THREE NEW ALKALOIDS, CONVOLUTAMINES F AND G, AND CONVOLUTAMYDINE E, FROM THE FLORIDIAN MARINE BRYOZOAN *Amathia convoluta*

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Two new (2-phenylethyl)amine alkaloids, convolutamines F and G, and a dibromohydroxyoxindole alkaloid, convolutamydine E, have been isolated from the butan-1-ol soluble material of the Floridian marine bryozoan *Amathia convoluta*. Their structures were elucidated on the basis of spectroscopic data. Convolutamine F exhibited activity against KB, KB/VJ-300, and U937 cells. This compound also exhibited the inhibitory effects for cell division of fertilized sea urchin eggs.

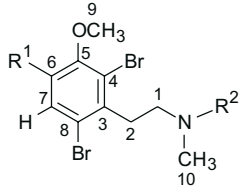
Key words: Marine natural products; Bryozoa; *Amathia convoluta*; (2-Phenylethyl)amine alkaloids; 3-Hydroxyindol-2(3*H*)-one; Convolutamine F; Convolutamine G; Convolutamydine E; Cytostatic activity; Isolation.

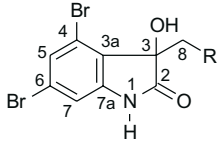
In our continuing search for bioactive substances, we previously isolated three series of new alkaloids, the γ-lactam alkaloids convolutamides A–F (ref.[1]), the (2-phenylethyl)amines, convolutamine A (**1**) (ref.[2]), and the bromohydroxyoxindoles convolutamydine A (**4**) (refs[3,4]) from the ethyl acetate-soluble material of the Floridian marine bryozoan *Amathia convoluta*. In this paper, we describe the isolation and structural elucidation of three new alkaloids, designated as convolutamines F (**2**) and G (**3**), and convolutamydine E (**5**), respectively, from the butan-1-ol-soluble material.

By column chromatography of the butan-1-ol-soluble material obtained by partition from the extract of *Amathia convoluta*, on various materials,

three new alkaloids, convolutamines F (**2**) and G (**3**), and convolutamydine E (**5**) were isolated.

Convolutamine F (**2**) was identified as $C_{10}H_{12}Br_3NO$ by HR FAB-MS (*m/z*): 401.9234 [M^+] for $C_{10}H_{12}Br_3NO$; Δ 0.2 mmu. By combination of FAB-MS spectra it showed the molecular ions [M^+] at *m/z* 400, 402, 404, and 406 in the ratio of 1 : 3 : 3 : 1, suggesting the presence of three bromine atoms. The ^1H and ^{13}C NMR chemical shifts, and the HMBC cross-peaks of **2** are shown in Table I. The ^1H NMR spectra of **2** revealed aromatic proton signals appeared at δ 7.74 (1 H, s), the ^{13}C NMR spectra at δ 116.0 s, 120.0 s, 121.8 s, 135.4 d, 139.9 s, and 154.0 s. This suggested the presence of 1,2,3,4,6-pentasubstituted benzene ring, which was also supported by the HMBC cross-peaks for H-7/C-3, H-7/C-6, and H-7/C-8. The quaternary aromatic carbons at δ 116.0 s, 120.0 s, and 121.8 s could be assigned to C-6, C-8, and C-4, respectively, substituted with bromine. The IR absorption at 2 853 and 1 456 cm^{-1} indicated the presence of CH_3–NH group in **2**. The ^1H NMR spectrum confirmed the presence of methyl (H-10), methoxy (H-9), and two methylene (H-1 and H-2) groups. The linkage of two methylene groups was supported by ^1H-^1H COSY spectrum. For the connectivity





Convolutamine A **1**, R^1= Br, R^2= $^{11}CH_2{}^{12}CH(OH)^{13}CH_3$

Convolutamine F **2**, R^1= Br, R^2= H

Convolutamine G **3**, R^1= H, R^2= H

Convolutamydine A **4**, R = $^{9}C^{10}OCH_3$

Convolutamydine E **5**, R = CH_2OH

of partial structures, HMBC experiments were carried out. A comparison of chemical shift of convolutamine A (**1**) (ref.[4]) and the HMBC cross-peak for H-9/C-5 indicated that the methoxy group was attached to C-5. The partial structure of aliphatic chain (C-2–C-1–C-10) in **2** having N–H group was deduced from the chemical shift at δ 2.51 s of *N*-methyl group and the HMBC cross-peak for H-10/C-1 (Table I). The tribromomethoxyphenyl unit (C-3–C-8) was shown to be connected to C-3 position by the HMBC cross-peaks for H-2/C-3, H-2/C-4, and H-2/C-8. These results led for

