Crotogossamide, a Cyclic Nonapeptide from the Latex of *Croton gossypifolius*

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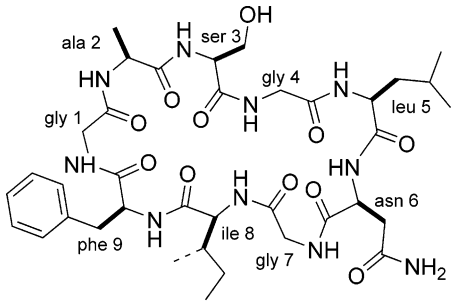
A new cyclic nonapeptide, crotogossamide (**1**), was isolated from the latex of *Croton gossypifolius*. Its structure was elucidated by use of 1D and 2D NMR and MS and by hydrolysis followed by GC-MS analysis as *cyclo*(-Gly[1]-Ala[2]-Ser[3]-Gly[4]-Leu[5]-Asn[6]-Gly[7]-Ile[8]-Phe[9]-). This is the first report of a cyclic peptide from a *Croton* species. The known flavonoids kaempferol 3-*O*-rhamnopyranoside, quercitrin, and myricitrin were also isolated from the plant latex.

Cyclic peptides have been isolated from marine sources,[1,2] as well as from fungi[3,4] and from higher plants[5] including members of the Caryophyllaceae,[6,7] Annonaceae,[8−10] and Amaranthaceae,[11] as well as from several species of the genus *Jatropha* of the family Euphorbiaceae.[12−21]

As part of a program aimed at discovering bioactive and structurally novel compounds of the Euphorbiaceae of Trinidad and Tobago, we have investigated the leaves and latex of *Croton gossypifolius* Vahl., which also occurs in Colombia, Martinique, Mexico, and Venezuela. The mature tree may grow up to 15 m in height and produces a red sap when cut. We report herein the isolation of a new cyclic peptide, crotogossamide (**1**), and three known flavonoids, kaempferol-3-*O*-α-L-rhamnopyranoside, quercitrin, and myricitrin, from the latex of the plant. While there have been several reports of the isolation of cyclic peptides from *Jatropha* species,[12−21] to the best of our knowledge this is the first report of the isolation of a cyclic peptide from a *Croton* species.

The red latex obtained from the trunk of *C. gossypifolius* was suspended in MeOH/H_2O and the suspension extracted successively with petroleum ether, $CHCl_3$, EtOAc, and n-BuOH. The precipitate obtained on concentrating the butanol fraction was chromatographed on Sephadex LH-20 ($CHCl_3$, $CHCl_3$/MeOH, H_2O) to yield crotogossamide (**1**).



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Crotogossamide (**1**) was obtained as white crystals (MeOH/H_2O). The HRESIMS spectrum displayed an $[M + H]^+$ ion at *m*/*z* 817.4221 (calcd for $C_{37}H_{57}N_{10}O_{11}$, 817.4202), indicating a molecular formula of $C_{37}H_{56}N_{10}O_{11}$. The FT-IR spectrum showed strong peaks at 3290, 1660, 1540, and 1250 cm^{-1}, pointing to the presence of amide groups. The ^1H NMR spectrum in DMSO-d_6 showed 11 N−H signals in the region δ 6.85−8.53. Two of these signals, at δ 6.85 and 7.39, were coupled to each other, and each gave an HMBC correlation to a methylene carbon signal at δ 36.0. This indicated the presence of an $-NH_2$ group. The ^{13}C NMR spectrum showed 10 carbonyl signals (δ 168.9 to 173.8) and nine carbon resonances in the region δ 42.6−58.0. The ^{13}C DEPT 135 spectrum revealed that six of these represented methine and three represented methylene carbons. All these data suggested that **1** was a peptide consisting of nine amino acids, of which three are glycine residues and another is an asparagine residue. The ^1H and ^{13}C NMR data of **1** also gave signals indicating the presence of a monosubstituted aromatic ring and a hydroxymethyl group, suggesting the presence of phenylalanine and serine residues. The $^1H-^1H$ COSY spectrum along with $^1H-^{13}C$ HMQC and HMBC spectra allowed the determination of the amino acids Leu, Ile, and Ala as well as Asn, Phe, and Ser and confirmed the presence of three Gly units in compound **1**. Since the molecular formula indicated 15 double-bond equivalents, the data summarized above indicated the presence of a cyclic nonapeptide.

