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Hemiasterlin and Geodiamolide TA; Two New Cytotoxic Peptides from the Marine Sponge Hemiasterella Minor (Kirkpatrick)

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Abstract: Three cytotoxic peptides, Jaspamide (1) and the two new peptides hemiasterlin (2) and geodiamolide TA (3), have been isolated from the sponge *Hemiasterella minor*. The structures of the three were determined by interpretating the NMR and mass spectra. Hemiasterlin (2) is a novel linear tripeptide embodying two unique, new natural aminoacids and geodiamolide TA (3) is a higher homologe of geodiamolides A-F.

The sponge *Hemiasterella minor* (Kirkpatrick) (class, Demospongiae; order, Hadromerida; family, Hemiasterellidae) collected in Sodwana Bay, north to Durban, South Africa, contained a variety of bioactive compounds. The major metabolite in four examined specimens was found, on the basis of its spectral data, to be the earlier reported bioactive cyclic depsipeptide jaspamide (jasplkinolide) (1) isolated from *Jaspis sp.*(order, Astrophoride (Choristida))[1] (0.2%, dry wt). Two of the sponge samples studied contained minute amounts of a second peptide, hemiasterlin (2) and one specimen a third compound, geodiamolide TA (3).

Freshly collected *Hemiasterella minor* was frozen on site and kept frozen until needed. Freeze-dried sponge tissue (60g, dry wt.) was extracted sequentially with hexane, ethyl acetate and ethyl acetate/MeOH, 1:1. The latter two extracts were subsequentially partitioned between aqueous methanol (1:1, MeOH/H_2O) and chloroform and the chloroform soluble portion was fractionated by sequential application of Sephadex LH-20 (2:1:1 hexane/MeOH/$CHCl_3$) and reversed phase HPLC (MeOH/H_2O,90:10) chromatographies to give samples of jaspamide (1)[1], hemiasterlin (2) and geodiamolide TA (3) (120 mg, 6 mg and 2 mg, respectively, from one of the specimens which contained all three).

Structure elucidation of **2**[2] was begun by intensive study of spectrometric data. The molecular formula, $C_{30}H_{47}N_4O_4$ (MH^+) was established by HRFABMS ("magic bullet") and by the C and H count obtained from NMR. The ^{13}C NMR ($CDCl_3$) revealed its functional groups, as follows: (a) an indole heterocycle (δ 121.0s, 127.5d, 139.2s, 109.5d, 122.3d, 119.1d, 121.2d and 127.0s); (b) three carbonyls (δ 172.1, 172.5, 173.1); (c) a double bond (δ 131.1s, 140.1d); and (d) eleven methyls including three N-Me's, one vinyl-methyl, a *gem*-dimethyl group, one iso-propyl and one *tert.* butyl group (Table 1). According to the ten degrees of unsaturation of **2** and the above functionalities, hemiasterlin has to be linear. 2D NMR spectra, i.e. COSY, HMQC, HMBC and TOCSY experiments (Table 1) revealed the three substructures A-C containing the above functional groups). Furthermore, CH-correlations from and to the CH(19)$NHCH_3$ moiety (B) (δ_C 73.2, δ_H 3.60) suggested the latter group

to be the linkage between substructures A and C.

Unequivocal proof for the linear tripeptide structure of **2** was achieved from the MS/MS data. Mass measurement of the parent ion gave MH^+ 527.3579 (calc. 527.3579 $C_{30}H_{47}N_4O_4$). The regular FAB mass spectrum and the B/E linked scan spectrum of the protonated parent ion (see Figure) were similar and the major ions were assignable to the structure. Loss of methylamine (see "-31") dominated much of the fragmentation observed, and is first observed from the parent ion at 496. The base peak at 172 arises from the methylated tryptamine moiety and is, as expected, quite stable so no significant further fragmentation is observed below this. The complementary species was also observed at 354, but smaller as expected. Further pairs of ions were observed where the break was α to the carbonyl and β to the nitrogen. These are at 215/311 and 328 (observed as 297-31)/198. The remaining significant fragments at 356 and 387 add further proof to the structure. In all cases the ions containing the methylamine fragment lost this as a neutral fragment (387→356 [indistinguishable from the 356 fragment], 356→325 and 215→184 [this was far more predominant in the normal FAB spectrum]). The ion at 354 does not loose 31 which is expected due to the methylamine nitrogen being involved in the stabilizing of the ion[3].

