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Convolutamines A-E, Novel β-Phenylethylamine Alkaloids from Marine Bryozoan *Amathia convoluta*

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Convolutamines A-E, novel cytotoxic alkaloids, have been isolated from Floridian bryozoan *Amathia convoluta* and the structures have been elucidated on the basis of extensive spectroscopic data.

Chemical studies of marine bryozoans have provided a variety of interesting bioactive secondary metabolites including alkaloids from *Amathia wilsoni* [1] and from *Flustra foliacea* [2] as well as antineoplastic macrolides bryostatins from *Bugula neritina* [3] and from *Amathia convoluta*.[4] During our search for new biologically active substances from marine organisms, we have examined an extract of the Floridian bryozoan *Amathia convoluta* collected off the Northeasten Gulf of Mexico. The EtOAc-soluble material from the extract was subjected to repeated chromatographies on silica gel, ODS, and Sephadex LH-20 to afford five novel β-phenylethylamine alkaloids convolutamines A (**1**), B (**2**), C (**3**), D (**4**), and E (**5**), as colorless oils, in the yields of 5.5×10^{-4}%, 2.5×10^{-5}%, 1.7×10^{-6}%, 1.4×10^{-5}%, and 4.0×10^{-7}%, respectively. We describe herein the structural elucidation of them.

The EIMS spectrum of convolutamine A (**1**)[5] showed ions $(M-CH_3)^+$ at *m/z* 442, 444, 446, and 448 in the ratio of 1:3:3:1, suggesting the presence of three bromine atoms. The molecular formula of **1** was determined as $C_{13}H_{18}O_2NBr_3$ by HREIMS [*m/z* 456.8929 $(M)^+$ for $C_{13}H_{18}O_2N^{79}Br_3$; Δ +4.1mmu], indicating four degrees of unsaturation. The ^1H[5] and ^{13}C (Table 1) NMR spectra in combination with ^1H-^1H COSY, HMQC, HMBC, INADEQUATE experiments showed that **1** consisted of an aromatic ring and an aliphatic chain, as depicted in Fig. 1. The ^1H and ^{13}C NMR signals for the aromatic portion (C-6-C-11) in **1** suggested the presence of a 1, 2, 3, 4, 6-pentasubstituted benzene ring, which was verified by the HMBC cross-peaks for H-10/C-6, H-10/C-8, H-10/C-9, and H-10/C-11. This result was further confirmed on the basis of ^1H nondecoupling ^{13}C NMR data, which were found to be $^3J_{CH}$ = 7.4 Hz for H-10/C-8 and $^2J_{CH}$ = 4.4 Hz for both H-10/C-9 and H-10/C-11.[6] Therefore the quaternary aromatic carbons at δ_C 121.6s, 115.9s, and 119.7s could be respectively assigned to C-7, C-9, and C-11 to the result that bromine atoms were substitued at C-7, C-9, and C-11. On the other hand, the signal at 119.7 ppm must be located at C-11 position, because the correlations for C-9/C-10 and C-10/C-11 were observed in the INADEQUATE spectrum. The HMBC cross-peak for H-12/C-8 indicated that the methoxyl group was attached to C-8. The partial structure of the aliphatic chain (C-1-C-5) in **1**

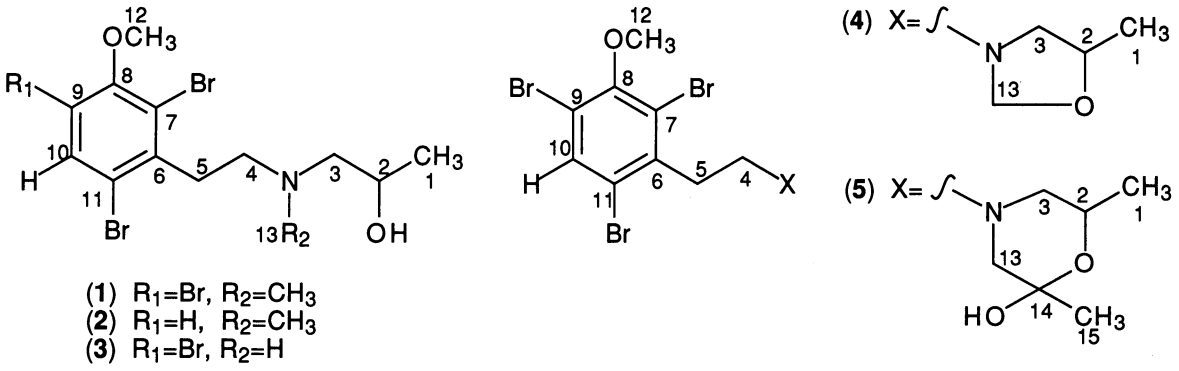

Fig. 1 The structures of convolutamines A (**1**), B (**2**), C (**3**), D (**4**), and E (**5**).

