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Cytotoxic Peptides from the Marine Sponge *Cymbastela* sp.

John E. Coleman, E. Dilip de Silva,[1] Fangming Kong and Raymond J. Andersen*
Departments of Chemistry and Oceanography
University of British Columbia
Vancouver, BC
CANADA V6T 1Z4

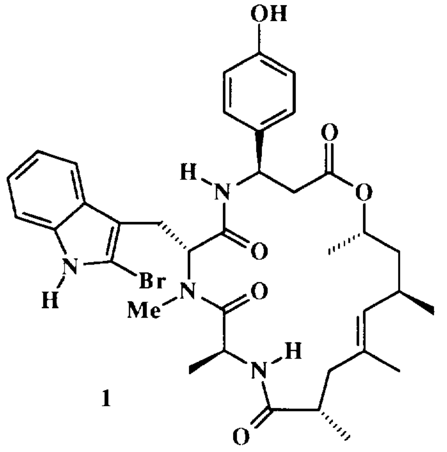
Theresa M. Allen
Department of Pharmacology
University of Alberta
Edmonton, Alberta
CANADA T6G 2H7

Abstract: Extracts of the sponge Cymbastela sp. have yielded the novel cytotoxic peptides geodiamolide G (11), hemiasterlin A (12), hemiasterlin B (13), criamide A (14) and criamide B (15). The structures of the new compounds were solved via spectroscopic analysis and chemical degradation.

Marine sponges continue to be a very rich source of novel cytotoxic peptides and depsipeptides that typically contain one or more D-amino acids and/or previously unknown amino acids.[2] Jaspamide (jasplakinolide) (**1**),[3,4] isolated from sponges of the genus *Jaspis* collected in Fiji and Palau, was the first reported example of a small family of four residue cyclic depsipeptides that share a common twelve carbon hydroxy acid fragment linked to three variable amino acids to give either 18 or 19 membered macrocylic rings. The closely related geodiamolides A (**2**) and B (**3**) were subsequently isolated from a Caribbean species of *Geodia*[5] and our group reported the isolation of geodiamolides A (**2**) to F (**7**) from a Papua New Guinea sponge identified as a *Pseudaxinyssa* sp.[6] Jaspamide and the geodiamolides were found to be potently cytotoxic to a number of human cancer cell lines *in vitro*.[7,8] Recently, Crews et al. reported that specimens of *Auletta c.f. constricta* collected in Papua New Guinea also contained jaspamide (**1**) and an unrelated cytotoxic peptide, milnamide A (**8**).[8] Shortly thereafter, Kashman and co-workers reported that the sponge *Hemiasterella minor* collected in South Africa contained jaspamide (**1**), geodiamolide TA (**9**) and hemiasterlin (**10**), which is closely related to milnamide A (**8**).[9]

Our original small collection (50 g dry wt.) of *Pseudaxinyssa* sp. did not provide the quantities of geodiamolides required for *in vivo* evaluation in animal models relevant to the observed *in vitro* cytotoxicities against human cancer cell lines.[6,7] In order to obtain sufficient amounts of the geodiamolides for *in vivo* testing, the source sponge was recollected from the original dive sites at Motupore and Madang in Papua New Guinea. Taxonomic identification of a voucher sample of the recollected sponge verified that it was identical to the original sample, however, in the period between collections the classification of the sponge had been changed from *Pseudaxinyssa* sp. to *Cymbastela* sp. (probably an undescribed species).[10] Bioassay-guided fractionation of extracts from the recollected *Cymbastela* sp. led to the isolation of the known metabolites geodiamolides A (**2**) to F (**7**) and hemiasterlin (**10**), and the novel metabolites geodiamolide G (**11**), hemiasterlin A (**12**), hemiasterlin B (**13**), criamide A (**14**) and criamide B (**15**). The structures of geodiamolide

G (**11**), hemiasterlin A (**12**), hemiasterlin B (**13**), criamide A (**14**) and criamide B (**15**) and their biological activities are described below.



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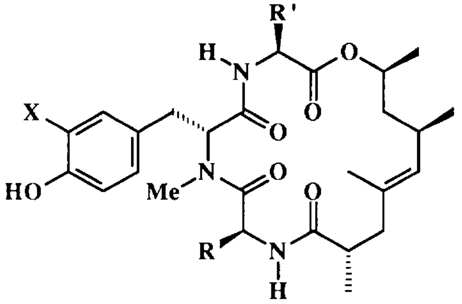
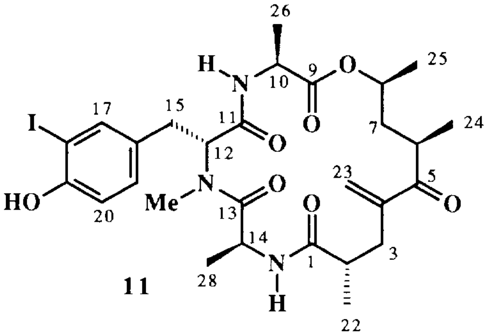