Besides t-Leu earlier discovered in the discodermins[4] and before only reported as a constituent of the actinomycete peptides bottromycins[5], hemisterlin (**2**) contains two other unique aminoacids i.e. the γ-amino acid 4-amino-2,5-dimethylhex-2-enoic acid and N,N',β,β-tetramethyltryptophan.

Of special interest in the IR spectrum of **2**, was the absence of a broad 3300-2600 cm^{-1} absorption of the CO_2H group, suggesting a carboxylate ion[6]. The presence of the carboxylic group, though, was confirmed by micro-scale methylation of **2** with CH_2N_2 to afford the corresponding methyl ester (δ_C 52.1, δ_H 3.75). The above observations together with the low-field NMR signals of the C19-methine group suggested a zwiterion between the spatially close carboxylic and amino groups. Indeed, acetylation of the C19-amine (Ac_2O/Pyr., rt) brought to the discovery of the CO_2H absorption at 3300b cm^{-1} and a 12 ppm up-field shift of the C19 resonance[7]. Final proof for the suggested cyclic conformation of the C1-C19 backbone of **2** was achieved from a NOE between Me-6 and Me-31[8].

Geodiamolide TA (**3**)[9], isolated as a colorless glass (2 mg), $[\alpha]_D$, +30 (c 0.027,$CHCl_3$), gave a parent ion in the HRMS at *m/z* 670.2335 (MH^+) corresponding to a molecular formula of $C_{30}H_{44}N_3O_6I$. Fragmentation of the structure is summarized in the Figure. The parent ion (I:X=I) dehalogenated under the FAB conditions to yield I:X=H at *m/z* 544.3379 (24%, calc. 544.3387 $C_{30}H_{46}N_3O_6$). Further fragmentation was observed from both species: they gave ions with structure II at *m/z* 421.0638 (17%, calc. 421.0625 $C_{15}H_{22}N_2O_4I$) and *m/z* 295.1667 (5%, calc. 295.1658 $C_{15}H_{23}N_2O_4$); and ions with structure III at *m/z* 275.9876 (31%, calc. 275.9886 $C_9H_{11}NOI$) and *m/z* 150 (12%, $C_9H_{12}NO$) (respectively X=I, X=H)[10]. Resonances in the ^1H and ^{13}C NMR spectra of **3** (Table 2) could be assigned to a 12-carbon polypropionate unit (C7 to C14 with the attached methyls) identical to the one found in geodiamolides A to F[11]. Additional NMR data could be assigned to an N-methyliodotyrosine residue (with a characteristic δ_C value of 86 ppm for the iodine bearing C-atom), one alanine and one valine residue, by comparison of their chemical shifts and coupling constants to those observed for geodiamolides A to F[11] and motuporin[12](for the Val residue). 2D NMR spectra, i.e. COSY, HMQC, HMBC and TOCSY established unequivocally the complete structure of **3**, that is, the Ala residue in geodiamolide D[9] being replaced by a Val residue in geodiamolide TA. The similarity in the NMR data of **3** and the geodiamolides implied that the chiral centers in geodiamolide TA had the same relative configurations as in geodiamolides A to F[11].

Table 1. NMR Data of hemiasterlin (2) in $CDCl_3$ (500 MHz)

