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Malaysiatin, the First Cyclic Heptapeptide from a Marine Sponge

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Abstract: Malaysiatin (**1**), the first homodetic cycloheptapeptide from a marine source, has been isolated from the cytotoxic and antimicrobial extracts of the marine sponge *Pseudaxinyssa sp.*, collected in Borneo. The unusual structure of malaysiatin, which contains homodipeptide and homotripeptide fragments, was elucidated by extensive spectroscopic analysis that suggests an all-L stereochemistry.

In the last decade, marine sponges have been a source of interesting cyclic peptides that frequently contain new L and D amino acids and have potent pharmacological activities. Typical examples of these structures are barettin[1] (from *Geodia baretti*), cyclotheonamides[2], which are potent thrombin inhibitors; keramamides[3] and motuporin[4], which are enzyme inhibitors; the highly cytotoxic orbiculamide A[5] and theonellamides[6]; theonellapeptolides[7] (all from *Theonella sp.*); the antimicrobial discodermins[8] and polydiscamide A[9], an antitumor and antibacterial agent (all from *Discodermia sp.*) ; the cytotoxic, antineoplastic and antimicrobial geodiamolides[10] (from *Geodia sp.* and *Pseudaxinyssa sp.*); and jaspamide[11] (jasplakinolide), an insecticidal and antifungal agent from *Jaspis sp.* All these compounds are heterodetic peptides, so called because they include not only α amino acids but also hydroxy acids, β and γ amino acids, and polyketide and polypropionate fragments, etc. They thus have a highly modified peptide skeleton featuring a variety of functional groups, including heterocycles and ester groups in addition to peptide bonds. Apart from diketopiperazines, marine sponges have yielded only a couple of homodetic cyclopeptides, i. e. cyclopeptides in which only recognizable peptide linkages participate in ring formation. These are the fenestins,[12] tetra and pentacyclopeptides isolated from *Leucophloeus fenestrata*, and hymenistatin 1[13] (from *Hymeniacidon sp.*), a strongly cytostatic all-L cyclooctapeptide; the fenestins and hymenistatin are composed exclusively of α-amino acids. In this paper we report the isolation and structure of malaysiatin (**1**)[14], the first homodetic cycloheptapeptide isolated from a marine source.

Pseudaxinyssa sp. (class *Demospongia*, order *Axinellida*) (2.2 kg fresh weight) collected off Borneo by scuba diving, was extracted with MeOH. The concentrated extracts (6.75 g), exhibited *in vitro* cytotoxicity (82% and 70% inhibition of P388 and KB respectively) and antimicrobial activity, and were partitioned between $MeOH/H_2O$ solutions with increasing concentrations of water, and hexane, CCl_4, CH_2Cl_2 and 1-butanol.

Repeated purification of the CH_2Cl_2 fraction on silica gel columns (CH_2Cl_2/MeOH) and reversed-phase HPLC on a μ-Bondapac C_{18} column (9:1 Cl_2CH_2/MeOH) afforded 3 mg of malaysiatin (**1**) as a colourless solid.

Structure elucidation of **1** was begun by intensive study of spectrometric data. The molecular formula, $C_{38}H_{56}N_8O_8$ (15 unsaturations) was established by a combination of FABMS and HREIMS. The positive ion FABMS of **1** showed peaks of *m/z*=753 $[M+H]^+$ with a glycerol matrix and of *m/z*=775 $[M+Na]^+$ with a glycerol/NaCl matrix. HREIMS showed an ion of *m/z* 752.4214, (requires 752.4220).

The peptide nature of **1** was evident from its ^1H and ^{13}C DEPT NMR spectra. The former showed the characteristic α-proton resonances for seven α-amino acids in the range δ 4.05-4.76 ppm, and seven amide NH groups at δ 5.42-8.22 ppm. The latter included eight carbonyl peaks at δ 172.5-169.1 ppm and the aforementioned seven CH carbons in the range δ 50.2-63.5 ppm.

