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NOVEL MARINE SPONGE DERIVED AMINO ACIDS 7. THE FENESTINS

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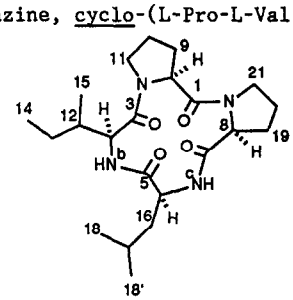
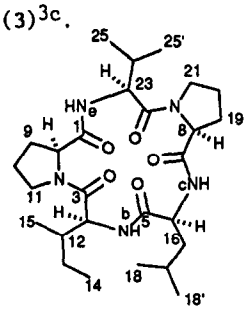
Abstract: Cyclic peptides, fenestins A (1), B (2) and a known diketopiperazine, cyclo-(L-Pro-L-Val) (3) are reported from the sponge Leucophloeus fenestrata. Fenestins A and B are respectively cyclo-(L-Pro-L-Pro-L-Leu-L-Ile) and cyclo-(L-Pro-L-Val-L-Pro-L-Leu-L-Ile).

A majority of the amino acids isolated from marine sponges seemingly arise from tyrosine or tryptophan biosynthesis[1]. Examples are respectively psammaplin A from Psammaplysilla sp.[2] and 5-bromo-N,N-dimethyltryptamine from Smenospongia sp..[3a] Much of the other sponge amino acid chemistry falls into three categories: simple derivatives, such as the dysidenin dipeptides from Dysidea herbacea[3b], the cyclic dipeptides from Tedania ignis[3c], or the cyclic peptide barettin from Geodia baretti[4]; mixed ketide-amino acids, such as jasplakinolide[5a] (jaspamide[5b,c]) from Jaspis sp, the bengamides[5d] from an undescribed Jaspidae sponge, or mycothiazole[5e] from Spongia mycofijiensis; and complex macrocyclic peptides, such as the discodermins from Discodermia Kiiensis[6a], or theonellapeptolide 1D[6b] and theonellamine B[6c] from Theonella sp.. We now expand this list by describing the first examples of medium ring cyclic polypeptides, the fenestins.

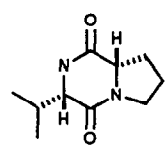
The Fiji collection of Leucophloeus fenestrata[7] (#87096, 3.80 Kg wet) was repeatedly extracted with methanol to yield a dark viscous oil (22.46 g). Solvent partition of the oil (aqueous MeOH against hexane, CCl_4 and CH_2Cl_2) afforded non-polar fractions, hexane (17.35 g) and CCl_4 (1.54 g), and a polar CH_2Cl_2 fraction (1.14 g). The portion of the oil which partitioned into the hexane and CCl_4 layers was rich in known amorphane sesquiterpenes possessing isocyanide, isothiocyanate or formamide groups[8]. Alternatively, purification of the CH_2Cl_2 fraction by flash chromatography on silica gel (Aldrich, grade 60, 60A), followed by repeated reversed phase HPLC [ODS, 25 x 1.0 cm; MeOH:H_2O, 80:20 (v/v)], afforded pure fenestins A (1), B (2) (21 mg and 36 mg, respectively) and a known diketopiperazine, cyclo-(L-Pro-L-Val) (3)[3c].



fenestin A (1)



fenestin B (2)



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Structure elucidations of **1** and **2** were begun by analyses of the NMR spectra (^{1}H, ^{13}C and 2D COSY NMR). A significant similarity was noted in the ^{1}H and ^{13}C NMR data of the two compounds (Table I). However, several NMR resonances present in **2** [^{13}C ($CDCl_3$) δ's at 60.5d, 28.9d, 19.2q and 16.2q; ^{1}H ($CDCl_3$) δ's at 3.90brs (1H), 2.59dh (1H), 1.06d (3H) and 0.88d (3H)] were absent in **1**. The ^{13}C NMR spectra also showed five amide carbonyls (170.5s, 170.5s, 170.4s, 166.4s and 165.1s) in **2**, but only four in **1** (170.6s, 170.6s, 166.5s and 166.3s). These data together with the occurrence of two amide (CONH) protons in **1** [δ's(C_6D_6) 7.76 and 7.66 ppm], but three such protons in **2** [δ's(C_6D_6) 7.83, 7.78 and 7.72 ppm], led to the assumption that both compounds were cyclic polypeptides which differed in a single amino acid moiety. Specific chemical shift assignments could be made from the various 2D homo and hetero COSY NMR data of both **1** and **2** which revealed the individual amino acids as valine, leucine, isoleucine and proline. Valine was not present in **1**, while two prolyl residues were present in both. This was verified by MS data which gave the molecular formula for **1** and **2** respectively as $C_{24}H_{36}N_4O_4$ (MW 420) and $C_{27}H_{45}N_5O_5$ (MW 519). The amino acid sequences in both compounds were established using the MS fragmentation patterns and the long range homo COSY NMR data. LRCIMS (isobutane) of **1** revealed fragments at m/z 421 (M+H), m/z 227 ($C_{12}H_{23}N_2O_2$), implying a Leu··Ile fragment, m/z 211 ($C_{11}H_{19}N_2O_2$), implying a Pro··Leu or a Pro··Ile fragment and m/z 195 ($C_{10}H_{15}N_2O_2$), indicating a Pro-Pro array. Long range COSY NMR correlations from a Pro-H_α(H-8) to Leu-H_α(H-6), and from an amide proton (NH-c) to H-6 and H-8 revealed the Pro-Leu. The combination of these data confirmed the structure of fenestin A (**1**) as cyclo-(Pro-Pro-Leu-Ile).

