Convolutindole A and Convolutamine H, New Nematocidal Brominated Alkaloids from the Marine Bryozoan *Amathia convoluta*

Christian K. Narkowicz,[†] Adrian J. Blackman,*[,†] Ernest Lacey,[‡] Jennifer H. Gill,[‡] and Kirstin Heiland[§]

School of Chemistry, University of Tasmania, PO Box 252-75, Hobart 7001, Tasmania, Australia, Microbial Screening Technologies Pty Ltd, PO Box 57, Kemps Creek 2171, NSW, Australia, and Novartis Animal Health Australasia Pty Ltd, Yarrandoo, 245 Western Road, Kemps Creek 2171, NSW, Australia

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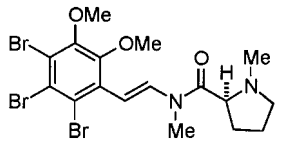
Nematocidal activity of an extract of the marine bryozoan *Amathia convoluta*, collected from Tasmania's east coast, was ascribed to two novel tribrominated alkaloids: convolutamine H (**2**) and convolutindole A (**5**), an indole possessing the unusual *N*-methoxy moiety. The structures were established by spectroscopic techniques.

Bryozoans (phylum Bryozoa) are sessile colonial invertebrates, with more species to be found in marine than in freshwater habitats. They can be significant fouling organisms on the hulls of vessels and on marine structures. The potent antineoplastic activity of bryostatin 1,[1] one of 20 macrolides isolated from the bryozoan *Bugula neritina*, has ensured continued interest in the chemistry and biological activity of compounds from bryozoans.

A collection of the cosmopolitan species *Amathia convoluta* Lamouroux (order Ctenostomata) from the Gulf of Mexico off the coast of Florida also yielded bryostatins.[2] However a collection of *A. convoluta* from southern Tasmania lacked significant antineoplastic activity, suggesting the absence of bryostatins. The lipid-soluble fraction from the Tasmanian collection yielded only one compound, amathamide G (**1**), a tribrominated proline-derived alkaloid.[3] This compound joined a series of amathamides (A−F) which were found in Tasmanian collections of *A. wilsoni*,[4] with amathamide C also being found in *A. pinnata*.[3]



1 amathamide G

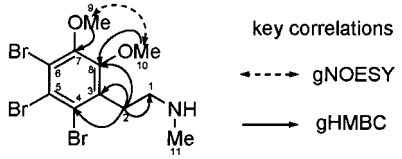
Further investigation of the *A. convoluta* extract, from the same Floridian collection that contained bryostatins, yielded the convolutamides, novel γ-lactam alkaloids;[5] the convolutamines, β-phenylethylamines;[6,7] a series of hydroxyoxindoles, the convolutamydines;[7−9] and the lutamides.[10] These alkaloids possessed varied biological activities. A collection of *A. convoluta* from the Atlantic coast off North Carolina yielded a further series of alkaloids, the leucine- and tyrosine-derived volutamides.[11]

As part of our continuing interest in the chemistry and pharmacology of bryozoans, we report investigation of a new population of *A. convoluta* from the east coast of Tasmania. Extracts from this population contained a highly potent and selective activity against the free-living larval stages of the parasitic nematode *Haemonchus contortus*, a pathogen of sheep and other ruminants. Freeze-dried *A. convoluta* was extracted with CH_2Cl_2 and fractionated by flash Si gel chromatography. Further bioactivity-guided fractionation led to the isolation of two compounds (**2** and **5**) possessing potent nematocidal activity (Table 1). These are the first nematocides reported from a bryozoan. In addition, amathamide G (**1**) was isolated as a minor component.

Table 1. In Vitro Bioactivity[a] of Convolutamine H (**2**), Convolutindole A (**5**), and Levamisole (**6**)

	test organism		
compd	*H. contortus*	*B. subtilis*	*S. cerevisiae*
2	0.20	NI (100)[b]	NI (200)
5	0.39	50	NI (200)
6	1.6	NI (50)	NI (50)

[a] LD_{99} (μg/mL): concentration to inhibit growth or development of test organism by 99%. [b] NI: no inhibition, highest concentration tested is indicated in parentheses.



2 convolutamine H

Compound **2** was isolated as a pale yellow oil. High-resolution LSIMS gave the molecular formula $C_{11}H_{14}Br_3NO_2$. This assignment was supported by a positive Mayer's alkaloid test and by the mass spectral isotope pattern, which was consistent with a tribrominated compound. The ^{1}H NMR spectrum (Table 2) included three three-proton singlets: two singlets at 3.86 and 3.82 ppm attributable to two methoxy groups and a broadened signal at 2.47 ppm consistent with an *N*-methyl group. Signals from two mutually coupled methylenes at 3.05 and 2.75 ppm and a broad signal at 1.95 ppm completed the spectrum. Exchange with D_2O eliminated the signal at 1.95 ppm and sharpened both the *N*-methyl signal and the methylene signal at 2.75 ppm.