The sequence of amino acids in **1** was determined by judicious use of a combination of $^1H-^1H$ COSY, NOESY, and $^1H-^{13}C$ HMBC data, and this enabled the determination of the structure of **1** as *cyclo*(-Gly[1]-Ala[2]-Ser[3]-Gly[4]-Leu[5]-Asn[6]-Gly[7]-Ile[8]-Phe[9]-). The absolute configurations of the amino acids of **1** were unambiguously shown to be all L by comparison of the chromatograms from GC-MS analysis (using a chiral column) of suitably derivatized L- and D-amino acid standards with those obtained from the corresponding derivatives of the acid hydrolysate of the peptide.[22] This finding is consistent with the observation that all cyclic peptides isolated to date from Euphorbiaceae species consist exclusively of L-amino acids.[12−21]

The known flavonoids kaempferol 3-*O*-rhamnopyranoside, quercitrin, and myricitrin were also isolated and were identified on the basis of their ^1H and ^{13}C NMR spectra and comparison of these data and mp's with those reported in the literature.[23−26]

Experimental Section

General Experimental Procedures. Melting points were determined on a Reichert hot-stage apparatus and are uncorrected. Optical rotations were measured on a Polatronic D digital polarimeter in H_2O. FT-IR spectra were obtained in $CHCl_3$ using a Perkin-Elmer Spectrum RX1 FT-IR spectrophotometer. NMR spectra were recorded on a Bruker Avance DRX-400 spectrometer in DMSO-d_6 or CD_3OD using standard Bruker 1D (^1H, ^{13}C) and 2D ($^1H-^1H$ COSY, HMQC, HMBC, NOESY) pulse sequences. Chemical shifts in DMSO-d_6 are reported in ppm with reference to the solvent residual proton and carbon signals (δ_H 2.49 and δ_C 39.5). Mass spectra were obtained on a MDS Sciex QStar mass spectrometer. TLC was performed on silica gel 60 $PF_{254+366}$ using 0.25 mm thick plates for analytical TLC and 1.0 mm thick plates for preparative work. Column chromatography was conducted using silica gel 60 (70−230 mesh ASTM). GC analyses were carried out using a Hewlett-Packard 5890 gas chromatograph equipped with a deactivated

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Table 1. NMR Spectroscopic Data of Crotogossamide (**1**)[a]

