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Medusamide A, a Panamanian Cyanobacterial Depsipeptide with Multiple β-Amino Acids

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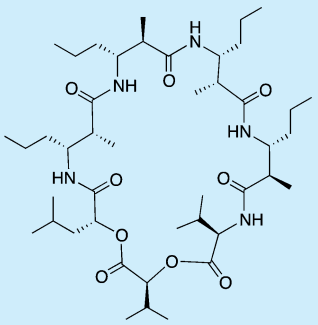
Supporting Information

ABSTRACT: From a collection of marine cyanobacteria made in the Coiba National Park along the Pacific coast of the Republic of Panama a novel cyclic depsipeptide, given the trivial name medusamide A, has been isolated and fully characterized. Medusamide A contains four contiguous β-amino acid (2*R*,3*R*)-3-amino-2-methylhexanoic acid (Amha) residues. This is the first report of multiple Amha residues and contiguous β-amino acid residues within a single cyclic peptide-type natural product. Stereochemical assignment of the Amha residues was completed following the synthesis of reference standards for this β-amino acid and the subsequent derivatization with Marfey's reagent and LC−MS analysis.



Marine cyanobacteria produce a wide variety of secondary metabolites that are noted for their unique chemical scaffolds that have a diversity of biomedically relevant properties, most notably in the areas of anticancer and anti-inflammation.[1−3] A prevalent theme in their natural products is the creation of cyclic lipopeptides with a mixture of proteogenic as well as unusual amino acids along with polyketide sections. Understanding the mechanisms by which these organisms produce and incorporate these unusual structural units is an area of great scientific interest.[4−6] Moreover, this understanding is critical to the manipulation and engineering of these biosynthetic pathways to create analogues as well as transmission of the genetic machinery to expression hosts to produce metabolites in large quantities. Thus, discovery of unique structures from cyanobacteria that do not appear to result from known biosynthetic pathways provides exciting avenues for research related to cyanobacterial biosynthesis, chemical ecology, and drug discovery.[7−9]

Here, we report the isolation and structure elucidation of the marine cyanobacterial metabolite medusamide A (**1**), a depsipeptide containing four contiguous β-amino acid, 3-amino-2-methylhexanoic acid (Amha) units. The trivial name reflects the four Amha residues that give it a "snake-hair" like appearance. Single Amha units have been found in several cyanobacterial natural products, including the depsipeptides malevamide B,[10] kulokekahilide-1,[11] ulongamides A−F,[12] guineamide D,[13] lyngbyastatin 3,[14] and carriebowmide.[15] Medusamide A (**1**) is the first cyanobacterial compound to have adjacent β-amino acids, a structural motif which suggests a fundamentally different biosynthetic pathway for their incorporation than those previously described.

Medusamide A (**1**) was isolated from the organic extract of an encrusting cyanobacterium collected subtidally at four meters near Coiba Island on the Pacific coast of Panama. Based on 16S rRNA sequence analysis, the cyanobacterium does not clade with any known genera of marine cyanobacteria (Figure S5) and is thus likely a new genus. It is most closely related to members of the genera *Trichodesmium*, *Okeania*, and *Oscillatoria*. A methanolic fraction of the extract was moderately inhibitory to cancer cell growth in MCF-7 hormone-dependent breast cancer cells (73% inhibition at 10 μg/mL). Medusamide A (**1**) was isolated from this fraction as a white solid by HPLC and was found to be inactive in the assays (S19; a second compound was isolated and determined to be the active component of the original fraction but was in such small quantity that structure elucidation was not possible).

