Celogenamide A, a New Cyclic Peptide from the Seeds of *Celosia argentea*

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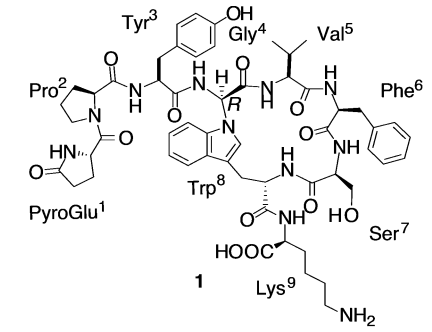
A new cyclic nonapeptide, celogenamide A (**1**), has been isolated from the seeds of *Celosia argentea*, and the structure including absolute stereochemistry was determined by using extensive 2D NMR and MS-MS methods and chemical means.

The seeds of *Celosia argentea* L. (Amaranthaceae) are used as a herbal drug for eye and hepatic diseases in China and Japan.[1] Previous studies have shown that the new bicyclic peptides, celogentins A−H and J,[2,3] and moroidin[4] from the seeds of *C. argentea* exhibited remarkable tubulin polymerization inhibition, whereas a new monocyclic peptide containing an oxindole ring, celogentin K,[5] did not. During our continued search for new compounds from *C. argentea*, a new cyclic peptide, celogenamide A (**1**), was isolated together with the known related peptides, lyciumins A and C methylates (**2** and **3**, respectively).[6] In this paper we describe the isolation and structure elucidation of **1**.

The seeds of *C. argentea* were extracted with MeOH, and the MeOH extract was in turn partitioned with hexane, EtOAc, and *n*-BuOH. The *n*-BuOH-soluble materials were subjected to a Diaion HP-20 column (MeOH−H_2O, 0:1 → 1:0), in which each fraction, eluted with 80% MeOH, was purified by an amino silica gel column ($CHCl_3$−MeOH−H_2O, 7:3:0.5 → 6:4:1) followed by C_{18} HPLC (30% CH_3CN−0.1% CF_3CO_2H) to give celogenamide A (**1**, 0.0002%) as a colorless solid together with lyciumins A and C[6] methylates (**2** and **3**, respectively).

The molecular formula of celogenamide A (**1**) was established as $C_{55}H_{69}N_{11}O_{13}$ by its HRFABMS data (*m/z* 1092.5190, $[M + H]^+$, Δ +3.6 mmu). The peptide nature of **1** was suggested from the IR (3090 and 1680 cm^{-1}) and NMR data (Table 1). Amino acid analysis of the hydrolysates of **1** showed the presence of 1 equiv each of Glu, Pro, Tyr, Val, Phe, Ser, and Lys. The ^{13}C NMR data included 10 carbonyl signals containing a PyroGlu residue, and the ^1H NMR data showed the presence of eight amide protons, indicating the nonapeptide nature of **1**. Detailed 2D NMR analysis of **1** revealed almost the same ^1H and ^{13}C signals as lyciumin C,[6] isolated from *Lycium chinense*, except for the Lys residue. The sequence of Pro[2]-Tyr[3]-Gly[4]-Val[5]-Phe[6]-Ser[7]-Trp[8]-Lys[9] in **1** was elucidated by NOESY correlations between the amide proton and each adjacent amino acid residue as shown in Figure 1. The HMBC correlation of NH of Lys[9] to the carbonyl carbon of Trp[8] (δ 170.63) also provided the sequence of Trp[8]-Lys[9]. The nitrogen atom of indole ring in Trp[8] was suggested to be connected to C-α in Gly[4] from NOESY correlations of H-α of Gly[4]/H-2 and H-7 of Trp[8] (Figure 1). Furthermore, the NOESY correlation of H-δ of Pro[2]/H-α of PyroGlu[1] allowed the sequence to be extended to PyroGlu[1]-Pro[2]-Tyr[3]-Gly[4]-Val[5]-Phe[6]-Ser[7]-Trp[8]-Lys[9]. The remaining NOESY correlations (Table 1) observed for **1** also supported the proposed sequence.

Further evidence supporting the proposed structure of **1** was provided by tandem mass spectrometry through examination of the collision-induced dissociation (CID) mass spectrum of the $[M + H]^+$ ions.[7] Positive-ion FABMS-MS spectra of **1** showed product ion peaks generated by fission at some amide bonds (Figure 2). The absolute configuration of each amino acid in **1** was assigned with all L-configurations by chiral HPLC analysis of the hydrolysates of **1**. The remaining configuration of the C−N linkage at the C-α in Gly[4] was assigned *R* by the ^1H and ^{13}C NMR data and NOESY relationships (Table 1) compared with those of a known related peptide, lyciumin A.[8] Therefore, the structure of **1** was assigned as shown.



