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Mahafacyclin A, a cyclic heptapeptide from *Jatropha mahafalensis* exhibiting β-bulge conformation

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Abstract

The structure of mahafacyclin A (**1**), a cyclic heptapeptide isolated from *Jatropha mahafalensis* latex was elucidated by a combination of chemical degradation, LSIMS data and 2D NMR experiments. Mahafacyclin A solution conformation was shown to have β-bulge characteristics.

Jatropha species (Euphorbiaceae) have been shown to be a rich source of bioactive cyclic peptides which contain seven to ten residues with a high proportion of hydrophobic amino acids.[1–5] In our investigation for bioactive cyclic peptides from plants, we studied the latex of *J. mahafalensis*, a grandiose endemic bottle tree of Madagascar, which grows in the dried forest of the western part, and is perfectly adapted to extreme desert-like conditions. A new cyclic heptapeptide named mahafacyclin A, with β-bulge characteristics was exhibited (Fig. 1).

The dry latex of *J. mahafalensis* (250 g) was extracted by a CH_2Cl_2/MeOH (9/1) mixture to give 2.72 g of crude extract. This material was chromatographed on Sephadex LH20 (MeOH) to yield a crude peptide fraction (673 mg) which was then analyzed by C18 reverse phase HPLC, exhibiting one major component. The pure compound, mahafacyclin A (328 mg), was obtained by multi-step semi-preparative HPLC. It showed a positive reaction with the chlorine/*o*-tolidine reagent, indicating the presence of amide groups, and a negative reaction with ninhydrine, suggesting that **1** is a cyclic peptide. The amino acid composition was determined from the acid hydrolysis of **1** (HCl 6N, 110°C, 24 h) followed by HPLC analysis: Gly (2), Ile (1), Leu (1), Phe (1), Thr (1) and Val (1). The absolute configuration of the chiral amino acid was shown to be L by derivatization of the acid hydrolysate to *N*-trifluoroacetyl isopropyl esters, followed by GC analysis on a chiral capillary column. The molecular weight M=687 was deduced from the positive LSIMS spectrum where the protonated molecule MH^+ and the adduct ion $[M+Na]^+$

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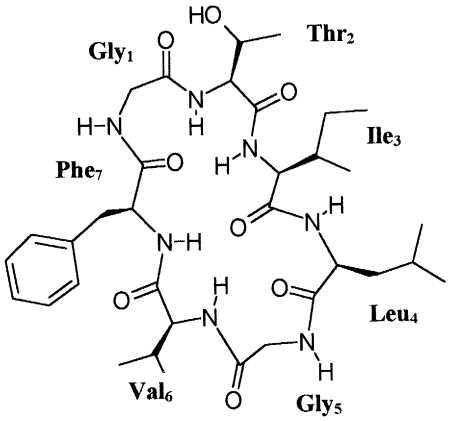

Fig. 1. Structure of mahafacyclin A (**1**)

were observed at *m/z* 688 and 710, respectively. In the high-resolution mass spectrum, the protonated molecule MH^+ at *m/z* 688.4016 corresponding to the molecular formula $C_{34}H_{54}N_7O_8$ (calcd 688.4034), was in agreement with the above amino acid composition in a cyclic heptapeptide.

NMR data were recorded in DMSO-d_6 as in this polar solvent, the 1D NMR spectra of **1** gave well resolved sharp signals. Complete amino acid sequence assignment for **1** was determined by 2D ^{1}H NMR spectroscopy. Assignment of ^{1}H chemical shifts to specific protons of individual residues was obtained by 2D homonuclear COSY and TOCSY experiments, showing the complete spin systems of two Gly, one Ile, one Leu, one Phe, one Thr and one Val. The corresponding carbon resonances were determined on the basis of *J*-modulated ^{13}C, HMQC and HMBC experiments (Table 1).