2 X=I, R=R'=Me
3 X=Br, R=R'=Me
4 X=Cl, R=R'=Me
9 X=I, R=Me, R'=isopropyl

5 X=I, R=H, R'=Me
6 X=Br, R=H, R'=Me
7 X=Cl, R=H, R'=Me



11

Geodiamolide G (**11**) (2mg) was obtained as a clear glass that gave a parent ion in the EIHRMS at m/z 655.1760 appropriate for a molecular formula of $C_{28}H_{38}N_3O_7I$, which differed from the molecular formula of geodiamolide A (**2**) simply by the addition of one oxygen atom and the loss of two hydrogen atoms. Examination of the ^{1}H NMR, COSY, HMQC and HMBC spectra of **11** revealed that it contained a tripeptide fragment identical to that present in geodiamolide A (**2**) (Table1), but that it differed in the polyketide fragment. Absent from the ^{1}H NMR spectrum of **11** were resonances that could be assigned to the H5 olefinic methine and the H23 olefinic methyl protons found in geodiamolide A (**2**). In their place, the ^{1}H NMR spectrum of **11** contained a pair of resonances at δ 5.78 (bs: H23') and 5.90 (bs: H23) that were both correlated to the same olefinic methylene carbon resonance at δ 127.8 (C23) in the HMQC spectrum. The HMBC spectrum of **11** contained strong correlations from both of the olefinic methylene proton resonances at δ 5.78 (H23') and 5.90 (H23) to a ketone carbonyl resonance at δ 205.1 (C5) and to an aliphatic methylene carbon resonance at δ 37.7 (C3). A series of additional HMBC correlations between the methylene carbon resonance (δ 37.7: C3) and a methyl proton resonance at δ 1.15 (H22), between the methyl proton resonance at δ 1.15 (H22) and a carbonyl carbon resonance at δ 174.5 (C1), and between the carbonyl carbon resonance (δ 174.5: C1) and the alanine α-methine (δ 4.72: H14) and NH (δ 6.19) proton resonances, located the ketone at C5 and the olefin between C4 and C23 in **11** as shown. COSY and HMBC data (Table 1) showed that the remaining portion of the polyketide fragment in **11** (C6 to C8 and C24, C25) had a constitution identical to the same portion of geodiamolide A (**2**). Difference NOEs observed between H23 (δ 5.90) and H6 (δ 2.95), and between H23' (δ 5.78) and H3' (δ 2.14), demonstrated that H23 was *cis* and H23' was *trans* to the C5 ketone. The configurations at C2, C6 and C8 in geodiamolide G (**11**) were assumed to be identical to the configurations at the same centers in geodiamolide A (**2**).

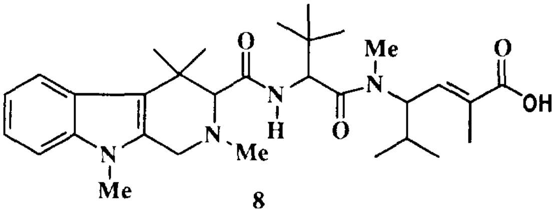
Table 1. NMR Data for geodiamolide G (11). Recorded in $CDCl_3$ at 500MHz.

Carbon No.	δ $^{13}C^a$	δ ^1H	COSY	HMBC^b
1	174.5			H3, H3', H14, H22, NH(14)
2	41.0	2.46, m	H3, H3', H22	H3', H22
3	37.7	2.55, dd,J=12.3, 3.7Hz	H2, H3'	H22, H23, H23'
3'		2.14, t,J=11.9Hz	H2, H3	
4	143.6			H3, H23'
5	205.1			H3', H23, H23', H24
6	36.2	2.95	H7', H24	H24
7	38.8	1.82, ddd,J=14.6, 9.5, 2.8Hz	H7', H8	H24, H25
7'		1.61, ddd,J=14.6, 10.9, 2.8Hz	H6, H7	
8	69.7	5.11, m	H7, H25	H25
9	170.6			H10, H26
10	49.1	4.51, dq,J=7.3,7.2Hz	H26, NH(10)	H26
NH(10)		6.35, d,J=7.3Hz	H10	
11	168.9			H12, H15'
12	57.1	5.06, dd,J=7.9,8.9Hz	H15, H15	H15, H15', H27
13	174.4			H12, H14, H27, H28, NH(14)
14	45.1	4.72,dq,J=7.0,6.9Hz	H28, NH(14)	H28
NH(14)		6.19, d,J=7.0Hz	H14	
15	33.2	3.12, dd,J=14.6,7.9Hz	H12, H15'	H12, H17, H21
15'		2.90, dd,J14.6,8.9Hz	H12, H15	
16	130.3			H15, H15', H20
17	138.2	7.45, d,J=1.4Hz	H21	H15, H15', H21
18	85.2			H17, H20
19	154.5			H17, H21
20	115.6	6.88, d,J=8.2Hz	H21	
21	130.6	7.04, dd,J=8.2, 1.4Hz	H17, H20	H17
22	17.9	1.15, d,J=6.4Hz	H2	
23	127.8	5.90, s		H3'
23'		5.78, s		
24	17.8	1.09, d,J=7.1Hz	H6	
25	20.8	1.28, d,J=6.3Hz	H8	
26	18.3	1.32, d,J=7.2Hz	H10	H10
27	30.7	2.97, s		H12
28	19.2	1.04, d,J=6.9Hz	H14	

