Cyclogossine B, a Cyclic Octapeptide from *Jatropha gossypifolia*

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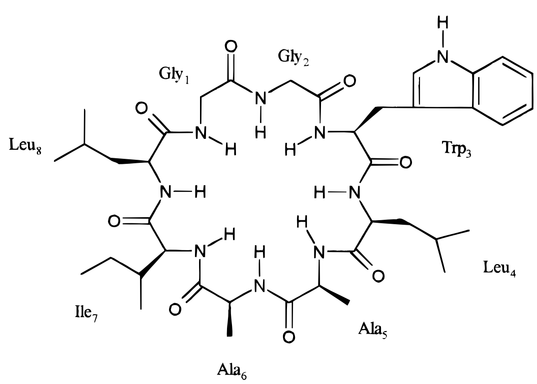
From the EtOAc extract of the latex of *Jatropha gossypifolia* (Euphorbiaceae), the new cyclic octapeptide, cyclogossine B (**1**), was isolated together with the known cyclic heptapeptide cyclogossine A. The structure of **1** was elucidated by chemical degradation, mass spectrometry, and homonuclear and heteronuclear NMR experiments.

Jatropha gossypifolia is a pantropical species of Euphorbiaceae originating from South America and used in folk medicine. The fresh latex is applied to infected wounds[1,2] and ulcers,[3] and decoction of the plant is used to treat fever in Togo and Senegal, where it is known as "lumulum" (vernacular name in Volof).[4] Previous chemical study on the latex of *J. gossypifolia* from Indonesia led to the characterization and sequence of the cycloheptapeptide cyclogossine A [cyclo(−Leu$_1$-Ala$_2$-Thr$_3$-Trp$_4$-Leu$_5$-Gly$_6$-Val$_7$-)][5].

From the latex of *J. gossypifolia* L. (Euphorbiaceae) collected in Senegal, a new cyclic octapeptide, named cyclogossine B (**1**), together with cyclogossine A, was isolated. In this paper, we report the isolation and the structure of **1**.



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The latex of *J. gossypifolia* was partitioned between EtOAc and H_2O.[6] The EtOAc-soluble materials chromatographed on Sephadex LH20, yielded a crude peptide fraction that was analyzed by C-18 reversed-phase HPLC to reveal two peaks. The mixture was resolved into pure peptides by multi-step semi-preparative HPLC separation. The less hydrophobic compound was characterized as cyclogossine A, followed by cyclogossine B. Both compounds showed a positive reaction with chlorine−*o*-tolidine reagent, suggesting the presence of amide groups. The amino acid composition was determined from the acid hydrolysis of **1** followed by HPLC analysis: Ala (2), Gly (2), Ile (1), Leu (2), and Trp (1). The absolute stereochemistry of the residues was shown to be the L-configuration by derivatization of the acid hydrolysate to *N*-trifluoroacetyl isopropyl esters followed by GC analysis on a chiral capillary column. The molecular weight of 781 was deduced from the positive LSIMS spectra, which displayed the protonated molecule MH^+ and the adduct ion $[M + Na]^+$ at *m/z* 782 and 804, respectively. The molecular formula $C_{39}H_{59}N_9O_8$ for **1** resulted from HRLSIMS (found 782.4589 MH^+, calcd 782.4565). This formula fits well with the above amino acid composition in a cyclic octapeptide.

The sequence determination of **1** was obtained by assignments of different spin systems to residue types from $^1H-^1H$ COSY, HOHAHA and ROESY experiments, and then sequential assignments were afforded by exploitation of long-range $^1H-^{13}C$ HMBC experiments and dNN (i, i + 1) and dαN (i, i + 1) ROESY connectivities. Complete assignments of ^1H and ^{13}C NMR signals were obtained in a polar solvent such as DMSO-d_6. The ^1H-NMR spectrum of **1** showed resonances of one indole NH (δ 10.86 ppm) and eight protons in the amide proton region, which disappeared upon addition of D_2O, in addition to five aromatic protons, suggesting the presence of one Trp and seven amino acids, in agreement with the amino acid composition. The COSY and HOHAHA spectra assigned the spin systems of the unique Trp and Ile, the two Gly, and the two Ala but not the spin systems of the two Leu residues because their NH signals overlapped. Combination of *J*-modulated ^{13}C, HMQC for direct $^1J_{H-C}$ connectivities and HMBC for long-range $^2J_{H-C}$ and $^3J_{H-C}$ ones allowed the assignment of all ^1H and ^{13}C resonances (Table 1). The connectivities between neighboring amino acids, except for that between Gly$_1$ and Gly$_2$, were unequivocally determined for long-range $^2J_{H-C}$ and $^3J_{H-C}$ correlations. The remaining connectivity between Gly$_1$ and Gly$_2$ was supported by the ROESY cross peak observed between the two glycine amide protons. Other dNN (i, i + 1) and dαN (i, i + 1) ROESY connectivities confirmed the amino acid sequence (Figure 1).

