Alternatamides A−D: New Bromotryptamine Peptide Antibiotics from the Atlantic Marine Bryozoan *Amathia alternata*

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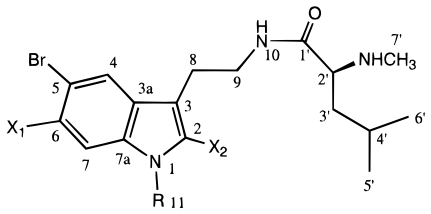
Four new bromotryptamine peptides, alternatamides A−D (**1**−**4**), have been isolated from the Atlantic bryozoan *Amathia alternata*. The structures of the alternatamides were assigned primarily on the basis of 2D NMR data. The absolute stereochemistry of the *N*-methylleucine amino acid was shown to be L (2′*S*) by hydrolysis and comparison with standards. The alternatamides show modest antibacterial activities against several Gram-positive bacteria.

The bryozoans, or moss animals, are a generally less abundant but chemically rich group of marine invertebrates.[1,2] Bryozoans of the widely distributed genus *Amathia* are known to contain amino acid-derived metabolites of several interesting types,[3−10] in addition to antitumor agents of the bryostatin class.[11] In recent studies of several Australian *Amathia* species, the distributions of metabolites within the animal, their susceptibility to fouling, and the possibility that metabolites are produced by surface bacteria were investigated.[12−14]

Our recent investigation of *A. convoluta* from North Carolina[15] led to the conclusion that the volutamides, new peptide metabolites derived from bromotyramine, function as a chemical defense against generalist fish and sea urchin predators. We now report an extension of this work to the related North Carolina bryozoan *A. alternata* Lamouroux 1816. This animal contains a series of bioactive bromotryptamine peptides identified as the alternatamides A−D (**1**−**4**). Although the natural functions of the alternatamides have not been studied, their structural similarity to the feeding deterrent volutamides as well as their antibacterial properties suggest these molecules may also serve a defensive role.

Extraction of the freeze-dried animal with 1:1 $CHCl_3$−MeOH, followed by removal of solvent under vacuum and purification of the condensed extract first by Si gel vacuum flash chromatography using mixtures of $CHCl_3$ and MeOH and then LH-20 column chromatography with MeOH led to the isolation of alternatamides A−D (**1**−**4**) as mixtures. By use of repeated silica and reversed-phase HPLC methods, the alternatamides A−D (**1**, 0.11; **2**, 0.05; **3**, 0.05 and **4**, 0.002% dry wt) were obtained in pure form.

Alternatamide A (**1**) was obtained as a white amorphous powder that analyzed for $C_{18}H_{25}Br_3N_3O$ by HR-FABMS and ^{13}C-NMR methods (see Table 1). The IR spectrum of **1** showed absorption bands at 3350 and 1660 cm^{-1} characteristic of NH and amide carbonyl



1, Alternatamide A, R = CH_3, X_1 = X_2 = Br

2, Alternatamide B, R = H, X_1 = X_2 = Br

3, Alternatamide C, R = H, X_1 = H, X_2 = Br

4, Alternatamide D, R = H, X_1 = Br, X_2 = H

functionalities. UV absorption maxima at 236, 296, and 306 nm (ϵ 52 000, 10 700, and 9 400) were indicative of the presence of an indole chromophore. The ^{1}H-NMR spectrum of alternatamide A showed two singlet aromatic protons at δ 7.78 and 7.52, an *N*-methyl group at δ 3.67, two sets of aliphatic methylene protons [δ 2.91 (2H) and δ 3.46 (1H) and δ 3.52 (1H)], one exchangeable NH proton (δ 7.29), one methyl group at δ 2.24, and complex proton bands (integrating for 9 H) above δ 2.0. Analysis of the highfield bands by COSY NMR methods led to the assignment of these bands to a leucine residue. This assignment, and the linkage of the leucine amino acid with a tribromotryptamine residue, was accomplished by combined 2D NMR methods, particularly by interpretation of ^{13}C DEPT and HMQC and HMBC data (Table 1), which allowed all protons and carbons to be confidently assigned. Of particular importance were the long-range HMBC correlations of the C-4 and C-7 protons with carbons C-3a, C-5, C-6, and C-7a, which defined the 5,6-dibromoindole constellation. An *N*-methylindole was indicated by similar HMBC correlations from the *N*-methyl group (C-11) to C-2 and C-7a. An ethylamino chain was positioned at C-3, first for biogenetic reasons, and secondly after the observation of long-range HMBC correlations between the C-8 protons and C-2, C-3, C-3a, and C-9. Lastly, the leucine residue was established as *N*-methylleucine by direct correlation of the *N*-methyl protons with the C-2′ methine carbon. Curiously, HMBC correlations (at 8 Hz) were not obvious between the N-10 proton or the C-2′ proton and C-1′, the amide carbonyl. Thus, in alternatamide A the amide linkage could not be confirmed by heterocorrelation NMR methods. This link-