[a] Obtained from HMQC and HMBC spectra only. [b] Proton resonances that are correlated to the carbon resonance in the δ ^{13}C column.

Hemiasterlin (**10**) was isolated as an amorphous white solid that gave an (M + H) ion in the HRFABMS at m/z 527.3594 appropriate for a molecular formula of $C_{30}H_{47}N_4O_4$. Comparison of the NMR data collected on the sample of **10** obtained from *Cymbastela* sp. with the literature values[9] for hemiasterlin obtained from *H.*

minor showed that the two compounds had identical constitutions (Table 2). Hemiasterlin (**10**) isolated from *H. minor* was reported to have an $[\alpha]_D = -95°$ while the sample obtained from *Cymbastela* sp. was found to have an $[\alpha]_D = -76°$. Since the specific rotations for the two samples of hemiasterlin have the same sign and their NMR data are virtually identical, we have assumed that the two samples have the same absolute configuration and that the observed differences in magnitude of the specific rotations are the result of measurement errors.



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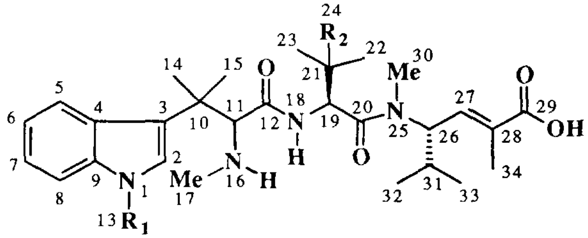


10 $R_1 = R_2 = Me$
12 $R_1 = H,\ R_2 = Me$
13 $R_1 = R_2 = H$

Hemiasterlin A (**12**) was isolated as an amorphous white solid that gave an (M + H) ion in the HRFABMS at m/z 513.3471 appropriate for a molecular formula of $C_{29}H_{44}N_4O_4$. Comparison of the ^1H, ^{13}C, COSY, HMQC and HMBC NMR data obtained for hemiasterlin A (**12**) with the data obtained for hemiasterlin (**10**) (Table 2) revealed that hemiasterlin A (**12**) differed from hemiasterlin (**10**) only by the absence of the indole N-methyl substituent. Thus, the resonance at δ 3.75 (Me13) in the ^1H NMR spectrum of **10**, assigned to the indole N-methyl substituent, was replaced in ^1H NMR spectrum of **12** by a broad resonance at δ 11.20 assigned to the indole NH. The ^1H resonance at δ 11.20 showed HMBC correlations to the ^{13}C resonances assigned to C3 (δ 117.2), C4 (δ 124.8) and C9 (δ 137.7) of the indole ring, confirming its assignment to the indole NH.

Hemiasterlin B (**13**) was isolated as an amorphous white solid that gave an (M + H) ion in the HRFABMS at m/z 499.3319 appropriate for a molecular formula of $C_{28}H_{43}N_4O_4$. Examination of the ^1H, COSY, HMQC and HMBC NMR data for hemiasterlin B (**13**) (Table 2) revealed that the molecule contained the trimethylated tryptophan and N-methyl homo vinylogous valine (MHVV)[8] residues present in hemiasterlin A (**12**). Further analysis of the NMR data showed that the tert-leucine residue in **12** had been replaced by a valine residue in **13**. Evidence in support of the valine residue was found in the COSY spectrum of **13** where a pair of methyl resonances at δ 0.88 (Me22; d, J = 6.7 Hz) and 0.92 (Me23; d, J = 6.7 Hz) were both correlated into an aliphatic methine resonance at δ 2.08 (H21; m) that was in turn correlated into an amino acid α-methine proton resonance at δ 4.62 (H19; t, J= 8.8 Hz). The HMBC spectrum contained correlations between both of the methyl resonances (δ 0.88 and 0.92) and a carbon at δ 54.8 (C19) that was also correlated into the valine α-methine proton resonance (δ 4.62: H19) in the HMQC spectrum. HMBC correlations were observed between the valine α-methine proton resonance at δ 4.62 (H19) and the valine carbonyl at δ 170.8 (C20), and between the proton resonance at δ 3.04 (Me30), assigned to the N-methyl protons of the MHVV residue, and the valine carbonyl resonance (δ 170.8: C20). The latter HMBC correlation verified that the valine carbonyl was linked to the nitrogen atom of the MHVV residue in a manner identical to the tert-leucine/ MHVV linkage in hemiasterlin (**10**).