TABLE I
NMR data of convolutamines F (**2**) and G (**3**), and convolutamydine E (**5**)

Position	**2**			**3**	**5**		
	δC[a]	δH[a]	HMBC	δH[a]	δC[b]	δH[b]	HMBC
1	49.6 t	2.80 m, 2 H	H-2,10	2.84 brs, 2 H	–	12.1 brs, 1 H	–
2	37.7 t	3.19 m, 2 H	H-1	3.23 brs, 2 H	180.6 s	–	H-8
3	139.9 s	–	H-2,7	–	77.3 s	–	H-8,9
3a					130.1 s	–	H-5,7,8
4	121.8 s	–	H-2	–	130.1 s	–	–
5	154.0 s	–	H-9	–	128.0 d	7.46 bd, 1 H, 1.5	–
6	116.0 s	–	H-7	6.50 d, 1 H, 8.8	120.8 s	–	H-5,7
7	135.4 d	7.74 s, 1 H	–	7.48 d, 1 H, 8.8	112.5 d	7.08 bd, 1 H, 1.9	H-5
7a					146.7 s	–	–
8	120.0 s	–	H-2,7	–	39.2 t	3.16 m, 2 H	H-9
9	60.6 q	3.86 s, 3 H	–	3.88 s, 3 H	58.0 t	4.01 m, 2 H	H-7
10	36.2 q	2.51 s, 3 H	H-1	2.54 brs, 3 H			
N–H	–	1.62	–	1.66			

[a] The chemical shift was observed in $CDCl_3$ at 400 Mhz for ^1H and 100 Mhz for ^{13}C. [b] The chemical shift was observed in C_5D_5N.

convolutamine F, to structure **2**, *N*-methyl-2-(2,4,6-tribromo-3-methoxyphenyl)ethylamine.

Convolutamine G (**3**) was formulated as $C_{10}H_{13}Br_2NO$ by FAB-MS data. The structure elucidation of **3** was based on the data of MS, UV, IR, and ^{1}H NMR spectra. Unfortunately, the ^{13}C NMR spectrum of **3** could not record by reason of a small quantity of preparation. The ^{1}H NMR spectral data of **3** suggested that the structure of **3** was close to that of **2**. In this spectrum of **3**, a new doublet signal at δ 6.50 due to aromatic H-6, has appeared. The coupling constant supported the connectivity of H-6/H-7 with no bromine atom at position C-6. Also, the FAB-MS spectrum revealed that **3** possessed one less bromine atom than **1**. Thus, the structure of convolutamine G (**3**) was determined to be 2-(2,6-dibromo-3-methoxyphenyl)-*N*-methylethylamine.

Convolutamydine E (**5**) was formulated as $C_{10}H_9Br_2NO_3$ by HR EI-MS, indicating six unsaturations degrees. The EI-MS data of **5** displayed a molecular ion [M^+] at *m/z* 349, 351, and 353 in the ratio of 1 : 2 : 1, suggesting the presence of two bromine atoms. The ^{13}C NMR spectrum (Table I) assisted with DEPT experiment, showed ten carbon signals attributable to two aromatic methines, two aliphatic methylenes, one oxygenated quaternary carbon, one carbonyl carbon, and four aromatic quaternary carbons. Interpretation of the ^{1}H and ^{13}C NMR spectral data (Table I) facilitated by application of 2D NMR spectra (^{1}H-^{1}H COSY, HMBC) suggested that **5** consisted of partial structure of a dibromohydroxyoxindole moiety and a CH_2CH_2OH group. Also, the absorption of 223 and 298 nm in the UV spectrum suggested the presence of the oxindole ring. Therefore, **5** was assumed to be an analogue of convolutamydine A (**4**) by comparison of the NMR (Table I), UV, and MS data of **5** and convolutamydine A (**4**). The structure of **5** was elucidated by the following experiments. The ^{1}H-^{1}H COSY spectrum showed only two cross-peaks, one of which was assignable to a correlation due to couplings H_2-8/H_2-9, and the other was assignable to long-range couplings for H-5/H-7 (J = 1.5 and 1.9 Hz) of *meta* aromatic protons. The ^{1}H and ^{13}C NMR spectra of **5** revealed four quaternary aromatic carbons and two aromatic methines. These suggested the presence of the 1,2,3,5-tetrasubstituted benzene ring, which was verified by the HMBC cross-peaks for H-5/C-3a, H-5/C-6, H-7/C-3a, and H-7/C-6. The ^{13}C NMR chemical shifts (δ) for quaternary aromatic carbons of C-3a (130.1 s), C-4 (130.1 s), C-6 (120.8 s), and C-7a (146.7 s) implied that bromine atoms were present at C-4 and C-6, and an N–H group was attached at C-7a. The assignments of C-2, C-3, and C-3a were determined by the HMBC correlations of H-8/C-2, H-8/C-3, and H-8/C-3a. The ^{1}H NMR signals due to the N–H pro-