The ^{13}C NMR spectrum of **2** (Table 2) indicated six downfield, aromatic carbons. Given the absence of any

* To whom correspondence should be addressed. Tel: +61 3 6226 2183. Fax: +61 3 6226 2858. E-mail: Adrian.Blackman@utas.edu.au.
[†] University of Tasmania.
[‡] Microbial Screening Technologies Pty Ltd.
[§] Novartis Animal Health Australasia Pty Ltd.

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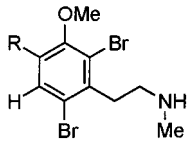
Table 2. NMR Data for Convolutamine H (**2**) and Convolutindole A (**5**)

	2			5		
pos.	^{13}C	^{1}H	gHMBC	^{13}C	^{1}H	gHMBC
1	50.4	2.75 brm, 2H	2, 3, 11			
2	32.6	3.05 m, 2H	1, 3, 4, 8	114.1		
3	135.4			112.2		
3a				124.1		
4	122.9			108.6[a]		
5	123.2[a]			128.5	7.43 s, 1H	3a, 4, 6, 7
6	120.4[a]			110.9[a]		
7	150.8			141.7		
7a				128.8		
8	151.6			23.5	3.12 m, 2H	2, 3, 3a, 9
9	60.5	3.82 s, 3H	7	60.2	2.53 m, 2H	3, 8, 12, 13
10	61.3	3.86 s, 3H	8	66.9	4.10 s, 3H	
11	36.0	2.47 brs, 3H	1	62.8	3.95 s, 3H	7
12,13				45.4	2.38 s, 6H	8
N-H		1.95[b] brs, 1H	11			

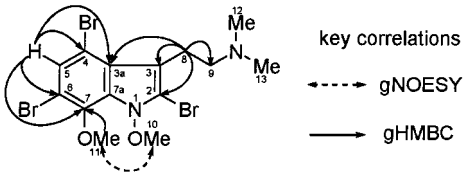
[a] Assignments within a column may be interchanged. [b] Variable chemical shift.

aromatic protons in the ^{1}H NMR spectrum, this implied a fully substituted benzene ring. A key gHMBC correlation, from the methylene protons at 3.05 ppm to one of the aromatic carbons bearing a methoxy group, fixed one methoxy substituent ortho to the ethylamine substituent on the ring. A strong gNOESY correlation between the two methoxy groups placed the second methoxy group adjacent to the first, meta to the ethylamine substituent. Three bromine substituents completed the structure **2**. This compound, obviously related to the Floridian convolutamines F and G (**3** and **4**), was named convolutamine H.



3 convolutamine F, R=Br
4 convolutamine G, R=H

Inspection of amathamide G (**1**) suggests the possibility that convolutamine H may be a precursor to its biosynthesis. A similar relationship was suggested for the *β*-phenylethylamine isolated from *A. wilsoni* and the corresponding amathamides found in that bryozoan.[12]



5 convolutindole A

The second nematocidal compound, **5**, was isolated as a pale yellow oil which crystallized after storage at −30 °C overnight, mp 61.5−62.5 °C. The molecular formula was determined by high-resolution LSIMS to be $C_{14}H_{17}Br_3N_2O_2$. In agreement with this assignment the isotope pattern was characteristic of a tribrominated compound, and **5** gave a positive Mayer's alkaloid test. A methanol solution of the compound gave absorption maxima at 235, 289, and 305 nm (log ϵ 4.7, 4.1, and 4.0 respectively), characteristic of an indole.[13] The ^{1}H NMR spectrum included two three-proton singlets at 4.10 and 3.95 ppm, attributable to two methoxy groups. A six-proton singlet at 2.38 ppm was consistent with an *N*,*N*-dimethyl group. Signals from a pair of mutually coupled methylenes at 2.53 and 3.12 ppm and a single aromatic proton, at 7.43 ppm, completed the spectrum.

The ^{13}C NMR spectrum was consistent with an indole, having eight downfield signals. Only one of these, at 141.7 ppm, corresponded to an aromatic carbon having a methoxy substituent. This carbon gave a gHMBC correlation to the methoxy protons at 3.95 ppm. The other methoxy protons, at 4.10 ppm, lacked gHMBC correlations to carbon. These results implied one methoxy group having an attachment to the indole nitrogen.