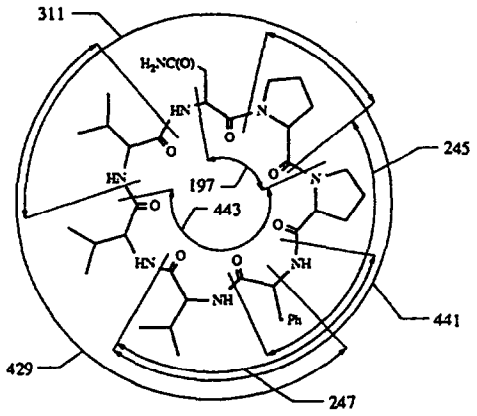
Table 1. ^1H and ^{13}C NMR chemical shifts of malaysiatin in $CDCl_3$ (298 K).

Atom #	δC^a	DEPT	δH^b, mult., J (Hz)	Atom #	δC^a	DEPT	δH^b, mult., J (Hz)
1	NH	–	7.30, d (9.5)	23	19.9[f]	CH_3	1.04, d (7.5)
2	53.3	CH	4.21, dd (9.5; 9.0)	24	19.8[f]	CH_3	1.03, d (6.5)
3	172.5[e]	C	–	25	29.1	CH	1.94[c], m
4	NH	–	6.76[g], bs	26	19.3[f]	CH_3	1.04, d (6.0)
5	58.5	CH	4.14, dd (7.5; 4.5)	27	18.7[f]	CH_3	0.95, d (6.0)
6	172.3[e]	C	–	28	29.5	CH	2.37, oct (7.5)
7	NH	–	8.22, d (7.5)	29	18.6[f]	CH_3	0.96, d (7.5)
8	62.5	CH	4.05, d (7.5)	30	18.4[f]	CH_3	0.96, d (7.5)
9	172.2[e]	C	–	31	36.3	CH_2	a=3.20, dd (15.0; 3.5) b=2.90, m
10	NH	–	8.02[g], d (5.0)	32	169.1	C	–
11	50.2	CH	4.62, d (5.0)	33	NH_2	–	a=6.56[g], bs b=5.42[g], bs
12	171.8[e]	C	–	34	48.0	CH_2	a=3.59, d (11.5) b=3.50, m
13	NH	–	–	35	21.7	CH_2	a=1.88[d], m b=1.70, m
14	63.5	CH	4.08, dd (10.5; 7.5)	36	29.5	CH_2	a=2.38, m b=1.33, m
15	171.5[e]	C	–	37	46.1	CH_2	a=3.70, ddd (12.5; 11.5; 8.5) b=3.52, m
16	NH	–	–	38	25.8	CH_2	a=1.96[c], m b=1.78, m
17	61.2	CH	4.53, d (7.5)	39	31.2	CH_2	a=2.56, dd (11.5; 6.0) b=1.90[d], m
18	171.2[e]	C	–	40	37.6	CH_2	a=3.24, dd (13.5; 5.0) b=2.94, dd(13.5; 11.5)
19	NH	–	7.53, d (11.5)	41	137.0	C	–
20	55.5	CH	4.76, ddd (13.5; 11.5; 5.5)	42	128.9	CH	7.18, d (6.9)
21	170.4[e]	C	–	43	128.3	CH	7.19, d (6.9)
22	30.1	CH	1.99[c], m	44	126.6	CH	7.24, d (6.9)

[a]Assignments based on HMQC. [b]Assignments based on COSY and TOCSY. [c, d]Overlapping signals. [e, f]Assignments for these signals may be interchanged. [g]D_2O interchange.

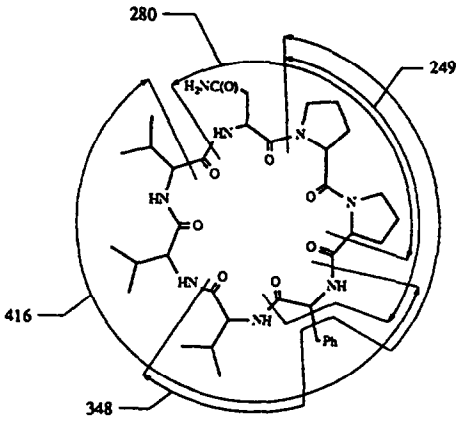
Extensive 2D NMR analysis, including ^1H-^1H COSY, HMQC and TOCSY, was used to determine the identity of the seven amino acids and to assign the NMR signals. TOCSY proved especially useful for

determining the network of mutually coupled protons within each amino acid unit. As a result of these studies, the amino acids were found to be one Phe, one Asn, two Pro and three Val units. Table 1 shows the proton and carbon NMR assignments.