was deduced from the COSY correlations for H-1/H-2, H-2/H-3, and H-4/H-5 as well as the HMBC cross-peaks for H-13/C-3 and H-13/C-4. The tribromomethoxyphenyl unit (C-6-C-11) was shown to be connected to C-5 position by the HMBC cross-peaks for H-4/C-6, H-5/C-6, H-5/C-7, and H-5/C-11. An ^{1}H NMR signal due to the hydroxyl group on C-2 was observed at δ_H 4.42d in DMSO-d_6 and disappeared by addition of D_2O. This was also supported by the IR absorption band at 3450 cm^{-1}. These results led to structure **1**, 3-[*N*-methyl-2-(2,4,6-tribromo-3-methoxyphenyl)ethylamino]-2-propanol, for convolutamine A, as shown in Fig. 1.

Table 1. ^{13}C NMR Data of Convolutamines A (**1**), B (**2**), C (**3**), D (**4**), and E (**5**) in $CDCl_3$

position	1	2	3	4	5
1	19.9 q	19.6 q	20.4 q	20.0 q	18.9 q
2	63.1 d	63.0 d	65.5 d	71.7 d	65.3 d
3	64.7 t	64.7 t	56.3 t	59.5 t	59.1 t
4	55.0 t	55.1 t	47.1 t	52.3 t	55.1 t
5	34.7 t	34.4 t	37.9 t	37.3 t	34.5 t
6	139.7 s	139.8 s	139.5 s	139.3 s	139.5 s
7	121.6 s	115.1 s	121.8 s	121.8 s	121.8 s
8	154.0 s	155.6 s	154.0 s	154.0 s	154.0 s
9	115.9 s	110.9 d	116.1 s	116.1 s	116.1 s
10	135.4 d	131.8 d	135.4 d	135.4 d	135.4 d
11	119.7 s	110.9 s	119.9 s	119.8 s	119.8 s
12	60.5 q	56.5 q	60.5 q	60.5 q	60.5 q
13	41.9 q	41.9 q		86.3 t	61.5 t
14					93.8 s
15					25.2 q

δ in ppm.

The spectral data of convolutamine B (**2**)[7] were similar to those of **1**. The EIMS spectrum revealed that **2** possessed one less bromine atom than **1**, since the molecular ions at *m/z* 379, 381, 383 showed a triplet in a ratio of 1:2:1, which suggested the presence of two bromine atoms. The molecular formula, $C_{13}H_{19}O_2NBr_2$, was deduced by HREIMS [*m/z* 378.9784, $(M)^+$ for $C_{13}H_{19}O_2N^{79}Br_2$, Δ +0.1mmu]. The ^{1}H NMR spectrum of **2** closely resembled that of **1** except the signals at δ_H 6.65d and δ_H 7.45d. The ^{13}C NMR spectral data (Table 1) of **2** were different from those of **1** only in the signals due to C-7, C-9, C-10, and C-11. Analysis of 2D NMR

spectral data revealed that **2** possessed the structure as follows. The ^1H-^1H coupling constant (J = 8.8 Hz) due to δ_H 6.65d and 7.45d implied that H-9 connected to ortho position of H-10, which was further confirmed by the COSY correlation between H-9 and H-10. Also, the position of H-9 was established by the NOESY correlation between H-9 and H_3-12 as well as the HMBC cross peaks for H-9/C-7, H-9/C-8 and H-9/C-11. The ^1H NMR spectral data of **2** in DMSO-d_6 also revealed the presence of an exchangeable proton due to OH-2 by addition of D_2O. Thus, the structure of convolutamine B was determined to be structure **2,** 3-[*N*-methyl-2-(2,6-dibromo-3-methoxyphenyl)ethylamino]-2-propanol.

Convolutamine C (**3**)[8] was isolated from a more polar portion than any of two compounds described above. The ^1H (δ_H 2.43 s) and ^{13}C (δ_C 41.9 q) NMR signals (Table 1) due to *N*-methyl group which observed in both **1** and **2** were absent in **3**. By the analyses of NMR spectral data, the structure of convolutamine C (**3**) was assigned to be structure **3,** 3-[2-(2,4,6-tribromo-3-methoxyphenyl)ethylamino]-2-propanol, as shown in Fig. 1.