No.	^{13}C,ppm	^{1}H, ppm (mult;J(Hz))	HMBC (to C#)	COSY (to H#)	No.	^{13}C,ppm	^{1}H, ppm (mult;J(Hz))	HMBC (to C#)	COSY (to H#)
1	172.1s				18	173.1s			
2	131.1s				19	73.2d	3.60(s)	33,32,31,21,20,18	
3	140.1d	6.73(d,9.8)	6,1	4	20	38.4s			
4	56.3d	5.11(t,9.8)	11,10,9, 8,7,3,2	7,3	21	121.0s			
6	14.6q	1.90(s)	3,2,1		22	127.0s			
7	30.3q	1.86(m)		9,8,4	23	121.2d	7.90(d,8.3)	27,25,22	24
8	19.5q	0.79(d,6.0)	9,7,4	7	24	119.1d	7.08(t,7.5)	26,22	25,23
9	19.0q	0.86(d,6.0)	8,7,4	7	25	122.3d	7.22(t,7.5)	27,23	26,24
10	31.4q	3.06(s)	11,4		26	109.5d	7.29(d,8.3)	24,22	25
11	172.5s				27	139.2s			
12	55.2d	4.88(d,9.8)	17,16,15 14,11	NH(13)	29	127.5d	6.86(s)	34,27,26,22, 21,20	
14	35.5s				31	36.0q	2.00(s)	19	
15	27.2q	1.00(s)	17,16,14,12		32	23.5q	1.44(s)	33,29,21, 20, 19	33
16	27.2q	1.00(s)	17,15,14,12		33	28.2q	1.60(s)	32,21,20,19	32
17	27.2q	1.00(s)	16,15,14,12		34	33.1q	3.75(s)	29, 27	
					NH(13)		7.90(d,8.3)	18,15	12

Table 2. NMR Data of geodiamolide TA (3) in $CDCl_3$ (500 MHz)

No.	^{13}C,ppm	^{1}H, ppm (mult;J(Hz))	HMBC (to C#)	COSY (to H#)	No.	^{13}C ppm	^{1}H, ppm (mult;J(Hz))	HMBC (to C#)	COSY (to H#).
1	170.0s				16	17.8q	0.75(d,6.5)	2,15,17	15,17
2	58.6d	4.25(dd,6,9)	1,15	15,NH(2)	17	18.7q	0.77(d,6.5)	2,15,16	15,16
3	169.5s				18	32.2t	2.82(dd,14.5,8)	3,4,20,24	4,18′
4	57.2d	5.18(t,7)	3,5,19	18,18′,20	18′		3.08(dd,14.5,8)	3,4,20,24	4,18
5	174.7s				19	130.5s			
6	46.8d	4.67(quin,6.5)	7,25	25,NH(6)	20	138.8d	7.42(d,2)	18,22,24	4,24
7	175.2s				21	86.2s			
8	42.1d	2.23(m)		9,9′,26	22	154.5s			
9	42.5t	1.98(m)	7,8,10,11,26	8,9′	23	115.1d	6.82(d,8)	19,21,22	24
9′		2.06(m)	7,8,10,11,26	8,9	24	131.0d	7.01(d,8)	18,20,22	20,23
10	133.5s				25	18.8q	1.04(d,6.5)	5,6	6
11	132.0d	4.86(d,8.5)	9,27	12	26	18.5q	1.09(d,6.5)	7,8,9	8
12	29.0d	2.06(m)	10,11	11,13,13′ 28	27	17.7q	1.46(s)	9,11	
13	43.6t	1.30(m)	12,14,28,29	12,13′,14	28	20.5q	0.79(d,6.5)	11,13	12
13′		1.60(m)	12,14,28,29	12,13 ,14	29	20.6q	1.16(d,6.5)	13,14	14
14	71.7d	4.78(sext,7)		13,13′,29	30	30.5q	2.97(s)	4,5	
15	32.0d	1.98(m)		2,16,17	NH(2)		6.32(d,6.5)	3	6
					NH(6)		6.44(d,6.5)	7	2

The very small amounts of 2 and 3 excluded a degradation of the molecules for absolute configuration assignments.

All three peptides (1-3) showed cytotoxicities against several cell lines; e.g. in concentration of ca.0.01 µg/mL against P388 cells, however, as 2 and 3 might have contained impurities of 1, the assays will have to be repeated when additional amounts of 2 and 3 will be available. Isolation of compounds 1 and 3 from taxonomically remote species (orders *Choristida, Axinellida* and now *Hadromerida*) together with the presence of the t-Leu residue in 2, *vide supra*, suggests, as already mentioned earlier[12], an involvement of symbionts in the production of peptide 1 and 3.

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References and Notes

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2. Glassy oil, $[\alpha]_D$ = -95° (c 0.06, MeOH), IR (neat) 2965-2929, 1664, 1655, 1648, 1630, 1619 cm^{-1}.