residue	δ_C	δ_H (mult., *J* (Hz))	$^1H-^1H$ COSY δ_H	HMBC δ_C	NOESY δ_H
Gly[1]					
NH		8.15 (br t, 6.0 Hz)	3.37, 3.89	171.4	3.37, 3.89, 4.28, 7.72, 8.53
α	42.8	3.37[b]	8.15		3.89, 8.15
		3.89[b]	8.15		8.15
C═O	168.9				
Ala[2]					
NH		7.72 (br d, 6.6 Hz)	4.37	168.9	1.27, 8.15
α	48.1	4.37 (qn, 6.6 Hz)	1.27, 7.72	16.9, 168.9, 173.8	1.27, 8.38
β	16.9	1.27 (d, 6.6 Hz)	4.37	48.1, 173.8	4.37, 7.72
C═O	173.8				
Ser[3]					
NH		8.38 (br s)	4.02		4.02, 4.37
α	57.0	4.02 (q, 6.0 Hz)	3.67, 8.38	60.6, 171.5, 173.8,	3.67, 8.33, 8.38
β	60.6	3.67[b]	4.02, 5.02	171.5	4.02, 5.02, 8.33
OH		5.02 (t, 6.0 Hz)	3.67	60.6, 57.0	3.67
C═O	171.5[c]				
Gly[4]					
NH		8.33 (br t, 6.0 Hz)	3.62, 3.72	171.5	3.62, 3.72, 4.02, 7.50
α	42.6	3.62 (dd, 6.0 Hz)[b]	3.72, 8.33	169.2,171.5	8.33
		3.72 (dd, 6.0, 17.0 Hz)	3.62, 8.33	169.2, 171.5	8.33
C═O	169.2				
Leu[5]					
NH		7.50 (br d, 7.0 Hz)	4.30		4.30, 8.25, 8.33
α	50.8[d]	4.30[b]	1.50, 1.57, 7.50	36.0,171.4	1.50
β	40.2	1.50[b]	1.57, 4.30	22.9, 24.0, 21.6	0.85, 4.30
		1.57[b]	1.50, 4.30		
γ	24.0	1.50[b]	0.80, 0.85	22.9, 21.6	0.85, 4.30
δ	21.6	0.80 (d, 5.5 Hz)	1.50	24.0, 40.2	
δ′	22.9	0.85 (d, 5.5 Hz)	1.50	21.6, 24.0, 40.2	1.50
C═O	172.7[e]				
Asn[6]					
NH		8.25 (br d, 7.2 Hz)	4.30	172.7	4.30, 7.50, 8.05
α	51.0[d]	4.30[b]	2.52, 2.72, 8.25	36.0	2.52, 2.72
β	36.0[f]	2.52[b]	2.72, 4.30	51.0, 171.8, 170.2	2.72, 4.30
		2.72 (dd, 5.5, 15.0 Hz)	2.52, 4.30		2.52, 4.30
C═O	171.0				
NH_2		6.85 (s)	7.38	36.0	7.38
		7.38 (s)	6.85		6.85
γ C═O	171.8				
Gly[7]					
NH		8.05 (br t, 6.0 Hz)	3.34, 3.85	171.0	3.34, 3.85, 4.30, 7.33, 8.25
α	42.8	3.34[b]	3.85, 8.05		8.05
		3.85[b]	3.34, 8.05		8.05
C═O	170.2				
Ile[8]					
NH		7.35 (br d, 6.6 Hz)	3.92	170.2	1.75, 3.92, 8.05
α	58.0	3.92 (d, 6.6 Hz)	1.75, 7.35	34.6	0.57, 7.35, 8.53
β	34.6	1.75 (m)	0.57, 1.00, 1.27, 3.92		0.57, 7.35
γ	24.6	1.00 (m)	0.73, 1.27, 1.75	10.4, 34.6	0.73, 1.27
		1.27[b]			1.00
γ′	14.8	0.57 (d, 6.6 Hz)	1.75	24.6, 34.6, 58.0	1.75, 3.92
δ	10.4	0.73 (t, 7.2 Hz)	1.00, 1.27	24.6, 34.6	1.00, 1.27
C═O	172.5[e]				
Phe[9]					
NH		8.53	4.28		4.28, 3.92, 8.15
α	55.4	4.28	2.97, 3.15	35.8	3.15, 8.53, 8.15
β	35.8[f]	2.97 (dd, 5.5, 15.0 Hz)	3.15, 4.28	55.4, 129.0, 138.1, 171.5	3.15, 4.28
		3.15 (dd, 5.5, 15.0 Hz)	2.97, 4.28	55.4, 129.0, 138.1, 171.5	2.97, 4.28
Ph: C-1	138.1				
C-2,6	129.0	7.18[b]	7.24	126.5, 129.0, 35.8	
C-3,5	128.2	7.24 (t, 6.5 Hz)	7.18	35.8, 129.0, 138.1	
C-4	126.5	7.18[b]	7.24		
C═O	171.4[c]				

[a] Recorded in CD_3SOCD_3 at 400 MHz (^1H NMR) and 100 MHz (^{13}C NMR). [b]Signal partially obscured. [c−f]Signals with the same letters may be interchanged.

glass capillary column (20 m × 0.31 mm) coated with Chirasil-L-Val (0.2 μm) and an HP 5973 mass selective detector operated in the SIMS mode.

Plant Material. The red latex of *Croton gossypifolius* Vahl. was collected in October 2002 from trees growing on the slopes alongside Lopinot Rd., Arouca, Trinidad, between the 8 and 8.5 km marks. The plant was identified by Mr. Winston Johnson of the National Herbarium of Trinidad and Tobago, where a voucher specimen [TRIN 34620] is deposited.

