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# PSEUDOAXINELLIN, A CYCLIC HEPTAPEPTIDE ISOLATED FROM THE PAPUA NEW GUINEA SPONGE *PSEUDOAXINELLA MASSA*

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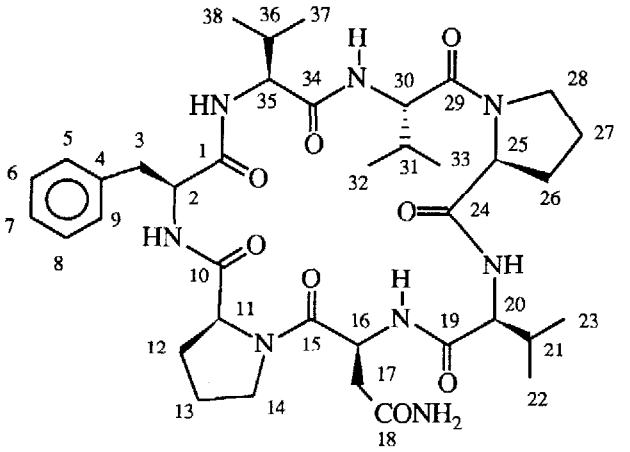
*Abstract: Pseudoaxinellin (1), a new cyclic heptapetide, has been isolated from the marine sponge Pseudoaxinella massa collected in Papua New Guinea. The structure of 1 was solved by spectroscopic analysis and chemical degradation. Pseudoaxinellin is the first cyclic heptapeptide reported from a marine source. One of the amide bonds in 1 is cis.*

Cyclic peptides and cyclic depsipeptides are rapidly emerging as an important class of metabolites present in extracts of marine sponges.[1,2] Motuporin,[1a] orbiculamide A,[1b] keramides B-D,[1c] the theonellapeptolides,[1d] cyclotheonamides A and B,[1e] and theonellamine B[1f] have all been isolated from sponges belonging to the genus *Theonella*. The cyclic depsipeptides discodermins A to D[2a] were isolated from the sponge *Discodermia kiiensis*, the cyclic tetra- and pentapeptides fenestins A and B[2b] were isolated from *Leucophloeus fenestrata*, the cyclic octapeptide hymeninstatin 1 was isolated from *Hymeniacidon* sp.,[2c] and the cyclic depsipeptides jaspamide[2d] and geodiamolides A to G[2e] were isolated from *Jaspis*, *Geodia* and *Pseudaxinyssa* sp. Many of the sponge peptides and depsipeptides contain unusual new amino acids and the majority are potently bioactive. As part of our ongoing search for biologically active metabolites in extracts of marine sponges collected off Papua New Guinea,[1a,3] it was found that crude methanol extracts of *Pseudoaxinella massa*[4] exhibited considerable antibacterial (*Staphylococcus aureus*) and cytotoxic (murine leukemia P388) activity. Bioassay-guided fractionation revealed that all of the the active constituents were water soluble.[5] As part of this investigation, a routine examination of the dichloromethane soluble materials from *Pseudoaxinella massa* by TLC and $^1$H nmr resulted in the discovery of a new cyclic heptapeptide, pseudoaxinellin (1), whose structure is reported in this communication.

Specimens of *P. massa* were collected by hand using SCUBA on nearshore reefs off of Motupore Island, Papua New Guinea. The freshly collected sponge was frozen on site and transfered to UBC over dry ice. Frozen sponge was homogenized with methanol and extracted at room temperature for two days. The methanolic filtrate obtained from the homogenate was evaporated in vacuo to give a residue that was suspended in water and sequentially extracted with hexanes, dichloromethane and ethyl acetate. $^1$H nmr analysis of the individual crude organic extracts indicated that the dichloromethane soluble portion contained a moderately complex peptide.

Purification of the dichloromethane soluble materials by sequential application of Sephadex LH20 (eluent: hexanes/$CHCl_3$/MeOH 10:10:1), silica-gel flash column (eluent: ethyl acetate to MeOH, step gradient) and silica-gel preparative thin layer (eluent: ethyl acetate/MeOH 4:1) chromatographies gave pure pseudoaxinellin (**1**) as an optically active ($[\alpha]_D$ -100.1°, c 0.34, $CHCl_3$) clear glass.

