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Curcacycline B , a cyclic nonapeptide from *Jatropha curcas* enhancing rotamase activity of cyclophilin

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Abstract : The structure of curcacycline B (1), a cyclic nonapeptide isolated from *Jatropha curcas* latex was elucidated by combination of chemical degradation, LSIMS data and 2D NMR experiments. Curcacycline B was shown to enhance the rotamase activity of human cyclophilin B. © 1997 Published by Elsevier Science Ltd.

Most of the known naturally cyclic peptides with various bioactivities have been isolated from marine sponges[1] or microorganisms[2], but until now, only a limited number of cyclic peptides have been found in higher plants[3]. However, *Jatropha* species (Euphorbiaceae) have been shown to be a source of cyclic peptides[4] with immunomodulatory activity. In our investigation for bioactive peptides, we isolated the known curcacycline A[4b,5] and a new cyclic nonapeptide **1** named curcacycline B from the latex of *Jatropha curcas* L. collected near Dakar (Senegal). We observed that curcacycline B enhanced the rotamase activity of cyclophilin B.

The latex of *Jatropha curcas* (250 ml) was partitioned between ethyl acetate and water. The EtOAc soluble material (2.1 g) was chromatographed on Sephadex LH20 (MeOH) to yield a crude peptide fraction (417 mg) which was then analyzed by C18 reversed phase HPLC, exhibiting two major components. The mixture was resolved into pure peptides by multi-step semi-preparative HPLC to yield curcacycline A (187 mg) and curcacycline B (37 mg), which showed a positive reaction with chlorine / o-tolidine reagent, suggesting the presence of amide

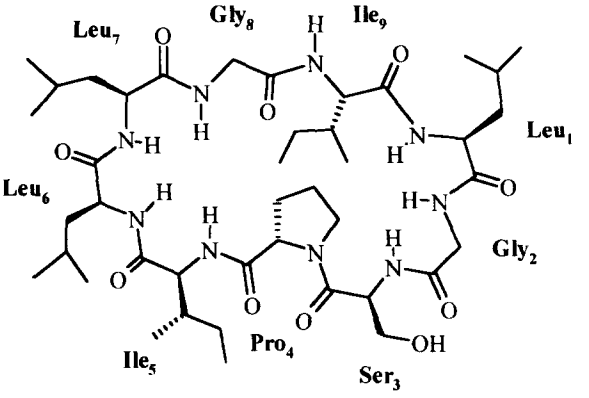

Figure 1 : structure of curcacycline B (**1**)

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groups, and a negative reaction with ninhydrine, indicating **1** to be 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 (2), Leu (3), Pro (1) and Ser (1). The absolute stereochemistry of the amino acids was shown to be *L*-configuration by derivatization of the acid hydrolysate to *N*-trifluoroacetyl isopropyl ester derivatives, followed by GC analysis on a chiral capillary column. The molecular weight M=863 was deduced from the positive LSIMS spectrum where the protonated molecule MH^+ and the adduct ion $[M+Na]^+$ were observed at *m/z* 864 and 886, respectively. The molecular weight of **1** fits well with the amino acid composition in a cyclic nonapeptide structure.

In spite of the presence of a proline residue, a single stable conformer (>95%) was displayed in a polar solvent such as DMSO-d_6, on the NMR time scale, by the occurrence of well-resolved sharp ^1H and ^{13}C NMR signals. Complete amino acid sequence assignment for **1** was determined by two-dimensional ^1H NMR spectroscopy. Assignment of ^1H chemical shifts to specific protons of individual residues was obtained by 2D homonuclear COSY and TOCSY experiments, showing complete spin systems of two Gly, two Ile, three Leu, one Pro and one Ser. 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 doublet NH proton of a leucine at 8.70 ppm was assigned to Leu_1. Inter-residue dαN(i,i+1) connectivities were found between each adjacent residue, extending from Leu_1 to Ser_3 and from Pro_4 to Leu_1, as well as the ROE connectivities between Ser_3-Hα and Pro_4-Hδ. In addition, dNN(i, i+1) connectivities were exhibited between Gly_2 and Ser_3, from Ile_5 to Leu_7 and from Gly_8 to Leu_1. Accordingly, the structure of curcacycline B (**1**) was determined as cyclo (-Leu_1-Gly_2-Ser_3-Pro_4-Ile_5-Leu_6-Leu_7-Gly_8-Ile_9-). Chemical shifts of β and γ carbons of Pro were δ 29.4 and 24.8 ppm, respectively, which gave evidence for the presence of a *trans*-proline amide bond; strong ROE enhancement between Ser_3-Hα and Pro_4-Hδ gave further evidence of the *trans*-geometry[6].

LSIMS spectrum gave small, but significant ions supporting the amino acid sequence derived from the NMR spectra. The data were consistent with initial cleavage of the ring at the Leu_1-CO/Gly_2-NH bond to give a linear protonated acylium ion corresponding to the nonapeptide which further sequentially lost Leu_1 (*m/z* 751), Ile_9 (*m/z* 638), Gly_8 (*m/z* 581), Leu_7 (*m/z* 468), Leu_6 (*m/z* 355) and Ile_5 leading to the protonated tripeptide Gly_2-Ser_3-Pro_4 acylium ion at *m/z* 242. LSIMS peak at *m/z* 324 could be assigned to the protonated Pro_4-Ile_5-Leu_6 fragment.

Curcacycline B is a novel cyclic nonapeptide, containing mostly hydrophobic residues and one proline, different therefore from all the cyclic peptides isolated from *Jatropha* species latex which do not contain proline. The structure of **1** was suggested to be a peptidyl-prolyl *cis-trans* isomerase (PPIase) substrate, as it has some structural features in common with cyclosporin A, the well known inhibitor of cyclophilins A and B.