The sequential assignment of the backbone NH proton signals arose from the ROESY spectrum and was completely carried out by using inter-residue dαN(i, i+1) and dNN(i, i+1) connectivities. The lowest-field triplet NH proton of a glycine at 8.54 ppm was assigned to Gly_1. Inter-residue dαN(i, i+1) connectivities were found between each adjacent residue. In addition, dNN(i, i+1) connectivities were exhibited from Gly_1 to Ile_3 and from Leu_4 to Phe_7. Accordingly, the structure of mahafacyclin A (**1**) was determined as cyclo (-Gly_1-Thr_2-Ile_3-Leu_4-Gly_5-Val_6-Phe_7-).

The solution conformation of **1** was studied by DG calculations[6] using distance constraints derived from adiabatic off resonance ROESY experiments[7] obtained with a mixing period τ_m=150 ms and a strong off resonance spin lock (8000 Hz) pulse using adiabatic rotations (Fig. 2). Solution conformations of cyclic heptapeptides such as segetalins D and E,[8] pseudostellarin D,[9] yunnanin A,[10] evolidine[11] and stylostatin 1[12] which contain at least one proline, were characterized by a two β-turns in one β-bulge[13] structure. In order to analyze the solution conformational preference of **1**, the temperature coefficients of NH protons, the coupling constant values and the distance geometry (DG) calculations based on nuclear Overhauser effect (NOE) constraints were used. The mean structure adopts a type I β-turn at Gly_1-Thr_2 [Gly_1 Φ, Ψ (−61°, −55°); Thr_2 Φ, Ψ (−87°, +11°)] stabilized by a 4→1 hydrogen bond between Ile_3-NH and Phe_7-CO, in agreement with the low temperature coefficient observed for the Ile_3 amide proton (Fig. 3). The proposed solution conformation also showed a turn at Leu_4-Gly_5 [Leu_4 Φ, Ψ (−59°, +91°); Gly_5 Φ, Ψ (+76°, +4°)] stabilized by a bifurcated 4→1 hydrogen bond between Val_6-NH and Ile_3-CO and between Phe_7-NH and Ile_3-CO. The hydrogen bond pattern was consistent with low temperature coefficients of the Val_6 and Phe_7 amide protons, involved in the β-bulge motif (Tables 2 and 3).

Mahafacyclin A is the first cyclic heptapeptide, without proline, which exhibits β-bulge characteristics. As it has been recently shown that some natural cyclopeptides such as apicidins[8] or cyclosporins[9] have

Table 1
^{1}H (500 MHz) and ^{13}C (75 MHz) NMR spectral data for mahafacyclin A (DMSO-d_6, 298 K)

Residue		$\delta^{1}H$	$\delta^{13}C$	Residue		$\delta^{1}H$	$\delta^{13}C$
Gly_1	NH	8.54 (dd, 4.7, 7.3)		**Leu_4**	NH	7.79 (d, 6.6)	
	CO		169.4		CO		172.4
	α	3.85 (dd, 4.7, 15.7)	43.3		α	3.96	52.2
	α'	3.34 (dd, 7.3, 15.7)			ββ'	1.50	40.0
Thr_2	NH	7.71 (d, 8.7)			γ	1.50	24.3
	CO		171.1		$CH_3(\delta_1)$	0.81 (d, 5.2)	21.3
	α	4.37	57.9		$CH_3(\delta_2)$	0.88 (d, 6.3)	22.8
	β	4.32	68.1	**Gly_5**	NH	8.30 (t, 5.5)	
	γ	1.11 (d, 6.2)	19.5		CO		168.4
	OH	4.93 (d, 8.5)			α	3.96	42.4
Ile_3	NH	8.16 (d, 6.9)			α'	3.35	
	CO		170.8	**Val_6**	NH	7.10 (d, 8.4)	
	α	4.10	58.0		CO		171.4
	β	1.96	35.1		α	4.14	58.0
	γ_1	1.43	24.2		β	1.88	31.2
	γ_1'	1.15			$CH_3(\gamma_1)$	0.73 (d, 6.5)	19.0
	$CH_3(\gamma_2)$	0.86 (d, 8.0)	15.6		$CH_3(\gamma_2)$	0.65 (d, 6.5)	17.7
	$CH_3(\delta)$	0.84 (t, 7.7)	11.4	**Phe_7**	NH	8.52 (d, 5.2)	
					CO		170.8
					α	4.17	
					ββ'	3.08 (d, 7.2)	36.2
					γ		137.5
					δ	7.16	129.0
					ε	7.25	128.2
					τ	7.20	126.4

Table 2
Distance geometry data for **1**

Structural parameters	
Number of constraints	
distance	58
torsion	7
Number of converted conformers	48
Mean RMS ROE	0.2
RMSD for backbone heavy atoms of mean structure (Å)	0.40

potent antiparasitic effects against *Plasmodium*, we examined the *P. falciparum* antiproliferative activity of the mahafacyclin A. We observed a moderate antimalarial activity for **1** with an IC_{50} value of 16 μM.