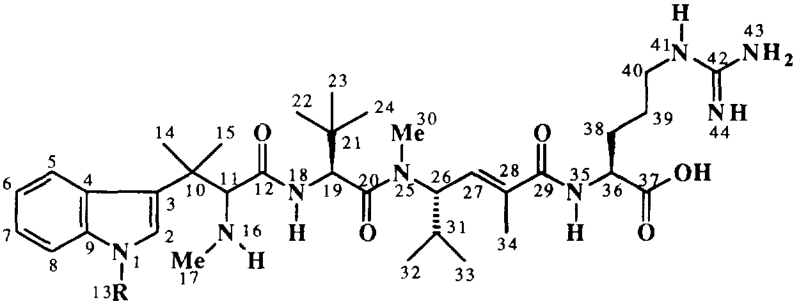
Table 2. NMR data for the hemiasterlins. Recorded in DMSO-d_6 at 500MHz.

	Hemiasterlin (10)		**Hemiasterlin A (12)**			**Hemiasterlin B (13)**		
Carbon no.	$\delta\ ^{13}C$	$\delta\ ^{1}H$	$\delta\ ^{13}C$	$\delta\ ^{1}H$	HMBC[a]	$\delta\ ^{13}C$	$\delta\ ^{1}H$	HMBC[a]
1-N				11.20,s			11.14,s	
2	128.7	7.16,s	124.4	7.15,d,J=1.5Hz		124.1	7.14,d,J=1.4Hz	
3	116.5		117.2		H1,2,14	117.0		H1,2,14,15
4	125.0		124.8		H1,2,6,8	124.5		H1,2,6,8
5	120.6	8.09,d,J=8 Hz	120.4	8.08,d,J=8 Hz	H7	120.0	8.08,d,J=8Hz	
6	118.4	7.07,t,J=8 Hz	118.4	7.02,t,J=8Hz	H8	118.3	7.03,t,J=8 Hz	
7	121.1	7.20,t,J=8 Hz	121.1	7.11,t,J=8 Hz	H5,8	121.0	7.12,t,J=8 Hz	H8
8	110.0	7.44,d,J=8 Hz	112.1	7.40,d,J=8 Hz	H6	112.0	7.41,d,J=8Hz	H6
9	137.7		137.7		H1,2,5,7	137.5		H1,2,5,7
10	37.5		37.7		H11,14,15	37.4		H11,14,15
11	67.5	4.44,d,J=6Hz	67.6	4.48,d,J=10.3Hz	H14	67.5	4.31,d,J=10Hz	H14,15
12	166.0		166.0		H11,18,19	165.4		H11,18,19
13	32.4	3.75,s						
14	27.0	1.41,s	27.2	1.42,s	H15	27.0	1.42,s	H15
15	22.5	1.38,s	22.6	1.40,s	H11,14	22.0	1.38,s	H11,14
16-N		7.38,bs		8.80,bs;7.38,bs			8.81,bs:7.35,bs	
17	33.4	2.24,s	33.6	2.23,t,J=4.6Hz	H11	33.4	2.21,t,J=4.8Hz	H11
18-N		8.87,s		8.90,bd,J=9 Hz			9.25,d,J=7.8Hz	
19	56.2	4.84,d,J=8 Hz	55.7	4.85,d,J=9 Hz	H22,23,24	54.8	4.62,t,J=8.8 Hz	H22,23
20	170.1		170.3		H19,26,30	170.8		H19,26,30
21	34.6		34.8		H22,23,24	30.0	2.08,m	H19,22,23
22	26.3	0.99,s	26.5	1.00,s	H23,24	18.4	0.88,d,J=6.7 Hz	H23
23	26.3	0.99,s	26.5	1.00,s	H22,24	18.5	0.92,d,J=6.7 Hz	H22
24	26.3	0.99,s	26.5	1.00,s	H22,23			
26	55.6	4.93,t,J=10 Hz	56.5	4.94,t,J=10 Hz	H32,33	56.5	4.91,t,J=10 Hz	H32,33
27	138.3	6.66,d,J=10 Hz	138.4	6.67,d,J=10 Hz	H26,34	137.9	6.66,d,J=9.4 Hz	H26,34
28	131.6		131.9		H26,34	131.7		H34
29	168.5		168.7		H27,34	168.5		H27,34
30	31.1	3.03,s	31.3	3.03,s	H26	30.5	3.04,s	H26
31	28.7	2.01,m	28.9	2.01,m	H32,33	28.8	2.01,m	H32,33
32	19.3	0.80,d,J=7 Hz	19.4	0.81,d,J=6.5 Hz	H33	19.2	0.81,d,J=6.3 Hz	H33
33	18.9	0.78,d,J=7 Hz	19.0	0.79,d,J=6.5 Hz	H32	18.8	0.80,d,J=6.3 Hz	H32
34	13.5	1.80,s	13.6	1.80,s	H27	13.3	1.79,s	H27

[a] Proton resonances that are correlated to the carbon resonance in the $\delta\ ^{13}C$ column.