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Table 1. NMR Data for Alternatamides A−D (**1**−**4**)[a]

atom no.	Alternatamide A (**1**)		Alternatamide B (**2**)		Alternatamide C (**3**)		Alternatamide D (**4**)	
	δ^{13}C*	δ^{1}H*	δ^{13}C†	δ^{1}H†	δ^{13}C†	δ^{1}H†	δ ^{13}C*	δ^{1}H*
N1				8.24 (1H, s)		8.25 (1H, s)		8.53 (1H, s)
C2	115.8 (C)		112.1 (C)		111.3 (C)		124.2 (CH)	7.08 (1H, s)
C3	111.6 (C)		112.4 (C)		111.9 (C)		112.6 (C)	
C3a	127.9 (C)		129.4 (C)		130.2 (C)		128.4 (C)	
C4	122.4 (CH)	7.78 (1H, s)	123.2 (CH)	8.00 (1H, s)	121.2 (CH)	7.80 (1H, d, 1.5)	123.0 (CH)	7.85 (1H, s)
C5	117.1 (C)		117.2 (C)		113.5 (C)		117.0 (C)	
C6	115.0 (C)		115.2 (C)		125.3 (CH)	7.25 (1H, dd, 8.5, 1.5)	114.5 (C)	
C7	114.1 (CH)	7.52 (1H, s)	116.5 (CH)	7.75 (1H, s)	113.4 (CH)	7.33 (1H, d, 8.5)	115.9 (CH)	7.66 (1H, s)
C7a	136.2 (C)		136.9 (C)		136.0 (C)		135.9 (C)	
C8	25.4 (CH2)	2.91 (2H, dd, 6.8, 6.8)	25.4 (CH2)	2.99 (2H, dd, 7.3, 6.8)	25.4 (CH2)	2.99 (2H, dd, 7.3, 6.8)	25.0 (CH2)	2.92 (2H, dd, 6.8, 6.4)
C9	38.6 (CH2)	3.46 (1H, m)	39.8 (CH2)	3.59 (1H, m)	39.8 (CH2)	3.63 (2H, dd, 7.3, 6.8)	39.3 (CH2)	3.58 (2H, m)
		3.52 (1H, m)		3.61 (1H, m}				
N10		7.29 (1H, s)		7.47 (1H, s)*		7.63(1H, s)*		7.58 (1H, s)
C11	31.7 (CH3)	3.67 (3H, s)						
C1′	174.8 (C)		168.6 (C)		168.5 (C)		171.4 (C)	
C2′	63.4 (CH)	2.91 (1H, m)	61.5 (CH)	3.85 (1H, m)	61.5 (CH)	3.92 (1H, t, 7.3)	62.5 (CH)	3.63 (1H, m)
C3′	42.6 (CH2)	1.25 (1H, m)	40.3 (CH2)	1.71(2H, m)	40.3 (CH2)	1.75 (2H, m)	41.4 (CH2)	1.25(1H, m)
		1.51 (1H, m)						1.56 (1H, m)
C4′	25.1 (CH)	1.62 (1H, m)	25.1 (CH)	1.62 (1H, m)	25.1 (CH)	1.66 (1H, m)	25.0 (CH)	1.56 (1H, m)
C5′	21.7 (CH3)	0.88 (3H, d, 6.4)	22.4 (CH3)	0.82 (3H, d, 6.0)	22.3 (CH3)	0.88 (3H, d, 5.9)	22.3 (CH3)	0.84 (3H, d, 6.4)
C6′	23.2 (CH3)	0.93 (3H, d, 6.4)	23.1 (CH3)	0.83 (3H, d, 6.4)	23.1 (CH3)	0.89 (3H, d, 5.4)	22.7 (CH3)	0.92 (3H, d, 6.4)
C7′	35.5 (CH3)	2.24 (3H, s)	32.6 (CH3)	2.70 (3H, s)	32.5 (CH3)	2.69 (3H, s)	33.9 (CH3)	2.31 (3H, s)

[a] ^{13}C-NMR data for **1** and **4** were obtained at 50 MHz, while for **2** and **3** data were obtained at 125 MHz. ^{1}H-NMR data were obtained at 500 MHz in the following indicated solvents: * = chloroform-*d*, † = acetone-d_6. Numbers of attached protons were determined by DEPT sequence experiments. Assignments of all protons and respective carbons were assisted by COSY, HMQC, and HMBC correlation methods.

age became secure, however, on the basis of other spectral features (chemical shifts and IR absorptions, in particular) and the requisite correlations were quite observable in the HMBC spectra of alternatamides B and C. On the basis of these data, alternatamide A was assigned as the tribromotryptamine-*N*-methylleucine amide **1**. At this point, no information defining the stereochemistry of the amino acid carbon, C-2′, could be obtained.