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Table 1. ^{1}H and ^{13}C NMR Data for 1

		^{13}C	^{1}H ppm, mult, Hz	COSY	$^{1}H-^{13}C$ HMBC	NOESY
OLeu	1	169.9, qC				
	2	73.6, CH	5.05, dd (3.6, 10.0)	3a, 3b	1, 3	*NH*-5, 3a, 3b
	3a	40.7, CH_2	1.77, m	2, 4	2, 4, 5, 6	*NH*-5, 4, 5, 6
	3b		1.63, m			*NH*-5
	4	24.7, CH	1.77, m	3a, 3b, 5, 6	2, 3	2, 3b, 5, 6
	5	22.9, CH_3	0.94, m	4	3, 4	4, 5
	6	21.2, CH_3	0.91, m	4	3, 4	4, 3b
Hiva	7	170.4, qC				
	8	68.1, CH	5.23, m	9, 10	7, 12	10
	9	33.9, CH	1.53, m	8, 11	8	8, 10, 11
	10	16.1, CH_3	1.42, d (6.8)	8, 11	7, 8, 9, 11	8, 9, 11
	11	14.1, CH_3	0.94, d (6.5)	9, 10	9, 10	9, 10
Valine	12	174.3, qC				
	13	50.9, CH	4.01, m	*NH*-1, 14	12, 17	14, 15, 16
	NH-1		7.10, s	13		18, 19
	14	43.0, CH	2.80, dq (3.7, 7.0)	13, 15, 16	12, 13	13, 15, 16
	15	12.8, CH_3	1.17, d (7.0)	14		13, 14
	16	12.8, CH_3	1.17, d (7.0)	14		13, 14
Amha-1	17	174.6, qC				
	18	44.9, CH	2.62, dq (7.3, 7.5)	19, 20	17, 19	*NH*-1, 19, 20, 21
	19	51.1, CH	4.12, m	*NH*-2, 18, 21	18, 21, 22, 23, 24	*NH*-1, *NH*-2, 18, 20, 21, 22
	NH-2		6.80, s	19		20, 21, 25, 27
	20	14.0, CH_3	1.06, d (6.9)	18	17, 19	*NH*-2, 18, 19
	21	35.1, CH_2	1.45, m	19, 22	19, 22, 23	18, 19, 22, 23
	22	19.7, CH_2	1.26, m	21, 23	19, 21, 23	18, 19, 21, 23
	23	13.7, CH_3	0.89 d (3.4)	22	21, 22	21, 22
Amha-2	24	174.0, qC				
	25	44.5, CH	2.52, m	26, 27	24, 26, 27	*NH*-2, *NH*-3, 26, 27
	26	51.3, CH	4.01, m	*NH*-3, 25, 28	31	*NH*-3, 25, 27, 28, 30
	NH-3		6.09, d (8.2)	26		25, 26, 27, 28, 32, 34
	27	14.7, CH_3	1.10, d (7.3)	25	24, 25, 26	*NH*-2, *NH*-3, 25, 26, 30
	28	34.4, CH_2	1.46, m	26, 29	36, 29, 30	*NH*-3, 26, 29, 30
	29	19.2, CH_2	1.28, m	28, 30	26, 28, 30	28, 30
	30	13−14, CH_3	0.9, m	29	28, 29	26, 28, 29
Amha-3	31	174.0, qC				
	32	45.9, CH	2.52, m	33, 34	32, 33, 34	*NH*-3, *NH*-4, 33, 34, 35
	33	51.2, CH	4.01, m	*NH*-4, 32		*NH*-4, 32, 34, 35
	NH-4		6.65, d (7.5)	33		32, 33, 35, 39, 40, 41
	34	14.8, CH_3	1.12, d (7.3)	32	31, 33	*NH*-3, *NH*-4, 33, 34, 35
	35	31.9, CH_2	1.46, m	33, 36	33, 36, 37	32, 33, 37
	36	20.0, CH_2	1.30, m	35, 37	33, 35, 37	37
	37	13−14, CH_3	0.9, m	36	35, 36	36
Amha-4	38	174.3, qC				
	39	47.2, CH	2.38, dq (7.1, 8.3)	40, 41	38, 40. 41	*NH*-4, *NH*-5, 40, 41, 42
	40	51.4, CH	4.12, m	*NH*-5, 39, 42	42, 43	*NH*-4, 39, 41, 42,
	NH-5		6.29, m	40		2, 3a, 3b, 39, 40, 41, 42
	41	15.5, CH_3	1.09, d (7.3)	39	38, 39	*NH*-4, 39, 40
	42	35.7, CH_2	1.43, m	40, 43	40, 43, 44	43, 44
	43	19.6, CH_2	1.25, m	42, 44	44	42, 44
	44	13−14, CH_3	0.85, d (6.8)	43	42, 44	42, 43