The LSIMS spectrum gave slight, but significant ions supporting the amino acid sequence derived from the NMR spectra. The data were consistent with the initial cleavage of the ring at the Leu$_8$/Gly$_1$ amide bond to give a linear protonated acylium ion corresponding to the octapeptide, which further sequentially lost Leu$_8$ (*m/z* 669), Ile$_7$ (*m/z* 556), Ala$_6$ (*m/z* 485), Ala$_5$ (*m/z* 414), and Leu$_4$, leading to the protonated tripeptide Gly$_1$−Gly$_2$−Trp$_3$ acylium ion at *m/z* 301.

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Table 1. ^{1}H- and ^{13}C-NMR Assignments of Cyclogossine B (**1**) in DMSO-d_6

assignment		δ^1H (int., mult., J in Hz)	δ^{13}C
Gly$_1$			
	NH	8.37 (1H, dd, 5.4, 7.1)	
	α	3.40 (1H, dd, 5.4, 16.9)	42.7
		3.85 (1H, dd, 7.1, 16.9)	
	CO		169.1
Gly$_2$			
	NH	7.70 (1H, dd, 4.4, 6.9)	
	α	3.58 (1H, dd, 4.4, 16.6)	41.7
		4.03 (1H, dd, 6.9, 16.6)	
	CO		169.2
Trp$_3$			
	NH	8.20 (1H, d, 7.1)	
	α	4.35 (1H, m)	54.9
	β	3.13 (1H, dd, 4.9, 14.8)	27.2
		2.97 (1H, dd, 8.6, 14.8)	
	1′(NH)	10.86 (1H, d, 2.2)	
	2′	7.17 (1H, d, 2.2)	123.5
	3′		109.7
	4′	7.50 (1H, m)	118.1
	5′	6.97 (1H, m)	118.3
	6′	7.05 (1H, m)	120.9
	7′	7.32 (1H, m)	111.3
	8′		136.1
	9′		127.1
	CO		171.4
Leu$_4$			
	NH	7.85 (1H, d, 6.8)	
	α	4.17 (1H, m)	51.6
	2β	1.45 (2H, m)	39.8
	γ	1.23 (1H, m)	24.1
	δ	0.75 (3H, d, 6.2)	21.2
		0.80 (3H, d, 6.2)	22.9
	CO		171.9
Ala$_5$			
	NH	7.55 (1H, d, 6.4)	
	α	4.19 (1H, m)	48.5
	β	1.18 (3H, d, 6.8)	17.0
	CO		172.7
Ala$_6$			
	NH	8.37 (1H, d, 5.3)	
	α	4.07 (1H, m)	50.2
	β	1.23 (3H, d, 7.3)	16.1
	CO		173.6
Ile$_7$			
	NH	7.92 (1H, d, 6.8)	
	α	4.05 (1H, m)	57.6
	β	1.75 (1H, m)	36.2
	γ	1.38 (1H, m)	24.4
		1.15 (1H, m)	
	CH$_3$(γ)	0.80 (3H, m)	15.1
	CH$_3$(δ)	0.80 (3H, m)	11.2
	CO		171.2
Leu$_8$			
	NH	7.85 (1H, d, 8.6)	
	α	4.12 (1H, m)	51.7
	2β	1.55 (2H, m)	39.0
	γ	1.40 (1H, m)	24.1
	δ	0.80 (3H, d, 6.0)	21.2
		0.86 (3H, d, 6.0)	22.7
	CO		172.5

Experimental Section

General Experimental Procedures. ^{1}H- and ^{13}C-NMR spectra were run in DMSO-d_6 using a Bruker AC 300 spectrometer, equipped with an Aspect 3000 computer. A 10-mg sample of cyclogossine B in a 5-mm tube (0.5 mL of DMSO-d_6 degassed) was used for homonuclear and heteronuclear measurements. A phase-sensitive ROESY NMR experiment was acquired with mixing time of 150 ms. The delay preceding the ^{13}C pulse for the creation of multiple quanta coherences through several bonds in the HMBC was set to 70 ms. Heteronuclear coupling-constant value used in the HMQC experiment to establish the delay needed to select the protons coupled to carbon was 135 Hz. Liquid secondary ion mass spectrometry (LSIMS) spectra were recorded on a ZAB-HF instrument. The $[\alpha]_D$ value was determined with a Perkin-Elmer Model 243 B polarimeter, and IR spectra registered on a Nicolet Model 205 FT-IR spectrometer. TLC and column chromatography were carried out on Merck precoated Si gel F_{254} plates and on Si gel 60 (Merck 230−400 mesh).