Alternatamide B (**2**) was also obtained as a colorless amorphous powder that analyzed for $C_{17}H_{23}Br_3N_3O$ by HRFABMS and ^{13}C-NMR methods. The IR, UV, and overall NMR data for this compound were almost identical to those from **1** with the exception that the indole *N*-methyl group (δ 3.67) was replaced by an indole NH (δ 8.45, 1H). As in **1**, comprehensive NMR studies allowed all protons and carbons to be assigned. HMBC data showed strong correlations between the C-2′ and C-3′ protons and the C-1′ carbonyl of the *N*-methylleucine constituent. Thus, alternatamide B (**2**) was assigned as the indole *N*-demethyl derivative of alternatamide A.

Alternatamide C (**3**) was also obtained as a colorless amorphous powder that analyzed for $C_{17}H_{24}Br_2N_3O$ by HRFABMS and ^{13}C-NMR methods. The IR, UV, and overall NMR data for this metabolite were almost identical to those from **1** and **2**, with the exception being the substitution of one less bromine on the indole ring. COSY NMR data showed a three-proton spin system that indicated one bromine substituent was placed at C-2 and one at either C-5 or C-6. Differentiating between these two latter positions was not straightforward. Changing NMR solvents from deuteriochloroform to deuterioacetone has been used to differentiate between the C-4 and C-7 protons in indole derivatives, by measuring an observed downfield shift of the C-7 proton.[16] This effect, which is apparently caused by acetone complexation with the indole nitrogen, was not observed in alternatamide C. Comparison of the NMR data for **3** with several literature analogues,[17] however, provided strong evidence of the C-5 bromoindole regiochemistry.

Alternatamide D (**4**) was also obtained as a white amorphous powder that analyzed for $C_{17}H_{24}Br_2N_3O$ by HRFABMS and ^{13}C-NMR methods. The IR, UV, and overall NMR data for this derivative were similar to alternatamide C except all aromatic proton resonances were observed as singlets at δ 7.08, 7.66, and 7.85, respectively. Because two aromatic bromine substituents were present, they were positioned at C-5 and C-6, leaving *para*-substituted (noncoupled) protons at C-4 and C-7, analogous to alternatamide A. The additional aromatic proton was positioned at C-2 based on its chemical shift and its HMBC correlations with adjacent carbons. Hence, the structure of alternatamide D was confidently assigned as 5,6-dibromotryptamine *N*-methylleucine amide (**4**).

The CD spectra of the alternatamides A−C (D was not measured) showed complex, multiple Cotton effects between 200 and 240 nm. Free L amino acids typically give a positive Cotton effect in the 210−240 nm range.[18] Because the CD spectra for the peptides did not seem to reflect the expected simple Cotton effects known for free amino acids, the absolute configurations of the leucine components of alternatamides A−C were confidently shown to be L (2′*S*) by hydrolysis (6 N HCl, 110 °C, 24 h) and chiral TLC analysis using authentic L-*N*-methylleucine as a standard. No data defining the absolute stereochemistry were obtained for alternatamide D. The L configuration of the *N*-methylleucine in **4** was assumed.

Alternatamides A−C show modest antibacterial activities against *Staphylococcus aureus* (NCCLS), *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*,

Bacillus subtilis, Enterococcus faecalis, Enterococcus faecium, and *Streptococcus pyogenes* with MIC values ranging between 4 and 32 μg/mL. Alternatamides A−C showed almost no activity against Gram-negative bacteria. Alternatamide D was not tested.