The original structure elucidation of hemiasterlin did not provide any information concerning the absolute configurations of the component amino acids.[9] In anticipation of undertaking a total synthesis of hemiasterlin and related analogs, we have determined the absolute configurations of the two aliphatic amino acids in the sample of hemiasterlin (**10**) obtained from *Cymbastela* sp. by chemical degradation followed by Marfey's Reagent analysis. Ozonolysis followed by oxidative workup using hydrogen peroxide converted the MHVV residue in **10** to a N-methyl valine residue. Hydrolysis of the ozonolysis product with 6N HCl followed by reaction with Marfey's Reagent[11] and HPLC analysis showed that the tert-leucine and MHVV residues in **10** both had the L configurations. Attempts to determine the configuration of the tetramethylated tryptophan residue in **10** by hydrolysis or CD analysis were unsuccessful. The partial absolute configurations of hemiasterlin A (**12**) and B (**13**) were determined in a similar manner. HPLC analysis of the Marfey's reagent derivatives of the N-methyl valine, tert-leucine and valine residues liberated from hemiasterlin A (**12**) and hemiasterlin B (**13**) by ozonolysis and hydrolysis revealed that all three amino acids had the L configuration.

Criamide A (**14**) was isolated as an optically active amorphous white solid that gave an (M + H) ion in the HRFABMS at m/z 669.4454 appropriate for a molecular formula of $C_{35}H_{57}N_8O_5$. Comparison of the NMR data obtained for criamide A (Table 3) with that obtained for the hemiasterlins (Table 2) revealed that criamide A contained a tripeptide substructure (N1 to C34) that was identical to the tripeptide structure of hemiasterlin A (**12**). The remaining atoms in criamide A ($C_6H_{13}N_4O_2$) could readily be accommodated by an arginine residue. Consistent with this assignment was the observation of a resonance at δ 156.7 in the ^{13}C NMR spectrum of criamide A that could be assigned to the guanidine functionality and COSY correlations that defined the spin system extending from the arginine α-amino proton (NH-35: δ 8.02) to the NH-41 (δ 7.53) proton. Confirmation of the presence of the arginine residue was obtained by acid hydrolysis of criamide A followed by reaction of the hydrolysate with 2,4-pentanedione to convert the arginine into its dimethylpyrimidine derivative. The arginine dimethylpyrimidine derivative was further treated with pentaflouropropionyl anhydride and isopropyl alcohol/acid to give a volatile derivative for GC analysis. Comparison of the profile of the derivatized criamide A hydrolysate on a chiral GC column with the GC retention times for the corresponding derivatives of authentic L and D arginine confirmed the presence of arginine in criamide A and showed that it had the L configuration. HMBC correlations observed between the NH-35 (δ 8.02), H27 (δ 6.30) and H34 (δ 1.82) resonances and the C29 (δ 168.5) resonance (Table 3) confirmed the peptide linkage between the arginine α-amino group and the carboxyl group of the MHVV residue as shown in **14**. We have assumed that the trimethylatedtryptophan, tert-leucine and MHVV residues in



14 R= H
15 R= Me

14 have the L configurations found in hemiasterlin A (**12**).

Criamide B (**15**) was isolated as an amorphous white solid that gave an (M + H) ion in the HRFABMS at m/z 683.4597 appropriate for a molecular formula of $C_{36}H_{59}O_5N_5$. Comparison of the NMR data obtained for criamide B (**15**) with that recorded for criamide A (**14**) (Table 3) showed that criamide B differed from criamide A only by the presence of an indole N-methyl substituent (δ 3.75, s: Me13).

Table 3. NMR data for the criamides. Recorded in DMSO-d_6 at 500MHz.