ton were observed at δ 12.1 (brs) in pyridine-d_5. The ^{13}C NMR chemical shifts were in good agreement with those of the indol-2(3*H*)-one system. The side chain unit was deduced from the ^{1}H-^{1}H COSY correlations and the HMBC cross-peak was indicated to attached at C-3 mainly by the HMBC cross-peak for H-9/C-3. The EI-MS fragmentation at *m/z* 306 supported the presence of the chain unit, $-CH_2CH_2OH$, having a hydroxy group. Thus, the structure of convolutamydine E (**5**) was determined to be 4,6-dibromo-3-hydroxy-3-(2-hydroxyethyl)indol-2(3*H*)-one.

Convolutamine F (**2**) exhibited activity against human epidermoid carcinoma KB cells and its vincristine-resistant KB/VJ-300 cells, and human monocyte-like lymphocytic leukemia U937 cells with IC_{50} values of 27, 9.6, and 13 μg/ml, respectively. Compound **2** also exhibited inhibitory effect for cell division of fertilized sea urchin eggs with IC_{50} value of 82 μg/ml. Biological evaluation of convolutamine G (**3**) and convolutamydine E (**5**) could not be achieved, due to a small quantity of preparation.

Since the (2-phenylethyl)amine structures of both convolutamine F (**2**) and G (**3**) were more simple than those of convolutamines A–E (refs[3,4]), these compounds might be appealed as intermediates in the biogenesis between convolutamines from *A. convoluta* and amathiamides from *A. wilsoni*[5]. The other (2-phenylethyl)amine alkaloids were isolated from *Amathia wilsoni*. Also, in interest, structure of convolutamydine E (**5**) just corresponded to a 2-hydroxyethyl analogue of 2-chloroethyl partial structure convolutamydine D (ref.[4]).

EXPERIMENTAL

All solvents used for chromatography were redistilled. Silica gel supplied by E. Merck was employed for column chromatography by using dry or wet-loading technique. HP-cellulofine (SEIKAGAKU CO., INC.) was used in gel chromatography. TLC was conducted on precoated Kieselgel 60 F254 (Merck) and the spots were detected by spraying with ninhydrin reagent and heating (hot plate). High-pressure liquid chromatography (HPLC) was performed on a Mightysil RP-18 250-20 column (20 mm i.d. × 250 mm, 5 mm, KANTO CHEMICAL CO., INC.) packed with 10 mm ODS. UV spectra were measured with a SHIMAZU, UV 2500PC spectrometer. Optical rotations were measured with a HORIBA, High Sensitive Polarimeter and the $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. EI, FAB, and their HR-MS mass spectra were taken with a JEOL JMS-AX505H mass spectrometer. IR spectra were recorded on a Jasco, FT/IR-300 spectrometer. The NMR spectra (Table I) were recorded on JEOL 400EX spectrometer at 303 K. The coupling constants (*J*) are given in Hz.