In the ^1H and ^{13}C NMR spectra of convolutamine D (**4**), the signals at δ_H 4.41d and 4.44d (H-13)[9] as well as the signal at δ_C 86.3t (C-13) (Table 1) were observed to be different from those of **1** and **2**. The HMBC cross peaks for H-13/C-4 and H-13/C-3 as well as the COSY correlation H-1-H-3 suggested the presence of an oxazolidine ring. The ^{13}C chemical shift (δ 86.3 t) for the methylene signal due to C-13 agreed well with that a oxazolidine system.[10] The EIMS spectrum showed the molecular ions (M^+) at *m/z* 454, 456, 458, 460 in a ratio of 1:3:3:1, suggesting the presence of three bromine atoms as **1**. Interpretation of the spectral data such as ^1H-^1H COSY, HMQC, HMBC led to the establishment of the structure of **4** as shown in Fig.1, assigned as 3-[2-(2,4,6-tribromo-3-methoxyphenyl)ethyl]-5-methyloxazolidine. To our knowledge, compounds with oxazolidine are rare in nature.

The EIMS spectrum for convolutamine E (**5**)[11] showed ions $(M-H_2O)^+$ at *m/z* 481, 483, 485, 487 in a ratio of 1:3:3:1, suggesting the presence of three bromine atoms. The molecular formula, $C_{15}H_{20}O_3NBr_3$, was estabilished by the HREIMS[$(M)^+$, *m/z* 498.8994, for $C_{15}H_{20}O_3N^{79}Br_3$]. With the aid of NMR techniques such as ^1H NMR, ^{13}C NMR (Table 1), HOMO decoupling and NOE difference spectral experiments, the structure of **5** was assigned as 4-[2-(2,4,6-tribromo-3-methoxyphenyl)ethyl]-2-hydroxy-2,6-dimethylmorpholine, as shown in Fig. 1.

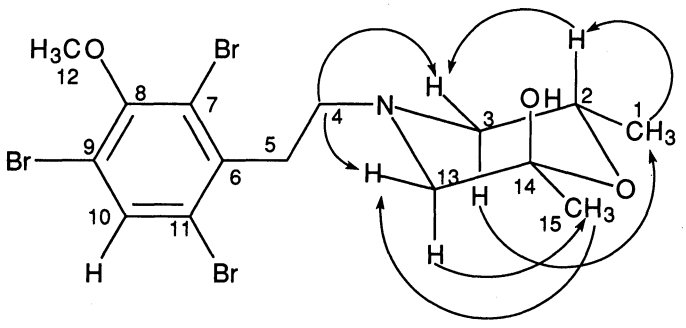

Fig. 2 NOEs of convolutamine E (**5**) in pyridine-d5.

The relative stereochemistry of convolutamine E (**5**) was illustrated in Fig. 2. Both convolutamines A(**1**)and B (**2**) were found to be a racemates, because the ^1H NMR spectra of their 2-O-(*S*)- or (*R*)-MTPA

derivatives showed the signals due to both (2*S*)-and (2*R*)-form in a ratio of 1:1.[12] The stereochemistry of convolutamines C and D remains unknown.

From other bryozoan *Amathia wilsoni*, a β-phenylethylamine has been isolated as a possible precursor of the brominated β-phenylethylamines, amathiamides, having the *N*-methylpyrrolidine groups.[1] This fact indicated that the biogenesis of convolutamines A–E (**1**-**5**) from *Amathia convoluta* is related to that of Amathiamides.

Convolutamines A (**1**), B (**2**), and D (**4**) exhibited cell growth inhibitory activity (IC_{50} 10.6, 4.8, and 8.6 μg/ml, respectively) against the murine P388 lymphocytic leukemia.