	Criamide A (14)				**Criamide B (15)**	
Carbon no.	δ ^{13}C	δ ^{1}H	COSY	HMBC[a]	δ^{1}H	COSY
1-N		11.14,s				
2	124.0	7.16,d,J=2.3Hz			7.17,s	
3	117.2			H1,2,14,15		
4	124.5			H1,2,6,8		
5	120.0	8.08,d,J=7.8Hz	H6	H7	8.10,d,J=7.9Hz	H6
6	118.1	7.03,t,J=7.8Hz	H5,7	H8	7.08,t,J=7.9Hz	H5,7
7	120.9	7.12,t,J=7.8Hz	H6,8	H5	7.21,t,J=7.9Hz	H6,8
8	111.7	7.41,d,J=7.8Hz	H7	H6	7.46,d,J=7.9Hz	H7
9	137.3			H2,5,7		
10	37.4			H14,15		
11	67.5	4.45,d,J=9.0Hz	H16	H14,15	4.44,d,J=9.2Hz	H16
12	165.8			H19		
13					3.75,s	
14	26.9	1.42,s		H15	1.42,s	
15	22.4	1.38,s		H14	1.38,s	
16-N		7.30,bs	H16',11,17		7.35,bs	H16',11,17
16-N'		8.77,bs	H16,17		8.84,bs	H16,17
17	33.4	2.23,t,J=4.8Hz	H16,16'		2.24,bs	H16,16'
18-N		8.86,d,J=9.2Hz	H19		8.87,d,J=6.1Hz	H19
19	55.3	4.88,d,J=8.2Hz	H18	H22,23,24	4.88,d,J=8.5Hz	H18,H21
20	169.8			H19,26,30		
21	34.6			H22,23,24		
22	26.5	1.00,s		H19,23,24	1.00,s	
23	26.5	1.00,s		H19,22,24	1.00,s	
24	26.5	1.00,s		H19,22,23	1.00,s	
26	55.6	4.98,t,J=9.8Hz	H27,31	H30,32,33	4.98,t,J=9.8Hz	H27,31
27	132.4	6.30,d,J=9.0Hz	H26	H26,34	6.30,d,J=8.5Hz	H26
28	134.8			H26,34		
29	168.5			H27,34		
30	30.6	3.04,s		H26	3.01,s	
31	28.9	2.01,m	H26,32,33	H32,33	1.94,m	H26,32,33
32	19.2	0.83,d,J=6.5Hz	H31	H33	0.83,d,J=7.7Hz	H31
33	18.7	0.78,d,J=6.5Hz	H31	H32	0.77,d,J=7.7Hz	H31
34	13.8	1.82,s		H27	1.82,s	
35-N		8.02,d,J=7.6Hz	H36		8.03,d,J=7.6Hz	H36
36	51.7	4.27,m	H35,38,38'		4.26, dd,J=4.0,9.0Hz	H35,38,38'
37	-*					
38	27.7	1.81,m	H36,38'		1.79,m	H36,38'
38'		1.67,m	H38,39		1.69,m	H38,39
39	25.2	1.51,m	H38',40		1.49,m	H38',40
40	40.2	3.10,dd, J=5.9,7.1Hz	H39,41		3.09,dd,J=6.0,7.0Hz	H39,41
41-N		7.53,t,J=5.9Hz	H40		7.59,t,J=5.0Hz	H40
42	156.7					

[a] Proton resonances that are correlated to the carbon resonance in the δ ^{13}C column. *Not observed due to small sample size.

Cymbastela sp. is the third sponge, along with *Auletta c.f. constricta*[8] and *Hemiasterella minor*,[9] that has been found to contain members of both the jaspamide/geodiamolide and hemiasterlin/criamide families of cytotoxic peptides. Geodiamolide G (**11**) represents the first member of the jaspamide/geodiamolide family with a modification in the polyketide fragment. Comparison of the cytotoxicities of jaspamide (**1**)[8] and the previously reported geodiamolides A (**2**) to F (**7**)[6,7] and TA (**9**)[9] shows that significant variation in the three amino acid residues causes only minor changes in the levels of cytotoxicity exhibited by the compounds. By contrast, geodiamolide G (**11**) (*in vitro* human glioblastoma/astrocytoma U373, ED_{50} 7.7 μg/mL; *in vitro* human ovarian carcinoma HEY, ED_{50} 8.6 μg/mL), with its modified polyketide fragment, is significantly less cytotoxic than the analog geodiamolide A (**2**) (*in vitro* human glioblastoma/astrocytoma U373, ED_{50} 0.016 μg/mL; *in vitro* human ovarian carcinoma HEY, ED_{50} 0.043 μg/mL).