Pseudoaxinellin (**1**) gave a parent ion in the EIHRMS at m/z 752.4218 appropriate for a molecular formula of $C_{38}H_{56}N_8O_8$ (ΔM -0.3mmu). The $^1H$ and $^{13}C$ nmr spectra of **1** (Table 1) contained resonances that were characteristic of peptides. A detailed analysis of the COSY and HOHAHA data recorded in $CDCl_3$ for pseudoaxinellin (**1**) showed that it contained one phenylalanine, one asparagine, two proline and three valine residues (Table 1). HMQC and HMBC data were used to assign the carbon resonances to the individual amino acids in the peptide. The molecular formula of pseudoaxinellin required fifteen sites of unsaturation. Since only fourteen of these sites of unsaturation could be accounted for by functionality present in the seven individual amino acids, it was apparent that **1** was a cyclic peptide. The failure of **1** to react with ninhydrin was consistent with a cyclic structure.




**Pseudoaxinellin (1)**

The amino acid sequence present in pseudoaxinellin (**1**) was determined from an analysis of HMBC, ROESY and difference nOe data. There were four HMBC cross peaks, viz., Phe-NH (δ 7.55) / $Pro_1$-CO (δ 170.31); $Val_3$-NH (δ 7.32) / Phe-CO (172.13); $Val_1$-NH (δ 8.22) / $Pro_2$-CO (δ171.5) and Asn-NH (δ 8.01) / $Val_1$-CO (δ172.26) that established the partial sequences $Pro_1$-Phe-$Val_3$ and $Pro_2$-$Val_1$-Asn. Difference nOes and ROESY correlations were observed between a $Pro_1$-δH (δ 3.58) and the Asn-αH (δ4.61), and between the $Val_3$-αH (δ 4.21) and the $Val_2$-NH (δ 6.80), completing the linear sequence $Pro_2$-$Val_1$-Asn-$Pro_1$-Phe-$Val_3$-$Val_2$. Joining the $Val_2$ and $Pro_2$ residues to generate the macrocyclic ring gave structure **1** for pseudoaxinellin. A long range COSY correlation between the $Val_1$-αH (δ4.05) and the $Pro_2$-αH (δ4.53) supported the $Pro_2$-$Val_1$ linkage. An intense ROESY correlation and a strong nOe (8.5%) were observed between the $Pro_2$-αH (δ 4.53) and the $Val_2$-αH (δ 4.16) resonances providing direct evidence for the $Val_2$-$Pro_2$ connectivity and demonstrating that the $Val_2$-CO / $Pro_2$-N amide bond had the cis geometry. Hydrolysis of pseudoaxinellin (**1**) with 6N HCl,

Table 1. $^{1}H$ and $^{13}C$ NMR data (recorded in $CDCl_3$) for pseudoaxinellin (1).