3. Linked scan analysis of the fragment ion at 325 (methylamine lost) yielded 297 from loss of CO and the ions at 172 and 184 (184=215-31) while 297 itself gave 184 and a pair of complementary ions at 212 (243-31) and 86 (fragmentation of the amide bond). The ion at 215 reluctantly fragmented to 184 due to the involvement of the nitrogen lone pairs in the 215 ion. Confirmation of the above assignments and hence the structure came from measurements of their accurate masses: meas. 387.2769 (calc. 387.2760 $C_{22}H_{35}N_4O_2$), meas. 325.1924 (calc. 325.1916 $C_{20}H_{25}N_2O_2$) meas. 297.1978 (calc. 297.1967 $C_{19}H_{25}N_2O$), meas. 215.1540 (calc. 215.1548 $C_{14}H_{19}N_2$) and meas. 172.1118 (calc. 172.1126 $C_{12}H_{14}N_2$).

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7. Glassy oil, IR(neat)3363b, 1654, 1648, 1638 cm^{-1}, ^1H NMR 2.26(s, NAc). Me's: 1.93(s), 0.91(d,6), 0.90(d,6), 2.99 (s, NMe), 0.47(s,tBu), 1.45(s). 1.63(s), 3.79(s, NMe); HRMS *m/z* 569.3725 (calc. 569.3703, $C_{32}H_{49}N_4O_3$), 369,398 (354 ,356 + Ac), 285, 172.

8. Other observed NOE's (d_6-DMSO) for 2 are between H-10/12,3; tBu/12, 32, 33; H-19/NH(13), 23, 31.

9. Colorless oil, IR(neat) 3420, 1720, 1670, 1655, 1630 cm^{-1}.

10. Verification of this fragmentation was performed by B/E linked scan analyses which also verified that the *m/z* 421 (II, X=I) fragmented to give *m/z* 276 (III, X=H). Additionally, this revealed a pair of fragmentations from the protonated parent ion and dehalogenated species (I, X=I and X=H) at (a) (571, 445 respectively) and (b) (553, 427 respectively) which further identified the valine. The FAB spectrum can be compared to that previously reported geodiamolide A^{11a} which had an alanine in place of the valine (*m/z* 670:642, 544:516, 421:393, 276:276, 150:150 for geodiamolide TA compared to geodiamolide A).

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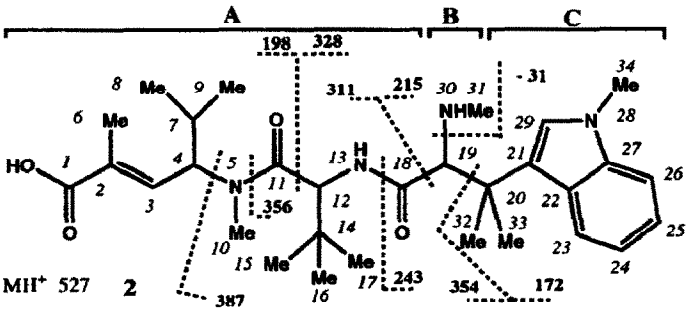

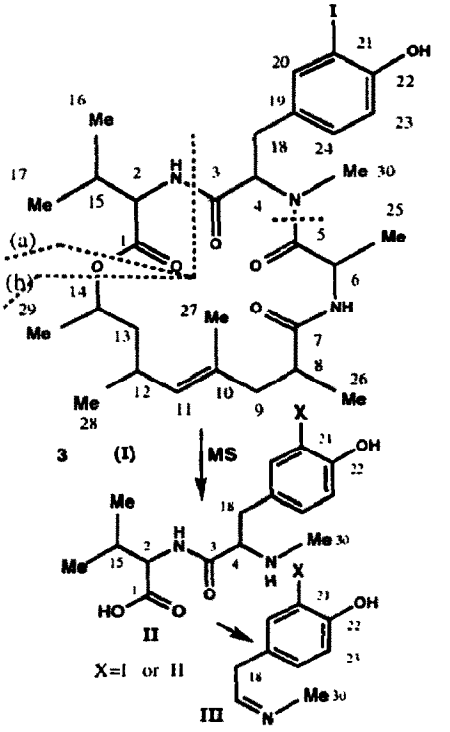

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