The hemiasterlins and criamides are potent *in vitro* and *in vivo* cytotoxins. Hemiasterlin (**10**) showed *in vitro* activity against: i) murine leukemia P388 (ED_{50} 4.57×10^{-5} μg/mL), ii) human breast cancer MCF7 (ED_{50} 0.089 μg/mL), iii) human glioblastoma/astrocytoma U373 (ED_{50} 0.012 μg/mL), and iv) human ovarian carcinoma HEY (ED_{50} 0.0014 μg/mL); hemiasterlin A (**12**) showed activity against: i) human glioblastoma/astrocytoma U373 (ED_{50} 0.0015 μg/mL) and ii) human ovarian carcinoma HEY (ED_{50} 0.0076 μg/mL); and hemiasterlin B (**13**) showed activity against murine leukemia P388 (ED_{50} 0.007 μg/mL), human breast cancer MCF7 (ED_{50} 0.066 μg/mL) and human ovarian carcinoma HEY (ED_{50} 0.016 μg/mL). In mice injected with 1×10^6 P388 leukemia cells, hemiasterlin (**10**) resulted in an increased life span of 123.4% after 5 daily doses of 0.45 μg begun 24 hours after implantation. Criamide B (**15**) showed *in vitro* activity against i) murine leukemia P388 (ED_{50} 0.0073 μg/mL), ii) human breast cancer MCF7 (ED_{50} 6.8 μg/mL), iii) human glioblastoma/astrocytoma U373 (ED_{50} 0.27 μg/mL), iv) human ovarian carcinoma HEY (ED_{50} 0.19 μg/mL), v) human colon LOVO (ED_{50} 0.15 μg/mL) and vi) human lung A549 (ED_{50} 0.29 μg/mL) cell lines .

EXPERIMENTAL

Specimens of *Cymbastela* sp. were collected by hand using SCUBA on reefs off of Madang, Papua New Guinea. Freshly collected sponge was frozen on site and transported to Vancouver over dry ice. The thawed sponge (260 g dry wt) was extracted exhaustively with a solution of CH_2Cl_2/ MeOH (1:1). Evaporation of the organic extract *in vacuo* gave an aqueous suspension. MeOH was added to give a 9:1 MeOH:H_2O solution (1L), which was extracted with hexanes (4 x 250 mL). Water was added to the MeOH solution to give a 1:1 MeOH/H_2O solution, which was extracted with $CHCl_3$ (4 x 250 mL). The combined $CHCl_3$ layers were concentrated *in vacuo* to yield an orange oil (3.5 g). Repeated size exclusion chromatography on Sephadex LH-20 eluting with MeOH yielded crude geodiamolides A (**2**) to G (**11**) and a mixture of crude hemiasterlin (**10**), hemiasterlin A (**12**) and hemiasterlin B (**13**). Pure geodiamolide G (**11**) (2 mg, 0.0007% dry wt) was obtained via isocratic reversed-phase HPLC (MeOH/H_2O 60:40). Reversed-phase isocratic HPLC (0.05% TFA:MeOH 1:1) also afforded pure hemiasterlin **10** (40 mg, 0.015 % dry wt), hemiasterlin A (**12**) (32 mg, 0.012 % dry wt), hemiasterlin B (**13**) (1 mg, 0.0004 % dry wt), criamide A (**14**) (3.3 mg, 0.0013 %dry weight) and criamide B (**15**) (3.3 mg, 0.0013 % dry weight)..

Hemiasterlin (10): white solid; $[\alpha]_D = -76°$ (c 0.07, MeOH); UV (MeOH) λ_{max} (ε) 216 (15,400), 273 nm (1,600); IR (neat) 3412, 2962, 1650, 1635 cm^{-1}; NMR data, Table 2; HRFABMS, MH^+ m/z 527.3594 ($C_{30}H_{47}O_4N_4$ ΔM -0.35 mmu).

Geodiamolide G (11): colorless glass; IR (neat) 3313, 1732, 1675, 1635 cm^{-1}; NMR data, Table 1; HREIMS, M$^+$ m/z 655.1760 ($C_{28}H_{38}N_3O_7I$ ΔM 0.6mmu).

Hemiasterlin A (12): white solid; $[\alpha]_D$ = -45° (c 0.25, MeOH); UV (MeOH) λ_{max} (ε) 218 (23,400), 280 nm (2,800); IR (neat) 3418, 2966, 1689, 1680, 1643 cm^{-1}; NMR data, Table 2; HRFABMS, MH$^+$ m/z 513.3471 ($C_{29}H_{45}O_4N_4$ ΔM 3.0 mmu).

Hemiasterlin B (13): white solid; NMR data, Table 2; HRFABMS, MH$^+$ m/z 499.3319 ($C_{28}H_{43}O_4N_4$ ΔM 3.4 mmu).

Criamide A (14): white solid; $[\alpha]_D$ = +97° (c 0.02, MeOH); NMR data, Table 3; HRFABMS, MH$^+$ m/z 669.4454 ($C_{35}H_{57}N_8O_5$ ΔM 0.74 mmu).

Criamide B (15): white solid; NMR data, Table 3; HRFABMS, MH$^+$ m/z: 683.45970 ($C_{36}H_{59}O_8N_5$ ΔM 0.6 mmu).

Ozonolysis of Hemiasterlins: Pure individual hemiasterlins were dissolved in 6 mL of MeOH and cooled to -78° C. A stream of ozone was bubbled through the solution until a deep blue color persisted. A stream of nitrogen was then bubbled through the solution to remove excess ozone and the reaction mixture was allowed to warm to room temperature. 3 mL of 30% H_2O_2 was added and the resulting solution was stirred for 40 min. Excess reagents were removed under high vacuum to yield the crude ozonolysis product.