References

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5) **1**: IR ($CHCl_3$) ν_{max} 3450, 1460, 1420, 1350, 1275, 1050, 936 cm^{-1}. UV (MeOH) λ_{max} 212.5 nm (ε 27, 900). ^1H NMR (400 MHz, $CDCl_3$) δ 1.15 (3H, d, *J* = 5.9 Hz, H-1), 2.34 (1H, dd, *J* = 12.1, 10.3 Hz, H-3a), 2.44 (1H, dd, *J* = 12.1, 2.9 Hz, H-3b), 2.44 (3H, s, H-13), 2.59 (1H, ddd, *J* = 12.5, 11.0, 5.5 Hz, H-4a), 2.71 (1H, ddd, *J* = 12.5, 11.0, 5.5 Hz, H-4b), 3.13 (1H, ddd, *J* = 12.5, 11.0, 5.5 Hz, H-5a), 3.21 (1H, ddd, *J* = 12.5, 11.0, 5.5 Hz, H-5b), 3.81 (1H, dqd, *J* = 10.3, 5.9, 2.9 Hz, H-2), 3.86 (3H, s, H-12), 7.74 (1H, s, H-10).
6) The C-H long range coupling constants of aromatic carbons: $^3J_{CH} > {}^2J_{CH}$, e.g. $^3J_{CH}$ = 7.4 Hz and $^2J_{CH}$ = 1.0 Hz (benzene ring). R. M. Silverstein, G. C. Bassler, and T. C. Morrill, "Spectrometic Identification of Organic Compounds", Fourth Edition (1981), p. 272.
7) **2**: IR ($CHCl_3$) ν_{max} 3420, 2970, 2910, 1570, 1460, 1420, 1070, 936 cm^{-1}. UV (MeOH) λ_{max} 211.5 nm (ε 20,100), EIMS *m/z* 89 $(C_4H_{10}ON+H)^+$, 103 $(C_5H_{12}ON+H)^+$, 275, 277, 279 [1:2:1, $(C_8H_7OBr_2\text{-}2H)^+$], 290, 292, 294 [1:2:1, $(C_9H_9OBr_2\text{-}H)^+$], 334, 336, 338 (1:2:1, $C_{11}H_{14}ONBr_2^+$). ^1H NMR (400 MHz, $CDCl_3$) δ 1.13 (3H, d, *J* = 6.3 Hz, H-1), 2.35 (1H, dd, *J* = 12.2, 10.7 Hz, H-3a), 2.43 (3H, s, H-13), 2.44 (1H, dd, *J* = 12.2, 2.9 Hz, H-3b), 2.59 (1H, ddd, *J* = 12.7, 11.2, 5.4 Hz, H-4a), 2.72 (1H, ddd, H-4b), 3.16 (1H, ddd, *J* = 12.7, 11.2, 5.4 Hz, H-5a), 3.25 (1H, ddd, *J* =12.7, 11.2, 5.4 Hz, H-5b), 3.79 (1H, dqd, *J* =10.3, 6.3, 2.9 Hz, H-2), 3.86 (3H, s, H-12), 6.65 (1H, d, *J* = 8.8 Hz, H-9), 7.45 (1H, d, *J* = 8.8 Hz, H-10).
8) **3**: ^1H NMR (400 MHz, $CDCl_3$) δ 1.17 (3H, d, *J* = 6.3 Hz, H-1), 2.49 (1H, dd, *J* = 12.1, 9.2 Hz, H-3a), 2.82 (1H, dd, *J* = 12.2, 2.9 Hz, H-3b), 2.83 (1H, dt, *J* = 11.7, 8.0 Hz, H-4a), 2.88 (1H, dt, *J* = 11.7, 8.0 Hz, H-4b), 3.19 (2H, t, H-5), 3.80 (1H, dqd, *J* = 9.2, 6.2, 2.9 Hz, H-2), 3.86 (3H, s, H-12), 7.74 (1H, s, H-10).
9) **4**: ^1H NMR (400 MHz, $CDCl_3$) δ 1.28 (3H, d, *J* = 6.4 Hz, H-1), 2.55 (1H, dd, *J* = 10.8, 7.8 Hz, H-3a), 3.24 (1H, dd, *J* = 10.8, 6.4 Hz, H-3b), 2.73 (2H, m, H-4), 3.19 (2H, m, H-5), 3.86 (3H, s, H-12), 4.15 (1H, dqt, *J* = 7.8, 6.4 Hz, H-2), 4.41 (1H, d, *J* = 4.9 Hz, H-13a), 4.44 (1H, d, *J* = 4.9 Hz, H-13b), 7.74 (1H, s, H-10).
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11) **5**: ^1H NMR (400 MHz, $CDCl_3$), δ 1.28 (3H, d, *J* = 6.2 Hz, H-1), 1.40 (3H, s, H-15), 1.96 (1H, t, *J* = 11.0 Hz, H-3a), 2.23 (1H, d, *J* = 11.0 Hz, H-13a), 2.58 (2H, t, *J* = 8.0 Hz, H-4), 2.79 (1H, dd, *J* = 11.0, 2.5 Hz, 13b), 2.87 (1H, dt, *J* = 11.0, 2.5 Hz, H-3b), 3.17 (2H, m, H-5), 3.87 (3H, s, H-12), 4.07 (1H, dqd, 11.0, 6.2, 2.5 Hz, H-2), 4.39 (1H, br s, OH-2), 7.74 (1H, s, H-10).
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