Experimental Section

Animal Collection and Extraction. Two collections of *Amathia alternata* were made, by hand, using scuba, at a depth of 3m off Morehead City, North Carolina, in June and September 1991. Voucher specimens, identified by Dr. William Kirby-Smith, Duke University Marine Laboratory, are available at the Institute of Marine Sciences in Morehead City. The animals were frozen separately, then freeze-dried to give 190 and 490 g dry animal, respectively. Extraction of the freeze-dried, powdered animals with 1:1 $CHCl_3$−MeOH, followed by removal of solvent under vacuum left crude extracts that were compared by diode array HPLC (C-18 RP column, MeOH−H_2O gradient) and by 500-MHz ^{1}H NMR. The extracts proved to be virtually identical. Purification of the September extract (36.4 g) first by Si gel vacuum flash chromatography using mixtures of $CHCl_3$ and MeOH, then by LH-20 column chromatography with MeOH, led to the isolation of alternatamides A−D (**1**−**4**) as complex mixtures. Using repeated silica (10% MeOH in $CHCl_3$ with 0.2% isopropylamine) and C-18 RP (isopropanol−MeCN−MeOH−H_2; 3:6:1:10 with 0.1% trifluoroacetic acid) HPLC methods, the alternatamides A−D (**1**, 0.11; **2**, 0.05; **3**, 0.05 and **4**, 0.002% dry wt) were obtained in pure form.

Alternatamide A (1): a colorless amorphous solid; CD (MeOH) $[\theta]_{236nm}$ +24 700, $[\theta]_{232nm}$ −10 500, $[\theta]_{222nm}$ −16 200, $[\theta]_{206nm}$ −22 200, $[\theta]_{202nm}$ +20 100; HRFABMS $(M + H)^+$ obsd 535.9553, calcd 535.9548 for $C_{18}H_{25}N_3O^{79}Br_3$, obsd 537.9556, calcd 537.9527 for $C_{18}H_{25}{}^{79}Br_2{}^{81}BrN_3O$; obsd 539.9542, calcd 539.9507 for $C_{18}H_{25}{}^{79}Br^{81}Br_2N_3O$; LREIMS (base) 538/540 (M^+, 54), 458/460 (15), 380 (9.2), 257 (4.0), 100 (100), 83/81 (44); IR ($CHCl_3$) 3350, 1600, 1518, 1462, 1425, 1369, 1308, 1093, 867 cm^{-1}; UV (MeOH) λ_{max} 236 nm (ϵ 52 000), 296 nm (ϵ 10 700), 306 nm (ϵ 9400).

Alternatamide B (2): a colorless amorphous solid; CD (MeOH) $[\theta]_{246nm}$ +7900, $[\theta]_{236nm}$ −15 300, $[\theta]_{228nm}$ +23 100, $[\theta]_{222nm}$ +24 500, $[\theta]_{206nm}$ +2700, $[\theta]_{204nm}$ +18 700; HRFABMS $(M + H)^+$ obsd 523.9388, calcd 523.9371 for $C_{17}H_{23}{}^{79}Br_2{}^{81}BrN_3O$; IR (film on NaCl) 3300, 3251, 3090, 2963, 1667, 1567, 1469, 1438, 1371, 1202, 1141, 922, 723 cm^{-1}; UV(MeOH) λ_{max} 236 nm (ϵ 54 000), 295 nm (ϵ 10 000), 305 nm (ϵ 9100).

Alternatamide C (3): a colorless amorphous solid; CD(MeOH) $[\theta]_{244nm}$ +20 200, $[\theta]_{236nm}$ −18 600, $[\theta]_{228nm}$ +16 600, $[\theta]_{196nm}$ +3700; HRFABMS $(M + H)^+$ obsd 446.0288, calcd 446.0265 for $C_{17}H_{24}{}^{79}Br^{81}BrN_3O$; IR (film on NaCl) 3400, 3258, 3075, 2963, 2874, 1665, 1570, 1438, 1202, 1141, 798, 723 cm^{-1}; UV(MeOH) λ_{max} 225 nm (ϵ 46 000), 290 nm (ϵ 10 000), 298 nm (ϵ 8300).

Alternatamide D (4): a colorless amorphous solid; HRFABMS $(M + H)^+$ obsd 444.0284, calcd 444.0286 for $C_{17}H_{24}{}^{79}Br_2N_3O$; IR (film on NaCl) 3417, 2959, 1663, 1560, 1453, 1400, 1227, 1167, 1097, 906, 862 cm^{-1}; UV (MeOH) λ_{max} 232 nm (ϵ 65 700), 295 nm (ϵ 8800), 305 nm (ϵ 8000).

Hydrolysis of Alternatamides A−D (1−4). In a typical experiment, the alternatamide isomer (2.5 to 5 mg) was treated, in a sealed glass tube, with 6 N HCl for 24 h at 110 °C. The hydrolysis product was concentrated under vacuum, taken up in Me_2CO-H_2O (9:1 with trace KOH), and chromatographed on chiral TLC plates (HPTLC-Fertigplatten, CHIR, Art 14285, EM Separations) with D- and L-*N*-methylleucine as standards. The *N*-methylleucine liberated in the hydrolysis migrated to the identical R_f as the L isomer.

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