Determination of amino acid sequence in **2** was accomplised using the same strategies. LRCIMS (isobutane) and LRFABMS both yielded, among others, the fragments at m/z 421 ($C_{24}H_{37}N_4O_4$), m/z 407 ($C_{23}H_{34}N_4O_4$), m/z 227 ($C_{12}H_{23}N_2O_2$), m/z 211 ($C_{11}H_{19}N_2O_2$) and m/z 197 ($C_{10}H_{16}N_2O_2$), which respectively, were assigned to fragments containing amino acids Pro··Pro··Leu··Ile, Pro··Pro··Val··Leu/Ile, Leu-Ile, Pro··Leu/Ile and Pro-Val. Important long range 2D COSY NMR peaks visible in benzene-d_6 and DMSO-d_6 were from Leu-H_α(H-6) to a Pro-H_α(H-8), and from an amide proton (NH-c) to H-6 and H-8. These data firmly established the existence of the Pro-Leu group, also present in the structure of **1**, and the expanded subunit Pro-Leu-Ile was proposed based on the m/z 227 MS fragment assigned above. The sequence Ile-Pro-Val was deduced from long range COSY correlations from a Pro-H_α(H-2) to Ile-H_α(H-4) and also to Val-H_α(H-23). These long range COSY results required the proline to be placed between the isoleucine and the valine residues. This is in agreement with the above MS fragments at m/z 211 (Ile-Pro + H) and m/z 197 (Pro-Val + H). Thus, the structure of fenestin B (**2**) could be assigned as cyclo-(Pro-Val-Pro-Leu-Ile).

The all L-absolute configuration of both cyclic peptides was determined by a derivatization method using the chiral reagent 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate (GITC)[9]. Fenestin B was first completely hydrolysed in 6N HCl (100° C, 5-7 hrs.), and the concentrated hydrolysate was then reacted with GITC according to the literature[9]. The mixture was eluted through an analytical reversed phase HPLC column (Alltech Spherisorb 5 μm ODS, 25 cm x 4.6 mm; 65% 0.05 M NaH_2PO_4 pH 3 & 35% acetonitrile). Retention times of elution peaks were compared with those of standard D- or L- amino acid GITC derivatives. The HPLC analysis of the hydrolysate revealed a ratio of L/D amino acids

Table I. ^{13}C and 1H NMR Data of 1 and 2 (δ in ppm from TMS, 300/75 MHz)