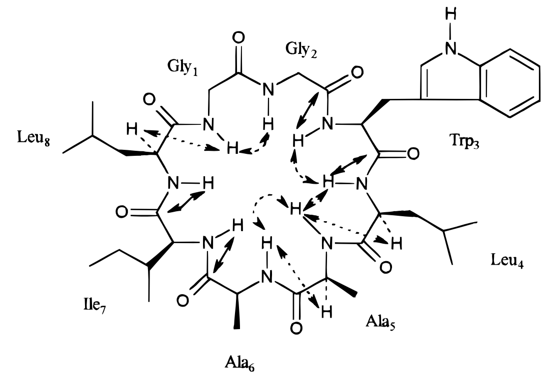

Figure 1. HMBC correlations (arrows) and ROE correlations (dashed arrows) for cyclogossine B (**1**) in DMSO-d_6.

Plant Material. *J. gossypifolia* L., growing in the region of Dakar (Senegal) and collected in September 1996, was identified, and a voucher specimen is on deposit in the Laboratoire de Phanérogamie, Muséum National d'Histoire Naturelle, Paris. Crude latex was obtained by cutting off leaf stalks and adding a few drops of EtOH to prevent the latex from excessive foaming. The latex was stored at −20 °C until use.

Extraction and Isolation. To 30 mL of crude latex, 30 mL of demineralized H_2O was added, and the mixture was extracted with 3 × 100 mL of EtOAc. The EtOAc was removed under reduced pressure, and the crude residue (293 mg) was dissolved in MeOH and chromatographed on Sephadex LH-20 (Pharmacia) with MeOH as eluent. Peptide-containing fractions were monitored by TLC with CH_2Cl_2−MeOH 9:1 as eluent system. Peptides were detected with Cl_2−*o*-tolidine reagent.[7] The peptide mixture (63 mg) was purified by reversed-phase HPLC (Kromasil C18, 250 × 7.8 mm, 5 μm, AIT France; 30% H_2O in MeOH; flow rate 2mL/min; detection 220 nm) to yield cyclogossine A (t_R 21 min, 17 mg) and cyclogossine B (t_R 23 min, 17 mg). Identification of cyclogossine A was made by amino acid composition exam and ^{1}H-NMR spectra comparison.[2]

Cyclogossine B (1): colorless amorphous solid; $[\alpha]_D^{22}$ −9° (*c* 0.1, MeOH); ^{1}H and ^{13}C NMR (see Table 1); LSIMS (thioglycerol matrice) *m/z* $[M + Na]^+$ 804 (100), $[MH]^+$ 782 (44), 669 (5), 556 (4), 485 (3), 414 (5), 301 (6).

Amino acid Composition. Cyclogossine B (1 mg) was hydrolyzed with 6 N HCl (0.5 mL) in a sealed tube at 110 °C under argon for 24 h. For the determination of tryptophan, hydrolysis was performed in 1 mL 6 N HCl containing 0.6% thioglycolic acid. After hydrolysis, the reagents were removed under reduced pressure. The residues were dissolved in 0.2 M Na−citrate buffer (pH 2.2). Amino acids were determined by cation-exchange chromatography on a Liquimat 2 Amino Acid Analyzer (Kontron) and detected with OPA reagent.

Absolute Configuration of Amino Acids. Hydrolysates of the peptide obtained with 6 N HCl or 6 N HCl− 0.6% thioglycolic acid as described above, were dried over KOH pellets. The crude residues were dissolved in an anhydrous solution of 3 N HCl in iPrOH and heated at 110 °C for 20 min. The reagents were evaporated under reduce pressure, the residues were dissolved in CH_2Cl_2 (0.5 mL), and 0.5 mL of trifluoroacetic anhydride was added. The mixtures were kept in a screw-capped tube at 100 °C for 5 min. The reagents were evaporated and the mixtures analyzed on a Chirasil-L-Val (*N*-propionyl-L-valine-*tert*-butylamide polysiloxane) quartz capillary column with He (1.1 bar) as carrier gas and a temperature program of 50−130 °C at 3 °C/min, then 130−190 °C at 10 °C/min. The retention times (min) of L-Ala (10.6), L-Ile (15.8), L-Leu (19.1) and L-Trp (37.1) were compared with those of commercial references.

References and Notes

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