| C# | $^{1}H$ (400 MHz) | COSY | nOes[a] | $^{13}C$ (125 MHz)[b] | HMBC[c] |
|---|---|---|---|---|---|
| Phe | | | | | |
| 1 | - | | | 172.13 | NH(7.32) |
| 2 | 4.77,m | H3,H3',NH(7.55) | H5,H9 | 55.4 | H3 |
| 3 | 2.94 | H2,H3' | | 37.7 | |
| 3' | 3.23 | H2,H3 | | | |
| 4 | - | | | 137.5 | H6,H8,H3,H3' |
| 5,9 | 7.17 | | | 128.9 | H3,H3',H7 |
| 6,8 | 7.24 | | | 128.3 | H8,H6 |
| 7 | 7.18 | | | 126.6 | H5,H9 |
| NH | 7.55, d(J=9.5 Hz) | H2 | | | |
| $Pro_1$ | | | | | |
| 10 | - | | | 170.31 | H11,NH(7.55) |
| 11 | 4.08 | H12,H12' | | 63.5 | |
| 12 | 2.28,m | H11,H12',H13,H13' | | 29.6 | |
| 12' | 1.36,m | H11,H12,H13 | | | |
| 13 | 1.80 | H12,H12',H14' | | 25.8 | |
| 13' | 1.92 | H12,H14,H14' | | | |
| 14 | 3.58, bt(J=7.3 Hz) | H13',H14' | H16 | 48.0 | |
| 14' | 3.48 | H13,H13',H14 | | | |
| Asn | | | | | |
| 15 | - | | | 172.6 | H17' |
| 16 | 4.61, bm | H17,H17',NH(8.01) | | 50.2 | H17' |
| 17 | 2.94 | H16,H17' | | 36.2 | NH2(5.43) |
| 17' | 3.20 | H16,H17 | | | |
| 18 | - | | | 169.0 | H17' |
| NH2 | 5.43,bs; 6.66,bs | 5.43 to 6.66 | | | |
| NH | 8.01, d(J=5.3 Hz) | H16 | | | |
| $Val_1$ | | | | | |
| 19 | - | | | 172.26 | H20,NH(8.01) |
| 20 | 4.05 | H21,H25,NH(8.22) | | 62.1 | |
| 21 | 2.36, m | H20,H22,H23 | | 29.5 | |
| 22 | 1.04 | H21 | | 18.8 | |
| 23 | 1.04 | H21 | | 18.7 | |
| NH | 8.22, d(J=8.0 Hz) | H20 | | | |
| $Pro_2$ | | | | | |
| 24 | - | | | 171.5 | H25,NH(8.22) |
| 25 | 4.53, d(J=6.9 Hz) | H20,H26' | H30 | 61.2 | |
| 26 | 2.57, m | H26',H27,H27' | | 31.2 | H25 |
| 26' | 1.90 | H25,H26 | | | |
| 27 | 1.93 | H26,H27' | | 21.8 | H25 |
| 27' | 1.70 | H26,H27 | | | |
| 28 | 3.70, m | H27,H27',H28' | | 46.0 | H25,H26 |
| 28' | 3.48 | H27,H27',H28 | | | |
| $Val_2$ | | | | | |
| 29 | - | | | 171.8 | H30 |
| 30 | 4.16, dd(J=4.4,7.3 Hz) | H31,NH(6.80) | H25 | 58.5 | |
| 31 | 1.95 | H30,H32,H33 | | 30.1 | |
| 32 | 1.04 | H31 | | 19.8 | |
| 33 | 0.95 | H31 | | 18.5 | |
| NH | 6.80, d(J=4.4 Hz) | H30 | H35 | | |
| $Val_3$ | | | | | |
| 34 | - | | | 171.18 | H36 |
| 35 | 4.21, t(J=9.4 Hz) | H36,NH(7.32) | NH(6.80) | 57.3 | |
| 36 | 2.01 | H35,H37,H38 | | 29.1 | H35 |
| 37 | 0.95 | H36 | | 19.9 | |
| 38 | 0.95 | H36 | | 19.3 | |
| NH | 7.32, d(J=9.4 Hz) | H35 | | | |

[a] Proton in C# column irradiated. [b] Assignments based on HMQC and HMBC data. [c] Listed protons are correlated to carbons in C# column.

followed by derivatization with Marfey's reagent and HPLC analysis,[6] confirmed the presence of Phe, Pro, Asn and Val and showed that all of the amino acids had the L (S) configuration.

There are only a small number of naturally occurring cyclic heptapetides reported in the literature. Included in this group are the plant peptide evolidine,[7a,9] and the microbial peptides rhizonin A,[7b] phalloidin[7c] and ilamycin $B_1$.[7d] Pseudoaxinellin (**1**) is the first cyclic heptapeptide isolated from a marine source.[8] Perhaps due to the limited number of natural examples of cyclic heptapetides, they have not been subjected to the same degree of conformational analysis as cyclic penta- and hexapeptides. A recent detailed examination of evolidine (cyclo(Ser-Phe-Leu-Pro-Val-Asn-Leu)) by X-ray,[9a] nmr[9b] and molecular mechanics[9b] analyses showed that it contains one cis amide bond at proline (Leu-CO/Pro-N) and a two-turn β-bulge backbone conformational motif. It is interesting to note that pseudoaxinellin (1) also contains a cis amide bond at proline ($Val_2$-CO/$Pro_2$-N) and that the partial sequence $Val_2$-$Pro_2$-$Val_1$-Asn in 1 is nearly identical to the partial sequence Leu-Pro-Val-Asn in evolidine. Efforts are currently underway to determine if the backbone conformation of pseudoaxinellin (1) fits the two-turn β-bulge motif found for evolidine.

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