound interconverting between two isomers on the NMR time-scale.[8] Comparison of the integrals for each of the signals within a pair signified that the two isomers were present in a 4:5 ratio. It could be deduced from the detailed analysis of the ^{1}H NMR spectrum that each isomer of **1** contained two *ortho* coupled aromatic methines, three *N*-methyl groups, three methylene groups, an aliphatic methine, two *cis* double bond protons (J = 9.0 Hz) and one broad exchangeable proton.

From analysis of the HSQC spectrum it was deduced that each isomer of **1** possessed 11 protonated carbons. The major isomer had methylene carbons at δ_C 55.4, 27.3 and 21.8 and the minor isomer at δ_C 55.5, 27.4 and 21.9. The pair of methylene carbons at δ_C 55.4 and 55.5 correlated to proton signals between δ_H 3.08 and 3.60 and this suggested that these carbons were substituted by nitrogen atoms. A pair of carbons at δ_C 67.1/66.4 that correlated to protons at δ_H 4.70/4.57 were assigned to either nitrogen or oxygen substituted methines. The six methyl proton signals correlated to carbons between δ_C 33 and 40 and this indicated that each was also attached to a nitrogen atom. Analysis of COSY correlations indicated that the three methylene groups in each isomer formed a chain, and one methylene (H_2-16) was vicinal to methine H-12. The presence of COSY correlations from the methylene protons, H_2-16, the methine proton, H-12 and the methyl protons 13-NCH_3 to the exchangeable proton H-13 suggested that a 2-substituted *N*-methylpyrrolidine was present in **1**.

COSY correlations between the two pairs of double bond protons (δ_H 6.10–6.80 in the major isomer and 6.09–6.87 in the minor isomer) in combination with a coupling constant of 9.0 Hz between these pairs indicated that these protons were part of a *cis* 1,2-disubstituted double bond. HSQC correlations showed that the protonated double bond carbons resonated at δ_C 109.8 and 128.8 in the major isomer and at δ_C 109.2 and 130.1 in the minor isomer and this suggested that the double bond was directly attached to a nitrogen atom.[4] The remaining two pairs of aromatic protons also showed mutual COSY correlations (δ_H 7.24–7.47 in the major isomer and 7.09–7.49 in the minor isomer) and shared a coupling constant of 8.4 Hz and this indicated that **1** contained a 1,2,3,4-tetrasubstituted benzene ring.

The methyl protons of 10-NCH_3 (δ_H 2.65 major isomer and δ_H 2.69 minor isomer) showed $^3J_{CH}$ correlations to the protonated double bond carbons at δ_C 128.8 (major isomer) and δ_C 130.1 (minor isomer) and downfield quaternary carbonyl carbons at δ_C 167.6 (major isomer) and 168.2 (minor isomer) in the HMBC spectrum, implying that **1** contained an *N*-methyl-enamide. A $^3J_{CH}$ correlation from H-16a to the carbonyl carbon C-11 established that a bond connected C-12 of the pyrrolidine to the amide carbonyl carbon C-11. C-8 of the enamide was directly attached to a non-protonated sp^2 hybridized carbon since H-9 showed a $^3J_{CH}$ correlation to a carbon at δ_C 111.1 (major isomer) and δ_C 110.6 (minor isomer). H-8 showed $^3J_{CH}$ correlations to two non-protonated sp^2 hybridized carbons at δ_C 119.5 and 127.8 (co-incident in both isomers). An HMBC correlation also signified that the carbon at δ_C 119.5 was three bonds away from the *N*-methyl protons that resonated at δ_H 4.15. These methyl protons showed an additional correlation to a quaternary carbon at δ_C 134.9. The aromatic protons of H-4 [δ_H 7.24 (major isomer) and δ_H 7.09 (minor isomer)] also showed $^3J_{CH}$ correlations to both δ_C 111.1 (major isomer) and 110.6 (minor isomer) and 134.9, while H-5 (δ_H 7.47 and 7.49) also correlated to the carbon at δ_C 127.8. In addition H-4 correlated to an unassigned non-protonated sp^2 hybridized carbon at δ_C 120.4 and H-5 correlated to an unassigned non-protonated sp^2 hybridized carbon at δ_C 106.3. In total these correlations were consistent with **1** containing a 2,3,6,7-tetrasubstituted-*N*-methylindole with enamide being attached at C-3 (Fig. 2).

All but the three bromine atoms from the molecular formula deduced from HRESIMS analysis were accounted for from the NMR analysis and since the enamide substituted the C-3 position of indole, the three bromine atoms must be attached to the three

Table 1
NMR data for both isomers of kororamide A (**1a** and **1b**)[a]

Position	1a		1b	
	^{13}C	^{1}H mult, (J in Hz)	^{13}C	^{1}H mult, (J in Hz)
1-NCH_3	35.2	4.15 s	35.2	4.15 s
2	119.5	—	119.5	—
3	111.1	—	110.6	—
3a	127.8	—	127.8	—
4	118.8	7.24 d, (8.4)	118.7	7.09 d, (8.4)
5	125.3	7.47 d, (8.4)	125.3	7.49 d, (8.4)
6	120.4	—	120.4	—
7	106.3	—	106.3	—
7a	134.9	—	134.9	—
8	109.8	6.10 d, (9.0)	109.2	6.09 d, (9.0)
9	128.8	6.80 d, (9.0)	130.1	6.87 d, (9.0)
10-NCH_3	33.6	2.65 s	34.7	2.69 s
11	167.6	—	168.2	—
12	67.1	4.70 q, (6.9)	66.4	4.57 q, (7.1)
13-NCH_3	39.8	2.81 s	39.8	2.79 s
13-NH	—	9.76 br s	—	9.67 br s
14α	55.4	3.60 m	55.5	3.58 m
14β		3.15 m		3.08 m
15α	21.8	1.95 m	21.9	1.89 m
15β		2.12 m		2.08 m
16α	27.3	2.64 s	27.4	2.47 s
16β		2.02 m		1.85 m

[a] Spectra were recorded at 600 MHz for ^{1}H and 150 MHz for ^{13}C in DMSO-d_6 at 30 °C.