775 $[M + Na]^+$
753 $[M + H]^+$

Fig. 1. (+) FAB fragmentations of 1.



695 M^+ - CH_2CONH_2
576 M^+ - Phe - CO
547 M^+ - Asn - $CH_2C_6H_5$

Fig. 2. EIMS fragmentations of 1.

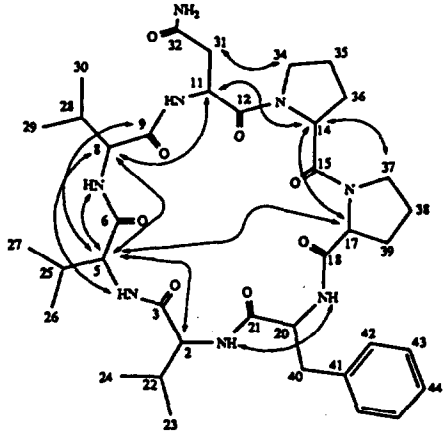


Fig. 3. Observed NOEs in ROESY for 1.

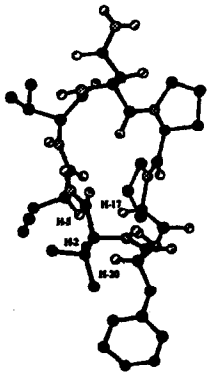


Fig. 4. Perspective view of 1 showing H-5 and H-17.

These data account for 14 unsaturations; the remaining one must correspond to ring closure. The relatively high intensity of the $[M+H]^+$ ion base peak observed in the FABMS spectrum also suggested that the heptapeptide 1 might be cyclic. The mass fragmentations under positive ion FAB and EIMS are indicated in Figures 1 and 2 and the combined data suggest that malaysiatin is cyclo-(Asn-Pro-Pro-Phe-Val-Val-Val).

Long range ^1H-^1H COSY and HMBC experiments were performed but, due to sample size limitations, no further information about the sequence could be extracted. However, results on the bonding between the amino acids and the conformation of the peptide ring were obtained from the ROESY spectrum, principally by

inspection of the signals produced by the NH and CH groups of each amino acid. Figure 3 shows a selection of the NOE cross peaks and the sequence obtained. In addition to the NOEs produced by protons belonging to vicinal amino acids, a strong NOE is observed between H-5 of a valine and H-17 of a proline, suggesting that in solution the ring adopts a folded, saddle like conformation. Independent evidence of this conformational preference comes from the absence of NOEs between H-20 of Phe and the neighbouring H-2 and H-17. Though this absence is at first sight puzzling, a Dreiding model of all-L malaysiatin in which the proximity of H-5 and H-17 allows the observed NOE effect across the ring (see Figure 4), shows that H-20 of Phe lies out of the ring plane and cannot exhibit NOEs with H-2 and H-17. Furthermore, ROESY shows NOE effects between the α-CHes of all the amino acids but one (the out of plane Phe H-20), which can be interpreted as evidence that all the α–protons are located on the same side of the ring, and therefore that all the amino acids might have the same configuration, either L or D. However, due to the conformational freedom exhibited by this molecule, further studies on the structure of malaysiatin are in progress. The range of the α-CHes chemical shifts also supports the all-L/D stereochemistry.[15] The all-D peptide would be an extremely rare find in view of the low abundance of natural D amino acids; thus the all-L enantiomer is the most probable.

Standard chiral GC amino acid analysis failed to provide information about absolute stereochemistry because of unexpected and extensive decomposition of the sample during hydrolisis. The small amount of **1** available prevented us from trying again. Besides being the first homodetic cycloheptapeptide from a marine source, malaysiatin is highly unusual in containing homotripeptide (Val-Val-Val) and homodipeptide (Pro-Pro) fragments.

Acknowledgements

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References and Notes

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14. $[\alpha]_D$ = -8° (*c* 2.5 Cl_3CH); UV (CH_3OH) λ_{max} 223 and 282 nm; IR ν_{max} (KBr) 3330-3300, 2980-2940, 1683, 1628 and 1522 cm^{-1}; ^{13}C and ^{1}H NMR, see Table 1; EIMS and positive ion FABMS, see Figures 1 and 2 respectively.
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