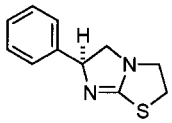
A key gHMBC finding was that only a single carbon had a correlation to both the aromatic proton and the methylene protons at 3.12 ppm. Taking cognizance of this result, the other gHMBC correlations and consideration of the ^{13}C chemical shifts allowed the placement of the aromatic proton on position 5 and the methylene on position 3. A gHMBC correlation between the aromatic proton and the carbon at 141.7 ppm allowed placement of the associated *C*-methoxy group on position 4, 6, or 7. A strong gNOESY correlation between the two methoxy groups and the lack of one between the aromatic proton and the *C*-methoxy group placed the methoxy group on position 7 of the indole. Thus the structure of this compound was determined to be **5**, for which we propose the name convolutindole A.

The *N*-methoxy moiety is unusual in marine natural products. A number of compounds having an acyclic *N*-methoxy group have been reported, such as kasarin from a marine microorganism[14] and several methoxyamino pyridines from marine sponges.[15−17] The sponge metabolites asmarines E and F contain a cyclic *N*-methoxy group.[18] The first marine-derived 1-methoxyindoles, **5** and pibocin B, were reported on the same day at an international symposium in Nago, Okinawa.[19,20] Pibocin B, an ergoline alkaloid, was isolated from a *Eudistoma* species ascidian.[21]

The occurrence of 1-methoxyindoles from natural products of terrestrial origin has been reported, for example stress metabolites of brassicas[22] and constituents of cruciferous plants.[23]

The results from this investigation further illustrate the variability in the metabolites obtained from a single species of bryozoan collected in different locations. Not only do the metabolites of *A. convoluta* from the east coast of Tasmania vary from those of Floridian and North Carolinian collections, they also vary from material collected in the south of Tasmania. Similar variability of alkaloidal metabolites from *A. wilsoni* has also been observed. One suggestion made to account for such variability is that the metabolites may actually be synthesized by bacterial symbionts of the

bryozoan, bacteria being more liable to mutate into different strains. Although some evidence exists to support this hypothesis, indisputable evidence is lacking.



6 levamisole

The LD_{99} values (in μg/mL) for **2** and **5** (Table 1) indicate that these metabolites are more potent nematocides than levamisole (**6**), a commercially available anthelmintic.[24] Despite having some structural similarities, **2** and **5** do not inhibit nematode development by the same mechanism as levamisole. While levamisole causes a highly characteristic paralysis of nematode larvae, **2** and **5** are lethal to the first and second stage larvae in the bioassay. However, like levamisole, **2** and **5** are highly selective nematocides showing little or no activity against *Bacillus subtilis* and *Saccharomyces cerevisae*, which are used as indicator species for antibacterial and antifungal activities.

Indole derivatives have previously been identified as anthelmintic agents. The bis(indole) amides from the red alga *Chondria atropurpurea*, for example, were reported to have moderate anthelmintic activity.[25] Investigation of further metabolites from *A. convoluta* is proceeding.

Experimental Section

General Experimental Procedures. All NMR spectra were recorded in $CDCl_3$ solvent with TMS as internal standard using a Varian INOVA spectrometer operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C. Standard pulse sequences were used. Infrared spectra were determined as thin films using a Perkin-Elmer PARAGON 1000 spectrometer. UV spectra were recorded in methanol on a Shimadzu UV-160 spectrophotometer. Mass spectrometry was performed on a Kratos Concept ISQ instrument. LSIMS was obtained in *m*-nitrobenzyl alcohol. Column chromatography was performed using Merck silica gel 60, 230−400 mesh. Preparative TLC utilized Merck silica gel 60 PF_{254}. Melting points were determined on a Yanagimoto micro melting apparatus.

Animal Material. The bryozoan was collected by scuba diving at a depth of 4−8 m from the piles of the woodchip loading facility at Triabunna, on Tasmania's east coast. An initial collection for bioactivity screening in July of 1998 was followed by a larger collection in July 1999. The collected material was transported to the laboratory and frozen within 3 h of collection. A voucher specimen (H2869) was deposited in the collection of the Tasmanian Museum and Art Gallery.

Bioassays. Nematode bioassays were performed using the McMaster strain of *Haemonchus contortus*, a reference susceptible strain which has had little, if any, exposure to any anthelmintic, by the method of Lacey et al.[26] Briefly, nematode eggs were added to the surface of the agar matrix containing the test compound, supplemented with a nutrient medium, and incubated at 26 °C until larvae in the control (no drug) wells developed to the L3 stage. A qualitative assessment of the larvae was made on day 5 of the assay to determine the lowest concentration of the test compound at which development was inhibited in 99% of the larvae present.