The molecular mass of **1** was measured by HRMS ($[M + H]^+$ m/z = 822.5955), representing a molecular formula of $C_{44}H_{79}O_9N_5$ with eight degrees of unsaturation. However, the ^{13}C NMR spectrum of **1** (Table 1; Figure S7) showed only 41 resonances, as a result of overlap of two valine methyl group resonances and overlap of carbonyl and methyl group resonances in two of the Amha residues (Amha-2 and -3). The most downfield region of the ^{1}H NMR spectrum (Table 1; Figure S6) contained five broad peaks (δ_H 6.09−7.10), suggesting five protonated amides. Four of these (δ_H 6.09−6.80) were part of overlapping AMX spin systems. In each case, these amide protons coupled to a four-proton resonance representing four β protons (δ_H 4.01−4.12). These latter resonances were in turn each coupled to a resonance representing four overlapping α protons (δ_H 2.62−2.38). The four α protons were correlated by HMBC to one of four amide carbonyls (δ_C 174.6−174.0) and, taken together with the additional alkyl substituents described below, afforded four

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identical β-amino acids subunits. The fifth amide proton (δ_H 7.10) was correlated by COSY to an α proton (δ_H 4.01 ppm) that correlated by HMBC to a carbonyl (C-12, δ_C 174.3), thus indicating that **1** contained a single α-amino acid. Two additional signals occurred in the midfield region of the ^{1}H NMR spectrum, corresponding to oxygenated α protons (δ_H 5.05, δ_C 73.6 and δ_H 5.23, δ_C 68.1). Compound **1** was thus confirmed to be a depsipeptide containing four β-amino acids, one α-amino acid, and two α-hydroxy acid residues.

Structures of these seven individual residues were determined using a series of 1D and 2D NMR experiments (Table 1 and SI). These residues were four Amha residues (numbered Amha-1−4 for clarity), valine (Val), leucic acid (OLeu), and α-hydroxyisovaleric acid (Hiva). COSY correlations were used to sequentially connect the resonances of Amha-1: a methyl group H_3-20 to a methine H-18 to a second methine H-19. H-19 was coupled to both an amide NH and methylene H_2-21, which was adjacent to a second methylene H_2-22 and then terminated with a methyl group H_3-23. Taken together with the HMBC correlations noted previously from H-18 to an amide carbonyl (C-17), these data defined an Amha residue. Deduction of the other three Amha residues followed a very similar logic with similar to identical chemical shifts (Table 1 and Figure 1).

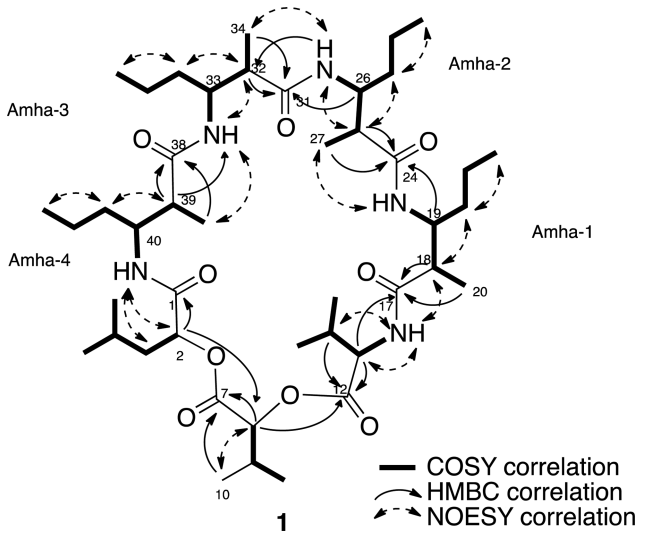

Figure 1. Planar structure of **1** with supporting 2D NMR correlations.