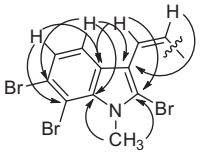

Figure 2. Key HMBC correlations for **1**.

remaining substituted carbons, C-2, C-6 and C-7. The chemical shifts of these carbons were consistent with those calculated for bromine substitution since bromine induces a ~6 ppm upfield shift of the aromatic carbon directly bonded to bromine and a ~3 ppm downfield shift of aromatic carbons *ortho* to bromine substituted aromatic carbons.[9] The planar structure of kororamide A (**1**) was therefore established.

It is well recognized that tertiary amides commonly exist in solution as interconverting *cis–trans* isomers about the amide bond.[10] This isomerism is slow on the NMR time-scale but quick enough to prevent purification of individual isomers. The doubled sets of signals present in the NMR spectra of **1** could therefore be assigned to interconverting amide bond tautomers. Comparison of the ^{13}C shifts of *N*-methyl groups *syn* and *anti* to the amide carbonyl have shown that *syn*-methyls always resonate upfield of the *anti*-methyl.[9] Therefore, the major isomer present in DMSO solutions of **1** is the *syn*-isomer **1a**. Correlations observed in a ROESY experiment confirmed this assignment since H-9 showed a cross peak with H-12 in the major isomer **1a** and 10-NCH_3 showed cross peaks to H-12 and 13-NCH_3 in the minor isomer **1b**.

The 2,6,7-tri-bromo substitution pattern of the indole in **1** is unique. The closest structures to **1** are the tribrominated indole alkaloids isolated previously from bryozoans from the sub-order Vesicularina.[11–13] Alternatamides A and B, isolated from the Atlantic bryozoan *Amathia alternata*, possess 2,5,6-tribrominated indoles and have been shown to have mild antibacterial effects against Gram-positive bacteria.[11] In addition two species from the genus *Zoobotryon* produce 2,5,6-tribrominated indole alkaloids that inhibit the settlement of barnacles and mussels.[12] Kororamide A is also similar to the 2,4,6-tribrominated alkaloid convolutindole A, isolated from *Amathia convoluta* from Tasmanian waters.[13] Convolutindole A has *anti*-nematode and anthelminthic parasitic effects. Kororamide A also possesses structural similarities to amathamide H isolated recently from *A. wilsoni*, since replacement of the 2,6,7-tribromo-1-methylindole in **1** with 2,4,6-tribromo-3-methoxyphenyl would yield amathamide H.[4] As **1** possessed a similar sign and magnitude for its optical rotation to those recorded for amathaminde H the absolute configuration of the stereogenic centre C-12 was assigned *S*.

Kororamide A (**1**) and convolutamine F (**2**) were tested for their ability to inhibit the growth of chloroquine-sensitive and resistant strains of the malarial parasite, *Plasmodium falciparum*. Kororamide A (**1**) was marginally active (72% inhibition at 20 μM) against the chloroquine-sensitive strain, but less active against the chloroquine resistant strain (50% inhibition at 20 μM). Convolutamine F (**2**) was only weakly active against both strains only reaching 80% inhibition at the highest dose tested (40 μM). Both compounds were also tested against normal human embryonic cells (HEK) and breast and pancreatic cancerous cells and were shown to be inactive up to a dose of 40 μM.[14]

Acknowledgment

The bryozoan was collected under the NSW DPI Fisheries Scientific collection permit P09/0031-1.1.

Supplementary data

Supplementary data (^1H, COSY, HSQC, HMBC and ROESY spectra for kororamide A (**1**) general experimental details, bryozoan collection details, extraction and isolation procedures for **1** and **2** associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2012.03.126. These data include MOL files and InChiKeys of the most important compounds described in this article.

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7. Kororamide A (**1**): yellow gum (5.1 mg, 0.0021%); $[\alpha]_D^{23}$ −3.1 (*c* 0.24, MeOH); UV (MeOH) λ_{max} (log ε) 282 nm (3.74), 212 nm (4.85) and 230 (sh) nm (4.49); IR (film) ν_{max} 3421, 2959, 1683, 1672, 1652, 1204, 1181, 1131 cm^{-1}; ^1H and ^{13}C NMR (see Table 1); (+)-LRESIMS *m*/*z* (35 eV) (rel int) 532 $[C_{18}H_{21}{}^{79}Br_3N_3O]^+$ (25), 534 $[C_{18}H_{21}{}^{81}Br^{79}Br_2N_3O]^+$ (100), 536 $[C_{18}H_{21}{}^{81}Br_2{}^{79}BrN_3O]^+$ (100), 538 $[C_{18}H_{21}{}^{81}Br_3N_3O]^+$ (20); (+)-HRESIMS *m*/*z* 531.92368 (Calcd for $C_{18}H_{21}N_3O^{79}Br_3$, 531.92292).
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