Antibacterial and antifungal activity were determined in agar-based, microtiter plate assays. Briefly, an aliquot of an overnight fermentation of *B. subtilis* (ATCC 6633) or *S. cerevisiae* (ATCC 9763) was applied to the surface of an agar matrix that contained the test compound, then incubated at 28 °C. A qualitative assessment of bacterial growth was made at 24 h with the LD_{99} determined as the lowest concentration of the test compound at which no growth of bacteria or yeast was observed.

Extraction and Isolation. Freeze-dried animal material (640 g) was broken up by hand and extracted repeatedly with dichloromethane. The combined extracts were evaporated under reduced pressure to obtain 14 g of a brown tarry residue. This material was fractionated by rapid vacuum column chromatography on silica gel. The fraction that eluted with 5% MeOH/CH_2Cl_2 (2.18 g) was further fractionated by flash column chromatography and then by MPLC on silica gel. Compound **5** (420 mg, 0.07% dry wt) eluted with 2% MeOH in EtOAc/CH_2Cl_2 20:80. Compound **1** (10 mg, 0.002% dry wt) eluted with 3% MeOH in EtOAc/CH_2Cl_2, 20:80. The fraction that eluted from the initial silica gel column with 10% MeOH/CH_2Cl_2 (2.8 g) was further fractionated by flash chromatography on silica gel and then by PTLC (development in 20% MeOH/CH_2Cl_2) to give compound **2** (240 mg, 0.04% dry wt).

Convolutamine H (2): pale yellow oil; UV (MeOH) λ_{max} (log ϵ) 217 (4.4) nm; IR ν_{max} (film) 3298, 2937, 2853, 1538, 1454, 1392, 1272, 1216, 1109, 1060, 1010, 953, 887, 786, 752, 691 cm^{-1}; LSIMS *m/z* 436 [$^{81}Br_3$] (31), 434 [$^{79}Br^{81}Br_2$] (95), 432 [$^{79}Br_2{}^{81}Br$] (100), 430 [$^{79}Br_3$] (38), 401 [$^{79}Br_2{}^{81}Br$] (15); EIMS *m/z*, 371 [$^{79}Br_2{}^{81}Br$] (37), 352 [$^{79}Br^{81}Br$] (100), 294 (28), 263 (15), 248 (11), 169 (15), 102(55); HRLSIMS *m/z* [M + H]$^+$ 429.86819 (calcd for $C_{11}H_{15}{}^{79}Br_3NO_2$, 429.86529); NMR refer to Table 2.

Convolutindole A (5): colorless solid; mp 61.5−62.5 °C; UV (MeOH) λ_{max} (log ϵ) 235 (4.7), 289 (4.1), 305 (4.0) nm; IR ν_{max} (film) 2937, 2856, 2818, 2768, 1548, 1470, 1383, 1329, 1261, 1261, 1177, 1141, 1084, 1043, 1020, 953, 870, 842, 718, 655, 633 cm^{-1}; LSIMS *m/z* 489 [$^{81}Br_3$] (27), 487 [$^{79}Br^{81}Br_2$] (88), 485 [$^{79}Br_2{}^{81}Br$] (100), 483 [$^{79}Br_3$] (43), 454 [$^{79}Br_2{}^{81}Br$] (53), 375 (37), 373 (36), 262 (21); EIMS *m/z* 371 [$^{79}Br_2{}^{81}Br$] (37), 352 [$^{79}Br^{81}Br$] (100), 294 (28), 263 (15), 248 (11), 169 (15), 102(55); HRLSIMS *m/z* [M + H]$^+$ 484.89100 (calcd for $C_{14}H_{18}{}^{79}Br_2{}^{81}BrN_2O_2$, 484.88992); NMR refer to Table 2.

Amathamide G (1): spectroscopic data have been published previously.[3]