Hydrolysis of the Ozonolysis Product of Hemiasterlins: The crude reaction product from the ozonolysis of hemiasterlins (see above) was dissolved in 1 mL of freshly distilled constant boiling HCl and the resulting solution was heated at 108° C with stirring for 16 hours in a threaded Pyrex tube sealed with a Teflon screw cap. The cooled reaction mixture was evaporated to dryness and traces of HCl were removed from the residual hydrolyzate by repeated evaporation from H_2O (3 x 3 mL).

Preparation of N-BOC-N-methyl-Valine: N-BOC-valine was selectively N-methylated using sodium hydride and methyl iodide in neat THF, without ester formation, according to the general procedure of McDermott and Benoiton.[12] The (D)- and (L)-N-BOC-N-methylvalines were obtained as clear oils after reversed-phase HPLC (70% MeOH/H_2O). The MS, ^{1}H and ^{13}C NMR data were consistent with the assigned structures.

Derivatization of Amino Acids with Marfey's Reagent and HPLC Analysis:[11] To a 1 mL vial containing 2 mmol of pure amino acid standard in 80 µl of H_2O was added 2.8 mmol of Nα-(2,4-dinitro-5-fluorophenyl)-L-alaninamide (FDAA) in 170 µL of acetone followed by 20 µL of 1N $NaHCO_3$. The mixture was heated for 1 hour at 40° C. After cooling to room temperature, 10 µL of 2N HCl was added and the resulting solution was filtered through a 4.5 µm filter and stored in the dark until HPLC analysis. To prepare FDAA derivatives of the amino acids in the hydrolyzate of the ozonolysis product of the hemiasterlins, a 90 µL aliquot containing 0.9 mg of amino acid mixture was reacted with 2.86 mmol of FDAA in 172 µL of acetone as described above. A 10 µL aliquot of the resulting mixture of FDAA derivatives was analyzed by reversed-phase HPLC. A linear gradient of (A) 9:1 triethylammonium phosphate (50 mM, pH 3.0) / MeCN and (B) MeCN, with 0% B at the start to 40% B over 60 minutes (flow rate 1 mL/min) was used to separate the FDAA derivatives, which were detected by UV at 340 nm. Each peak in the chromatographic trace was identified by comparing its retention time with that of the FDAA derivative of the pure amino acid standard and by coinjection. In all cases a peak was observed which was attributed to excess FDAA. The HPLC retention times are tabulated below:

HPLC Retention times in minutes.

	D-t-LEU	L-t-LEU	D/L-VAL	L-VAL	D-N-Me-VAL	L-N-Me-VAL
standards	61.30	55.66	L 49.19 D 55.06	49.40	59.10	55.81
hemiasterlin (10)		55.75				56.01
hemiasterlin A (12)		55.54				56.21
hemiasterlin B (13)				48.57		55.99

Gas Chromatography (GC): Capillary GC analyses were carried out on a Hewlett Packard 5880A gas chromatograph using a Chirasil Val column (0.25 mm x 50 m, film thickness: 0.16m, Alltech Associates, Deerfield, IL) and helium as a carrier gas (flow rate: 30 ml/min). The program rate for the analysis of amino acid derivatives was 90°C (4 min) to 220°C at 4°C/min, 220° for 30 min. The injector temperature was at 250°C and the detection temperature was set at 275°C. L and D arginine standards were converted to their dimethylpyrimidine derivatives prior to GC analysis. The arginines (500 ug) were treated with a mixture of H_2O (25 μl), EtOH (50 μl), triethylamine (25 μl), and acetylacetone (50 μl) at 110°C for 4 hours in a screw cap vial. The reaction mixture was evaporated to dryness under a stream of N_2. The arginine dimethylpyrimidine derivatives (500 μg) were treated with 250 μl of isopropyl acetate at 110°C for 75 minutes in a screw cap vial. The reaction mixture was evaporated under a stream of N_2, CH_2Cl_2 (250 μl) and pentafluoropropionic anhydride (PFPA) (100 μl) were added, and heated at 110°C for 15 minutes and then evaporated under N_2. The residual was dissolved in CH_2Cl_2 (200 μl) for GC analysis. Retention times: D 42.71 min, L 43.02 min.

Acid Hydrolysis of Criamide A (14) and Derivatization of the Hydrolysate: Criamide A (**14**) was hydrolyzed with 6 N HCl (500 ul) at 110°C for 22 hours. The reaction mixture was cooled to room temperature and then evaporated to dryness under a stream of N_2. The residue was converted to the dimethylpyrimidine derivative and then treated with isopropyl acetate and PFPA prior to GC analysis as described above.

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