Extraction and Isolation

The bryozoan *Amathia convoluta* was collected in the Northeastern Gulf of Mexico, Florida, in 1982. The animal (100 kg, wet weight) was extracted with EtOH. The EtOH extract

(639 g) was partitioned between 10% aqueous MeOH and hexane. The MeOH phase was diluted with water to give 70% MeOH solution, and this was partitioned with EtOAc to afford the EtOAc-soluble materials (150 g). Then, the MeOH phase was diluted with water to give 50% MeOH solution. By partition of this aqueous MeOH solution with BuOH, the BuOH-soluble material (5.41 g) was obtained. The BuOH extract was chromatographed on a column of HP-cellulofine (4.0 cm × 45.0 cm) in chloroform–methanol (1 : 1). The principal fraction (fraction 2, 4.71 g) was chromatographed on silica gel (4.0 cm × 42.1 cm) using a gradient of hexane–dichloromethane–methanol (8 : 10 : 1) to (2 : 3 : 1). Detailed separation of the fraction eluted with hexane–dichloromethane–methanol (6 : 8 : 1) (fraction 8) was performed by further chromatography on column of HP-cellulofine in hexane–dichloromethane–methanol (3 : 5 : 1) followed by HPLC on a C-18 reverse-phase column (a flow rate of 7 ml/min) with methanol–water (6 : 4) with 0.5% trifluoroacetic acid. It gave pure convolutamine F (**2**) (9.0 mg) and convolutamine G (**3**) (1.2 mg). Similarly, the fraction 4 (102.8 mg) gave by chromatography on a column of ODS (1.0 cm × 20.5 cm) in acetonitrile–water (85 : 15) with 1% AcOH followed by HPLC on a C-18 reverse-phase column with methanol–water (6 : 4) containing 0.5% trifluoroacetic acid pure convolutamydine E (**5**) (1.9 mg).

Biological Activity

Human carcinoma KB cells and KB/VJ-300 cells (resistant with vincristine) were maintained in tissue culture flasks and grown in 96-well microtiter plates for assay. Test samples appropriately diluted with DMSO were added to the culture at concentration 50 μg/ml. After 72 h incubation at 37 °C and 5% CO_2, the survival rates of cells in the cultures were evaluated by the MTT method. The effect was shown as IC_{50} values. On the other hand, the inhibtory effects for cell division of fertilized sea urchin eggs was tested by the use of *Pseudocentrolus depressus* (Japanese name: AKAUNI). The samples were dissolved in methanol and diluted with artificial sea water. For assay, to 96-well microtiter, 1 ml of artificial sea water, 4 ml of fertilized sea urchin eggs solution, and sample solution were added. The mixture was incubated at 17–20 °C. After about 100 min, the survival rates of cell division were counted by the aid of microscope. The effect was shown as IC_{50} values.

Convolutamine F (**2**)

Yield 9.0 mg, colorless oil, $[\alpha]_D^{20}$ +24.3 (*c* 0.4, $CHCl_3$). UV (MeOH): λ_{max} 212.6 (ε 21 800) nm. IR (KBr), ν_{max}: 3 361, 2 920, 2 852, 2 347, 1 677, 1 451, 1 260, 1 202, 798, and 722 cm^{-1}. FAB-MS (positive), *m/z*: 400, 402, 404, and 406 $[M^+]$ in ratio of 1 : 3 : 3 : 1 for $C_{10}H_{12}Br_3NO$.

Convolutamine G (**3**)

Yield 1.2 mg, colorless oil. UV (CH_3CN): λ_{max} 206.0 (ε 8 908), 229.0 (ε 1 932) nm. IR (KBr), ν_{max}: 3 644, 2 924, 2 853, 2 355, 1 682, 1 456, 1 360, 1 260, 1 204, 1 136, 800, and 722 cm^{-1}. FAB-MS (positive), *m/z*: 321, 323, and 325 $[M^+]$ in ratio of 1 : 2 : 1 for $C_{10}H_{13}Br_2NO$.

Convolutamydine E (**5**)

Yield 2.3 mg, colorless oil. UV (CH_3CN): λ_{max} 223.0 (ε 16 870), 298.0 (ε 850) nm. IR (KBr), ν_{max}: 3 342, 2 925, 2 853, 2 360, 1 732, 1 608, 1 260, 1 088, and 801 cm^{-1}. EI-MS (positive),

m/z: 349, 351, and 353 [M^+] in ratio of 1 : 2 : 1 for $C_{10}H_9Br_2NO_3$. EI-MS fragmentation, *m/z*: 306 (M – CH_2CH_2OH).

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