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Supporting Information Available: Spectroscopic data for **1**. ^{1}H, ^{13}C, and gHMBC NMR spectra for **2** and **5**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Pettit, G. R. *Pure Appl. Chem.* **1994**, *66*, 2271−2281.
(2) Pettit, G. R.; Kamano, Y.; Aoyagi, R.; Herald, C. L.; Doubek, D. L.; Schmidt, J. M.; Rudloe, J. J. *Tetrahedron* **1985**, *41*, 985−994.
(3) Blackman, A. J.; Eldershaw, P. D.; Garland, S. M. *Aust. J. Chem.* **1993**, *46*, 401−405.
(4) Blackman, A. J.; Green, R. D. *Aust. J. Chem.* **1987**, *40*, 1655−1662.
(5) Zhang, H.-P.; Shigemori, H.; Ishibashi, M.; Kosaka, T.; Pettit, G. R.; Kamano, Y.; Kobayashi, J. *Tetrahedron* **1994**, *50*, 10201−10206.
(6) Zhang, H.-P.; Kamano, Y.; Kizu, H.; Itokawa, H.; Pettit, G. R.; Herald, C. L. *Chem. Lett.* **1994**, 2271−2274.
(7) Kamano, Y.; Kotake, A.; Hashima, H.; Hayakawa, I.; Hiraide, H.; Zhang, H.-P.; Kizu, H.; Komiyama, K.; Hayashi, M.; Pettit, G. R. *Collect. Czech. Chem. Commun.* **1999**, *64*, 1147−1153.
(8) Kamano, Y.; Zhang, H.-P.; Ichihara, Y.; Kizu, H.; Komiyama, K.; Pettit, G. R. *Tetrahedron Lett.* **1995**, *36*, 2783−2784.
(9) Zhang, H.-P.; Kamano, Y.; Ichihara, Y.; Kizu, H.; Komiyama, K.; Itokawa, H.; Pettit, G. R. *Tetrahedron* **1995**, *51*, 5523−5528.
(10) Hashima, H.; Hayashi, M.; Kamano, Y.; Sato, N. *Bioorg. Med. Chem.* **2000**, *8*, 1757−1766.
(11) Montanari, A. M.; Fenical, W.; Lindquist, N.; Lee, A. Y.; Clardy, J. *Tetrahedron* **1996**, *52*, 5371−5380.
(12) Blackman, A. J.; Fu, S.-L. *J. Nat. Prod.* **1989**, *52*, 436−438.
(13) Scott, A. I. *Interpretation of the Ultraviolet Spectra of Natural Products*; Pergamon Press: Oxford, 1964; pp 172−178.
(14) Suenaga, K.; Aoyama, S.; Xi, W.; Arimoto, H.; Yamaguchi, K.; Yamada, K.; Tsuji, T.; Yamada, A.; Uemura, D. *Heterocycles* **2000**, *52*, 1033−1036.
(15) Quinoa, E.; Crews, P. *Tetrahedron Lett.* **1987**, *28*, 2467−2468.
(16) Sakemi, S.; Totton, L. E.; Sun, H. H. *J. Nat. Prod.* **1990**, *53*, 990−995.
(17) Nicholas, G. M.; Molinski, T. F. *Tetrahedron* **2000**, *56*, 2921−2927.
(18) Yosief, T.; Rudi, A.; Kashman, Y. *J. Nat. Prod.* **2000**, *63*, 299−304.
(19) Narkowicz, C. K.; Blackman, A. J. *Abstracts of Papers*; 10th International Symposium on Marine Natural Products: Nago, Okinawa, June 2001; Abstract OR1.

(20) Stonik, V. A.; Avilov, S. A.; Kalinin, V. I.; Levina, A. I.; Kalinovsky, A. I.; Kapustina, I. I. *Abstracts of Papers*; 10th International Symposium on Marine Natural Products: Nago, Okinawa, June 2001; Abstract IL4.
(21) Makarieva, T. N.; Dmitrenok, A. S.; Dmitrenok, P. S.; Grebnev, B. B.; Stonik, V. A. *J. Nat. Prod.* **2001**, *64*, 1559−1561.
(22) Monde, K.; Sasaki, K.; Shirata, A.; Takasugi, M. *Phytochemistry* **1991**, *30*, 3921−3922.
(23) Belkhiri, A.; Lockwood, G. B. *Phytochemistry* **1990**, *29*, 1315−1316.
(24) Thienpont, D.; Vanparijs, O. F. J.; Raeymaekers, A. H. M.; Vandenberk, J.; Demoen, P. J. A.; Allewijn, F. T. N.; Marsboom, R. P. H.; Niemegeers, C. J. E.; Schellekens, K. H. L.; Janssen, P. A. J. *Nature* **1966**, *209*, 1084−1086.
(25) Davyt, D.; Entz, W.; Fernandez, R.; Mariezcurrena, R.; Mombrú, A. W.; Saldaña, J.; Domínguez, L.; Coll, J.; Manta, E. *J. Nat. Prod.* **1998**, *61*, 1560−1563.
(26) Lacey, E.; Redwin, J. M.; Gill, J. H.; Demargheriti, V. M.; Waller, P. J. In *Resistance of Parasites to Antiparasitic Drugs*; Boray, J. C., Martin, P. J., Roush, R. P., Eds.; MSD AGVET: Rahway, NJ, 1990; pp 177−184.

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