Atom #	$\delta^{13}C$ (APT mult.) 1 ($CDCl_3$)	$\delta^{13}C$ (APT mult.) 2 ($CDCl_3$)	$\delta^{1}H$ (mult., J(Hz); # of H's) 1 ($CDCl_3$)	1 (C_6D_6)	2 ($CDCl_3$)	2 (C_6D_6)
1	166.3 (s)	165.1 (s)				
2	58.8 (d)	58.9 (d)	4.07 (dd,J=7.8;1H)	3.32 (dd, J=7.8;1H)	4.05 (dd, J=7.8;1H)	3.40 (dd, J=7.8;1H)
3	170.6 (s)	170.5 (s)				
4	60.6 (d)	60.5 (d)	3.95 (br s; 1H)	3.52 (br s; 1H)	3.94 (br s; 1H)	3.58 (br s; 1H)
5	170.6 (s)	170.5 (s)				
6	53.5 (d)	53.5 (d)	4.00 (dd,J=9.6,3.3;1H)	3.60 (dd,J=8.7,5.2;1H)	3.98 (dd,J=9.3,3.6;1H)	3.66 (dd,J=8.7,5.1;1H
7	166.5 (s)	166.4 (s)				
8	59.1 (d)	59.0 (d)	4.10 (dd,J=7.8;1H)	3.32 (dd,J=7.8;1H)	4.09 (dd,J=7.2;1H)	3.36 (dd,J=7.2;1H)
9	28.2 (t)	28.2 (t)	2.15 (c m; 2H)	1.80 (c m; 2H)	2.20 (c m; 2H)	1.80 (c m; 2H)
10	22.4 (t)	22.4 (t)	1.95 (c m; 2H)	1.15 (c m; 2H)	1.95 (c m; 2H)	1.12 (c m; 2H)
11	45.2 (t)	45.2 (t)	3.55 (c m; 2H)	3.26 (c m; 1H) 3.08 (ddd,J=12.3,8.7, 3.9;1H)	3.55 (c m; 2H)	3.32 (c m; 2H) 3.15 (ddd,J=12.3,8.7, 3.9;1H
12	35.4 (d)	35.4 (d)	2.31 (c m; 1H)	2.35 (c m; 1H)	2.30 (c m; 1H)	2.38 (c m; 1H)
13	24.1 (t)	24.1 (t)	1.40 & 1.20 (c m;2H)	1.64 & 1.37 (c m;2H)	1.40 & 1.20 (c m;2H)	1.65 & 1.20 (c m;2H)
14	12.2 (q)	12.2 (q)	0.90 (t,J=7.5; 3H)	0.91 (t,J=7.5; 3H)	0.91 (t,J=7.2; 3H)	0.90 (t,J=7.5; 3H)
15	15.9 (q)	15.8 (q)	1.05 (d,J=7.2; 3H)	1.08 (d,J=7.2; 3H)	1.04 (d,J=7.2; 3H)	1.10 (d,J=7.2; 3H)
16	38.6 (t)	38.6 (t)	2.00 (c m; 1H) 1.52 (ddd,J=14.4,9.6, 5.1; 1H)	2.19 (ddd,J=13.8,9.3, 4.5; 1H) 1.54 (ddd,J=13.8,8.4, 4.5;1H)	2.00 (c m; 1H) 1.51 (ddd,J=14.4,9.6, 5.1; 1H)	2.22 (ddd,J=13.8,9.3 5.1; 1H 1.60 (c m; 1H)
17	24.7 (d)	24.7 (d)	1.78 (c m; 1H)	1.86 (c m; 1H)	1.78 (c m; 1H)	1.85 (c m; 1H)
18	21.3 (q)	21.4 (q)	0.93 (d, J=6.6; 3H)	0.85 (d, J=6.6; 3H)	0.93 (d, J=6.3; 3H)	0.85 (d, J=6.3; 3H)
18'	23.3 (q)	23.5 (q)	0.98 (d, J=6.6; 3H)	0.91 (d, J=6.6; 3H)	0.93 (d, J=6.3; 3H)	0.90 (d, J=6.3; 3H)
19	28.6 (t)	28.6 (t)	2.15 (c m; 2H)	1.80 (c m; 2H)	2.15 (c m; 2H)	1.80 (c m; 2H)
20	22.8 (t)	22.8 (t)	1.90 (c m; 2H)	1.15 (c m; 2H)	1.95 (c m; 2H)	1.12 (c m; 2H)
21	45.6 (t)	45.5 (t)	3.55 (c m; 2H)	3.26 (c m; 1H) 3.08 (ddd,J=12.3,8.7, 3.9;1H)	3.55 (c m; 2H)	3.32 (c m; 1H) 3.15 (ddd,J=12.3,8.7, 3.9;1H
22		170.4 (s)			-	
23		60.5 (d)			3.90 (br s; 1H)	3.51 (br s; 1H)
24		28.9 (d)			2.59 (dh,J=7.2,2.7;1H)	2.70 (dh,J=7.5,3.0;1H
25		16.2 (q)			0.88 (d, J=6.3; 3H)	1.07 (d, J=6.9; 3H)
25'		19.2 (q)			1.06 (d, J=6.3; 3H)	1.07 (d, J=6.9; 3H)
NH-b			6.52 (br s; N-H)	7.76 (br s; N-H)	6.56 (br s; N-H)	7.78 (br s; N-H)
NH-c			6.52 (br s; N-H)	7.66 (br s; N-H)	6.60 (br s; N-H)	7.72 (br s; N-H)
NH-e					6.56 (br s; N-H)	7.83 (br s; N-H)

of approximately 10:1; therefore, we concluded that 2 contain all L-amino acids. It was assumed, on biogenetic grounds, that 1 also contained all L-amino acids.

We have investigated three collections of L. fenestrata from two geographical distinct areas, Fiji and Thailand. The fenestins were observed only in one of two Fiji collections which both contained overlapping amorphane sesquiterpenes. A collection from Thailand contained different isonitrile sesquiterpenoids, including axisonitrile-3. There is reason to suspect that the fenestins are a product of sponge symbiotic microorganisms. Simple diketopiperazines have been reported from terrestrial microorganisms[10], while medium ring cyclopeptides have also been observed from terrestrial microorganisms[10,11] as well as from marine dinoflagellates[12]. No activity was shown by either fenestins at 20 μg/mL (in vitro) against P388 or HT-29 (respectively, murine lymphoma and human colon tumor cells).

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