The valine residue was deduced from COSY correlations between the H-13 methine proton (δ_H 4.01) and both the remaining *N*H proton (δ_H 7.10) and a highly coupled H-14 methine proton (δ_H 2.80). The latter resonance was coupled to both the H-15 and H-16 methyl groups. Leucic acid (OLeu) was deduced from COSY correlations between the OLeu H-2 methine and the OLeu H-3a (δ_H 1.77) and H-3b (δ_H 1.63) methylene. Connection to the remainder of this residue was achieved through HMBC correlations from the OLeu H-3 protons as well as the two methyl groups, OLeu H-5 (δ_H 0.94) and OLeu H-6, (δ_H 0.91) to methine OLeu C-4 (δ_C 24.7). The Hiva unit was constructed based on COSY correlations between methine resonances Hiva H-8 (δ_H 5.23) and H-9 (δ_H 1.53). The latter resonance was coupled to both methyl groups Hiva H-11 (δ_H 0.94) and H-10 (δ_H 1.42). A rich network of HMBC correlations confirmed these seven partial structures (Figure 1 and Table 1).

Connectivity between residues was established using HMBC and NOESY correlation spectroscopy. HMBC correlations were observed between the H-8 α-proton of Hiva and the valine carbonyl (C-12, δ 174.3), the H-13 α-proton of valine and the Amha-1 carbonyl (C-17, δ 174.6), the H-19 α-proton of Amha-1 and Amha-2 carbonyl (C-24, δ 174.0), and the H-26 β-proton of Amha-2 and Amha-3 carbonyl (C-31, δ 174.0). Remaining connectivities were established by NOESY correlations which included valine *N*H-1 to Amha-1 H-18 and H-19, Amha-1 *N*H-2 (δ 6.80) to Amha-2 H-25 and H-27, Amha-2 *N*H-3 (δ 6.02) to Amha-3 H-32 (δ 2.52) and H-34 (δ 1.12), Amha-3 *N*H-4 (δ 6.65) to Amha-4 H-39 (δ 2.38) and H-41 (δ 1.09), and Amha-4 *N*H-5 (δ 6.29) to OLeu H-2 (δ 5.05) and weakly to OLeu H-3a (δ 1.77) and OLeu H-3b (δ 1.63). Cyclization of the structure provided the final degree of unsaturation and was corroborated by a weak HMBC correlation between OLeu H-2 and Hiva C-8.

Collisional-induced fragmentation by LC−MS/MS provided additional evidence for the planar structure of **1**. Fragmentation of the ESI-MS parent ion m/z = 822.31 $[C_{44}H_{80}O_9N_5 + H]^+$ after initial cleavage between Val and Amha-1, gave the following ions: m/z = 623.26 [OLeu-Amha-Amha-Amha-Amha + H]$^+$; m/z = 568.24 [Val-Hiva-OLeu-Amha-Amha + H]$^+$; m/z = 441.13 [Val-Hiva-OLeu-Amha + H]$^+$; and m/z = 314.02 [Val-Hiva-OLeu + H]$^+$ (Figure 2). The peak observed at m/z =

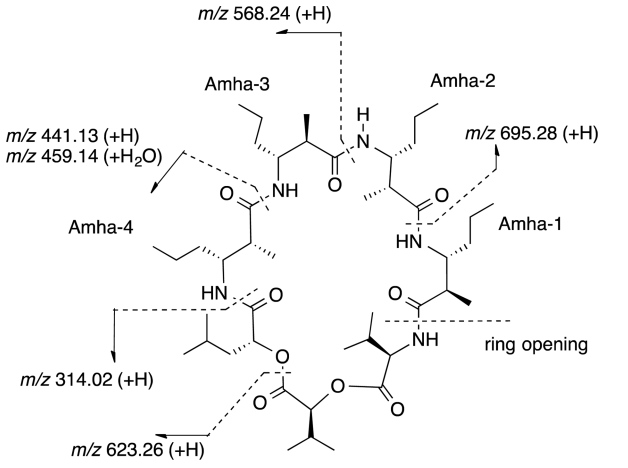

Figure 2. Collisionally induced fragments of **1** identified by LC-MS/MS from ring-opening site between Val and Amha-1.