Extraction and Isolation. The red latex was suspended in H_2O/MeOH (3:1) and the mixture extracted successively with petroleum ether, $CHCl_3$, EtOAc, and n-BuOH. A reddish-white solid precipitated on concentration of the n-BuOH extract. The solid, B2A, obtained after filtration was shown by TLC (silica gel, $CHCl_3$/MeOH, 3:1) to consist of three components of R_f 0.58, 0.43, and 0.24. The highest R_f component gave a nonwetting spot on spraying heavily with ammonium molybdate. The solid, B2A (55 mg), was suspended in MeOH and applied to a Sephadex LH-20 column. The column was eluted first

with 100% $CHCl_3$, then with 10% $CHCl_3$/MeOH and finally with H_2O to give 32 fractions. TLC analysis enabled combination into four major fractions, I to IV. Fraction III on evaporation gave a white crystalline substance, which was recrystallized from MeOH/H_2O to give 21 mg of pure crystalline compound **1**. Evaporation of the EtOAc fraction E gave a yellow solid, which by TLC (silica gel, $CHCl_3$/MeOH, 3:1) was seen, on spraying with ammonium molybdate and heating, to consist of three components (R_f 0.58, 0.43, and 0.24). Chromatography on Sephadex LH-20 (200 mg, 10% MeOH/$CHCl_3$) afforded two fractions, which gave solids after evaporation of the solvent, namely, solid A, consisting of the two components of higher R_f, and solid B, consisting of the lowest R_f component. Solid A was subjected to preparative TLC (silica gel, $CHCl_3$/MeOH, 4:1) followed by passage of the isolated bands over Sephadex LH-20, eluting with $CHCl_3$/MeOH mixtures of increasing polarity. This yielded two compounds, which were shown by mp and by ^{1}H and ^{13}C NMR methods to be kaempferol 3-*O*-rhamnopyranoside[23,24] (2 mg) and quercitrin[25,26] (20 mg). Solid B was subjected to chromatography over Sephadex LH-20, eluting with $CHCl_3$/MeOH mixtures of increasing polarity, to yield three fractions. The middle fraction was recrystallized from MeOH/H_2O to give 14 mg of a compound, which proved to be myricitrin on the basis of its mp and its ^{1}H and ^{13}C NMR spectra.[25,26]

Crotogossamide (1): white crystals (MeOH/H_2O); mp 300 °C; $[\alpha]^{25}_D$ −48 (*c* 0.08, H_2O); IR (nujol) ν_{max} 3280, 1660, 1540, 1250 cm^{-1}; ^{1}H and ^{13}C NMR (Table 1); LRESIMS *m*/*z* 817.4 $[M + H]^+$; HRESIMS *m*/*z* 817.4221 (calcd for $C_{37}H_{57}N_{10}O_{11}$, 817.4202) $[M + H]^+$.

Absolute Configuration of the Amino Acids of 1. Compound **1** (1 mg) was hydrolyzed with 6 M DCl in D_2O by heating in a sealed tube at 110 °C. After removal of excess reagent with a stream of nitrogen, the sample was esterified with a mixture of 4 M DCl and methanol for 15 min at 110 °C. After cooling to about 50 °C, the excess reagent was again removed by a gentle stream of nitrogen and the residue was dissolved in 250 μL of trifluoroacetic anhydride/ethyl trifluoroacetate (1:1) and heated for 10 min at 130−140 °C. The residue obtained after removal of the excess reagent was dissolved in 150 μL of toluene, and 0.5 μL was injected into the gas chromatograph (Chirasil-L-Val capillary column, H_2 carrier gas, 1.5 mL/min flow rate, oven temperature at 65 °C for 3 min then increased at the rate 4 °C/min to 190 °C). The peaks corresponding to the eluted amino acid derivatives were detected using SIMS at *m*/*z* 190, 232, 232, 188, 205, and 161 for the appropriate nondeuterated ions of the derivatives of alanine, leucine, isoleucine, serine, asparagine, and phenylalanine, respectively. Retention times were noted. Similarly derivatized D- and L-amino acid standards were subjected to GC-MS analysis under identical conditions and retention times obtained for the derivatives as follows: D- and L-alanine: 4.72 and 5.46 min; D- and L-leucine: 11.18 and 12.63 min; D- and L-isoleucine: 9.18 and 10.37 min; D- and L-serine: 11.53 and 12.00 min; D- and L-asparagine: 14.40 and 14.58 min; D- and L-phenylalanine: 20.77 and 21.25 min. The data obtained indicated that all the amino acids of the peptide had the L-configuration.

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