Table 3
Intramolecular hydrogen bonds in the mean structure of **1**

From	To	Distance (Å)	Angle (°)[a)]
I_3-NH	F_7-CO	2.37	167
F_7-NH	I_3-CO	2.55	170
V_6-NH	I_3-CO	2.59	171

[a)] Angles N-H···O

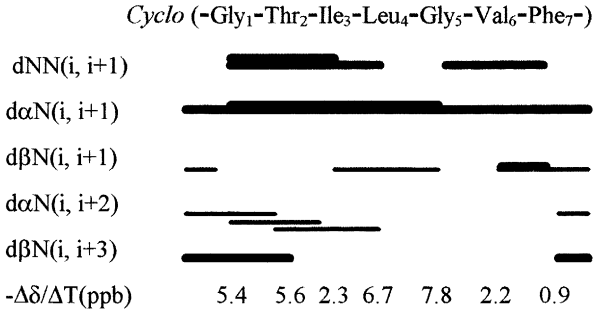

Fig. 2. Amino acid sequence of **1**, survey of the NOE connectivities involving NH and CαH and temperature coefficients of the amide protons. The observed NOEs are classified as strong, medium and weak and shown by thick, medium and thin lines, respectively

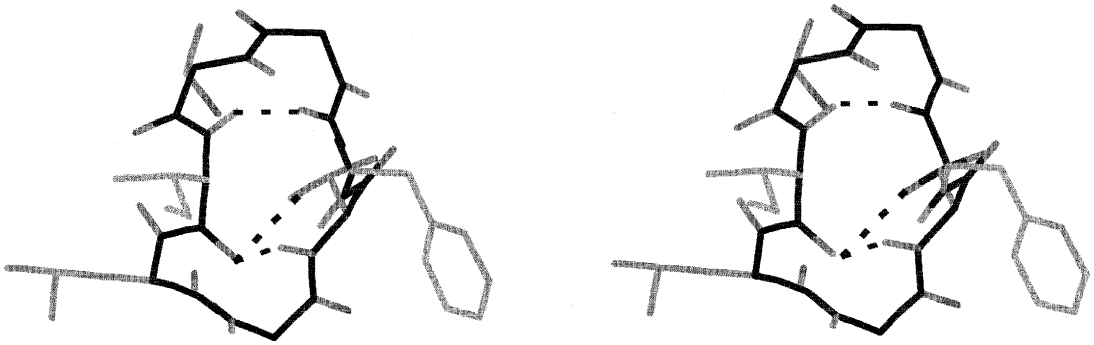

Fig. 3. Stereospecific view of the mean solution structure for **1**. The three broken lines represent the intramolecular hydrogen bonds

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6. Distances were classified into three ranges; 1.86–2.50, 1.86–3.50 and 2.50–4.50 Å corresponding to strong, medium and weak ROEs, respectively. The torsional constraints for the amide bond were taken into consideration, but no hydrogen bonds constraint was used. The initial structure satisfying the experimental constraints was generated by DG calculations with the program DISTGEOM (Hodsdon, M. E.; Ponder, J. W.; Cistola, D. P. *J. Mol. Biol*. **1996**, *264*, 585602). Finally, the produced conformers were subjected to energy minimization constraint with the AMBER all-atom force field. Among 289 structures embedded by the DG method, 48 structures were converged; the pair-wise root meansquare deviations (RMSD) for the backbone heavy atoms was less than 0.2. RMSD between the individual structures and the mean coordinate position were 0.40 Å for the backbone heavy atoms.
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