804.30 likely corresponds to $[M - H_2O + H]^+$. Ring opening between Amha-1 and Amha-2 produced the acylium ion m/z = 495.10 [OLeu-Amha-Amha-Amha]$^+$ (S14). Additional fragmentations following formation of the acylium ion at the initial ring opening site are included in S14. These fragmentation ions were annotated with the assistance of mMass prediction software and align perfectly with the planar structure predicted from NMR analysis.

Stereochemistry was determined using chiral chromatography for the hydroxy acids and advanced Marfey's method for valine and Amha residues. Co-elution on chiral chromatography with commercially available standards identified the presence of L-Hiva and D-OLeu in **1** (see S15). Following the advanced Marfey's procedure identified the valine residue in **1** as D-valine.[16] For the Amha residues, standards of the β-amino acid were synthesized in enantiomeric excess as previously reported.[14] The hydrogenation step was incomplete, but fully hydrogenated product was identified via LC−MS. The mixture

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of hydrogenated and nonhydrogenated enantiomers was reacted with either D-FDLA or L-FDLA, following the advanced Marfey's procedure, and the resulting Amha diastereomers were resolved into four distinct peaks by LC−MS (S18). Hydrolyzed **1** was reacted with L-FDLA, and the conjugated Amha coeluted as a single peak with the major peak of the L-FDLA Amha standard, indicating **1** contains only (2*R*,3*R*)-Amha residues (S18). In addition, we used advanced Marfey's technique to synthesize D/L-FDAA Amha conjugates, providing further evidence of the Amha stereochemistry. Hydrolyzed **1** conjugated with L-FDAA coeluted with the major peak of the L-FDAA Amha, confirming the presence of only (2*R*,3*R*)-Amha in medusamide A (**1**).

One of the more intriguing aspects of medusamide A's structure is the presence of four identical and adjacent Amha residues. To the best of our knowledge, multiple adjacent *β*-amino acids have not been previously observed in any cyanobacterial or other marine natural product. Moreover, only a very few marine natural products possess multiple *β*-amino acids (dihydrocyclotheonamide A, cyclocinamide A, theonellamides F, B, C, and onchidin),[17,18] and none have contiguous *β*-amino acids. This suggests involvement of a unique biosynthetic pathway to account for such an unusual structural motif as in medusamide A (**1**).

The known biosynthetic pathways for cyanobacterial metabolites containing *β*-amino acids, such as microcystin [contains a 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyl-4,6-decadienoic acid (Adda) residue] and nostophycin [contains a 3-amino-2,5-dihydroxy-8-phenyloctanoic acid (Ahoa) residue], involve the formation of *β*-amino acids from the pathway-initiating polyketide structural unit. Specifically, a *β*-ketocarboxylate is transaminated by a PLP-dependent aminotransferase to form the *β*-amino function. Following a series of NRPS-mediated incorporations of amino acids, the *β*-amino group nucleophile is employed in the macrocyclization event and completes the biosynthetic pathway.[19−21] However, the microcystin/nostophycin pathway for forming *β*-amino acids is inconsistent with the structure of **1**, as this would require multiple separate starting points or an iterative use of an NRPS module (as found in fungi[22] and bacteria[23,24]), and implies unprecedented reactions to interconnect these residues. Hence, it appears more likely that multiple Amha units are formed separately from the assembly line, and then incorporated as activated amino acids through an entirely NRPS-type mechanism, such as observed for the incorporation of *β*-alanine into jamaicamide A.[25] More research is needed to clarify the novel biosynthetic assembly illustrated by medusamide A (**1**).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b03110.