Table 1. ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectral data for curcacycline B (DMSO-d_6, 296 K). *,**,*** assignments may be reversed.

Residue		$\delta^{1}H$	$\delta^{13}C$	Residue		$\delta^{1}H$	$\delta^{13}C$
Leu_1	NH	8,70 (d, 3.8)		**Leu_6**	NH	7.35 (d, 8.4)	
	CO		172.3**		CO		172.3**
	α	3.75	53.4		α	4.41	50.6
	β	1.53	38.8***		β	1.65	39.6
	β'	1.45			β'	1.35	
	γ	1.50	24.2*		γ	1.53	24.1*
	$CH_3(\delta_1)$	0.83	22.0		$CH_3(\delta_1)$	0.79	21.0
	$CH_3(\delta_2)$	0.88	22.6		$CH_3(\delta_2)$	0.81	23.2
Gly_2	NH	8.59 (dd, 4.7, 7.3)		**Leu_7**	NH	7.97 (d, 5.3)	
	CO		169.2		CO		172.4**
	α	3.93	42.5		α	3.96	52.2
	α'	3.35			β	1.63	39.2***
Ser_3	NH	7.65 (d, 7.0)			β'	1.41	
	CO		171.5		γ	1.60	23.9*
	α	4.63	52.0		$CH_3(\delta_1)$	0.87	21.5
	β	4.07	61.6		$CH_3(\delta_2)$	0.83	23.0
	β'	3.50		**Gly_8**	NH	8.20 (t, 6.1[a])	
	OH	6.03			CO		169.0
Pro_4	CO		171.8		α	3.81	42.4
	α	4.28	60.7		α'	3.40	
	β	2.11	29.4	**Ile_9**	NH	6.85 (d, 9.0)	
	β'	1.93			CO		172.5
	γ	1.99	24.8		α	4.25	56.5
	γ'	1.87			β	1.88	35.1
	δ	3.85	47.1		γ_1	1.24	23.7
Ile_5	NH	7.47 (d, 9.7)			γ_1'	1.05	
	CO		169.9		$CH_3(\gamma_2)$	0.77	14.7
	α	4.12	58.1		$CH_3(\delta)$	0.71	9.7
	β	1.72	36.9				
	γ_1	1.37	24.3				
	γ_1'	1.05					
	$CH_3(\gamma_2)$	0.80	15.5				
	$CH_3(\delta)$	0.80	10.7				

[a] : determined at 313 K.

PPIase assays[7] using human cyclophilin B, based on α-chymotrypsine-rotamase coupled enzymatic experiment[8], were carried out to determine if curcacycline B could bind the human cyclophilin B. In the absence of cyclophilin B, **1** had no effect on the α-chymotrypsine activity. Surprisingly, in the presence of cyclophilin B **1** was shown to enhance the PPIase activity of cyclophilin-B of 60% at 30 μM (Figure 2) whereas no modification of cyclophilin B activity was observed in the presence of curcacycline A.

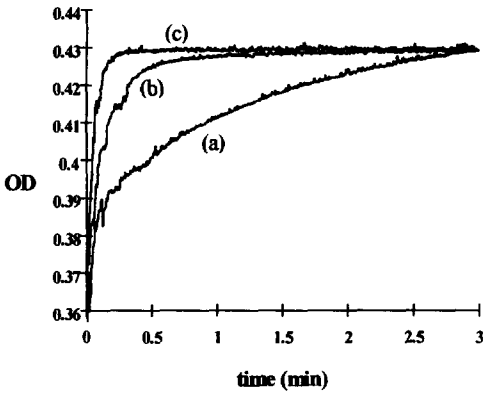

Figure 2 : Hydrolysis kinetics of Suc-Ala-Ala-Pro-Phe-pNA

(a) in the presence of α-chymotrypsin

(b) in the presence of cyclophilin-B and α-chymotrypsin

(c) in the presence of cyclophilin-B, 30 μM of **1** and α-chymotrypsin.

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5. Structure of curcacycline A: *cyclo* (-Gly_1-Leu_2-Leu_3-Gly_4-Thr_5-Val_6-Leu_7-Leu_8-).

6. Zabriskie, T.M.; Foster, M.P.; Stout, T.J.; Clardy, J.; Ireland, C.M. *J. Am. Chem. Soc.* **1990**, *112*, 8080-8084.

7. The PPIase activity was assessed with the α-chymotrypsin-coupled enzymatic assay. The substrate was the tetrapeptide Suc-Ala-Ala-Pro-Phe-*p*NA which is in an equilibrum between *cis* (~10%) and *trans* (~90%) forms in aqueous solution. The C-terminal blocking group (*p*NA) of the peptide substrate is cleaved instantaneously by α-chymotrypsin only if the Ala-Pro bond is in the *trans* form. The *cis* peptide spontaneously converts at a slow rate to the *trans* isomer which is cleaved by α-chymotrypsin. Release of the chromogenic group (*p*-nitroaniline) is monitored by measuring the absorbance at λ=410 nm as a function of time. In the presence of a PPIase the rate of *cis/trans* isomerization of peptides is accelerated, which in turn shortens the life time of the blocked peptide.

8. In a plastic cuvette were added 2 ml of ice cold assay buffer (40 mM Na-HEPES, 1 mM EDTA, 5 mM DTT, 150 mM NaCl, 0.015% Triton X-100, pH 7.9) 10 μL human cyclophilin-B (89 μM), 25 μL of peptide substrate (4.8 mg / 2 mL DMSO) and 6 μL of **1** at various concentration in MeOH. The reaction was initiated by addition of 20 μL of α-chymotrypsin (20 mg/mL in 1 mM HCl). The absorbance at 410 nm versus time was monitored for 180 s using an UVIKON 930 spectrophotometer.

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