Full experimental details and spectral data (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Gerwick, W. H.; Moore, B. S. *Chem. Biol.* **2012**, *19*, 85−98.
(2) Fattorusso, E.; Taglialatela-Scafati, O. *Mar. Drugs* **2009**, *7*, 130−152.
(3) Cragg, G. M.; Grothaus, P. G.; Newman, D. J. *Chem. Rev.* **2009**, *109*, 3012−3043.
(4) Daly, J. W. *J. Nat. Prod.* **2004**, *67*, 1211−1215.
(5) Jones, A. C.; Monroe, E. A.; Eisman, E. B.; Gerwick, L.; Sherman, D. H.; Gerwick, W. H. *Nat. Prod. Rep.* **2010**, *27*, 1048−1065.
(6) Mahmud, T.; Flatt, P. M.; Wu, X. *J. Nat. Prod.* **2007**, *70*, 1384−1391.
(7) Blunt, J. W.; Copp, B. R.; Hu, W.-P.; Munro, M. H. G.; Northcote, P. T.; Prinsep, M. R. *Nat. Prod. Rep.* **2007**, *24*, 31−86.
(8) Choi, J. Y.; Plummer, M. S.; Starr, J.; Desbonnet, C. R.; Soutter, H.; Chang, J.; Miller, J. R.; Dillman, K.; Miller, A. a; Roush, W. R. *J. Med. Chem.* **2012**, *55*, 852−870.
(9) Chang, Z.; Flatt, P.; Gerwick, W. H.; Nguyen, V.-A.; Willis, C. L.; Sherman, D. H. *Gene* **2002**, *296*, 235−247.
(10) Horgen, F. D.; Yoshida, W. Y.; Scheuer, P. J. *J. Nat. Prod.* **2000**, *63*, 461−467.
(11) Kimura, J.; Takada, Y.; Inayoshi, T.; Nakao, Y.; Goetz, G.; Yoshida, W. Y.; Scheuer, P. J. *J. Org. Chem.* **2002**, *67*, 1760−1767.
(12) Luesch, H.; Williams, P. G.; Yoshida, W. Y.; Moore, R. E.; Paul, V. J. *J. Nat. Prod.* **2002**, *65*, 996−1000.
(13) Tan, L. T.; Sitachitta, N.; Gerwick, W. H. *J. Nat. Prod.* **2003**, *66*, 764−771.
(14) Williams, P. G.; Moore, R. E.; Paul, V. J. *J. Nat. Prod.* **2003**, *66*, 1356−1363.
(15) Gunasekera, S. P.; Ritson-Williams, R.; Paul, V. J. *J. Nat. Prod.* **2008**, *71*, 2060−2063.
(16) Fujii, K.; Ikai, Y.; Mayumi, T.; Oka, H.; Suzuki, M.; Harada, K. *Anal. Chem.* **1997**, *69*, 3346−3352.
(17) Clark, W. D.; Corbett, T.; Valeriote, F.; Crews, P. *J. Am. Chem. Soc.* **1997**, *119*, 9285−9286.
(18) Romanova, N. N.; Tallo, T. G.; Rybalko, I. I.; Zyk, N. V.; Shvyadas, V. K. *Chem. Heterocycl. Compd.* **2011**, *47*, 395−417.
(19) Tillett, D.; Dittmann, E.; Erhard, M.; von Döhren, H.; Börner, T.; Neilan, B. A. *Chem. Biol.* **2000**, *7*, 753−764.
(20) Fewer, D. P.; Osterholm, J.; Rouhiainen, L.; Jokela, J.; Wahlsten, M.; Sivonen, K. *Appl. Environ. Microbiol.* **2011**, *77*, 8034−8040.
(21) Kudo, F.; Miyanaga, A.; Eguchi, T. *Nat. Prod. Rep.* **2014**, *31*, 1056−1073.
(22) Boettger, D.; Hertweck, C. *ChemBioChem* **2013**, *14*, 28−42.
(23) Blodgett, J. A.; Oh, D. C.; Cao, S.; Currie, C. R.; Kolter, R.; Clardy, J. *Proc. Natl. Acad. Sci. U. S. A.* **2010**, *107*, 11692−11697.
(24) Gaitatzis, N.; Silakowski, B.; Kunze, B.; Nordsiek, G.; Blöcker, H.; Höfle, G.; Müller, R. *J. Biol. Chem.* **2002**, *277*, 13082−13090.
(25) Edwards, D. J.; Marquez, B. L.; Nogle, L. M.; McPhail, K.; Goeger, D. E.; Roberts, M. A.; Gerwick, W. H. *Chem. Biol.* **2004**, *11*, 817−833.

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