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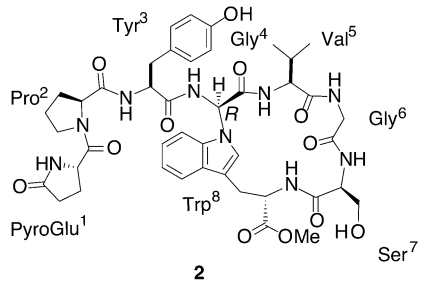

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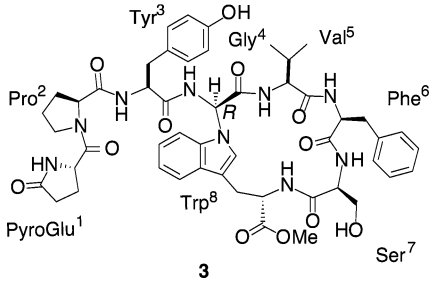

3

FABMS data of compounds **2** {$[\alpha]^{24}_D$ −6° (*c* 0.5, DMSO)} and **3** {$[\alpha]_D^{24}$ −13° (*c* 0.3, DMSO)} showed pseudomolecular ions at *m/z* 888 $[M + H]^+$ and 1016 $[M + K]^+$, respectively, and the molecular formulas $C_{43}H_{53}N_9O_{12}$ and $C_{50}H_{59}N_9O_{12}$ were established by HRFABMS (*m/z* 888.3883, $[M + H]^+$, Δ −0.9 mmu and 1016.3931, $[M + K]^+$, Δ +1.0 mmu,

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Table 1. ^{1}H (δ_H) and ^{13}C NMR Data (δ_C) of Celogenamide A (**1**) in DMSO-d_6

		δ_H [int. mult, J(Hz)]		δ_C	NOE relationship
PyroGlu[1]					
	α	4.35 (1H, m)	α	54.84	PyroGlu[1]: H-β, NH
	β	1.72 and 2.25 (each 1H, m)	β	23.66	PyroGlu[1]: H-γ; Pro[2]: H-δ
	γ	2.10 (2H, m)	γ	28.87	
	NH	7.69 (1H, s)	δ	177.13	
			C=O	170.80	
Pro[2]					
	α	4.36 (1H, m)	α	54.72	Pro[2]: H-β, H-γ, H-δ
	β	1.97 (2H, m)	β	52.51	Pro[2]: H-γ
	γ	1.81 (2H, m)	γ	28.27	Pro[2]: H-δ
	δ	3.58 (1H, m)	δ	46.02	
		3.37 (1H, m)			
			C=O	170.71	
Tyr[3]					
	α	4.38 (1H, m)	α	59.10	Tyr[3]: H-β, NH, H-2, H-6; Gly[4]: NH
	β	2.75 (1H, m)	β	35.69	Tyr[3]: NH, H-2, H-6; Gly[4]: NH
		2.67 (1H, dd, 14.2, 8.0)			
	H-2, 6	6.75 (2H, d, 8.4)	C-4	155.62	
	H-3, 5	6.39 (2H, d, 8.6)	C-3, 5	114.60	Tyr[3]: H-2, H-6
			C-2, 6	129.67	
			C-1	126.41	
	NH	7.91 (1H, m)	C=O	171.75	Trp[8]: H-7
Gly[4]					
	α	6.64 (1H, d, 8.8)	α	61.08	Gly[4]: NH; Val[5]: NH; Trp[8]: H-2
	NH	9.35 (1H, d, 7.8)	C=O	166.41	Val[5]: H-α, H-β, NH
Val[5]					
	α	3.68 (1H, m)	α	60.87	Val[5]: H-β, H-γ, NH; Phe[6]: NH
	β	2.00 (1H, m)	β	25.56	Val[5]: H-γ, NH; Phe[6]: NH
	γ	0.79 (3H, t, 6.5)	γ	18.83	Val[5]: NH; Phe[6]: NH
		0.68 (3H, t, 6.5)		18.41	
	NH	7.81 (1H, d, 7.2)	C=O	170.54	
Phe[6]					
	α	4.29 (1H, q, 8.0)	α	55.65	Phe[6]: H-β, NH, H-2; Ser[7]: NH
	β	3.05 (1H, m)	β	36.62	Phe[6]: NH
		3.23 (1H, dd, 4.7, 14.2)	C-1	137.35	
	H-2, 6	7.20 (2H, m)	C-2, 6	129.60	
	H-3, 5	7.25 (2H, m)	C-3, 5	128.83	
	H4	7.19 (1H, br s)	C-4	126.14	
	NH	8.25 (1H, d, 8.0)	C=O	172.92	Ser[7]: NH
Ser[7]					
	α	4.13 (1H, q, 6.5)	α	54.64	Ser[7]: H-β, NH; Trp[8]: NH
	β	3.70 (1H, m)	β	60.87	Ser[7]: NH; Trp[8]: NH
		3.56 (1H, m)			
	NH	8.14 (1H, d, 5.5)	C=O	166.41	Trp[8]: NH
Trp[8]					
	α	4.72 (1H, t, 9.4)	α	51.66	Trp[8]: H-β, NH, H-2; Lys[9]: NH
	β	3.29 (1H, m)	β	26.11	Trp[8]: H-2, H-4, H-5; Lys[9]: NH
		3.00 (1H, m)	C-2	123.87	
	H2	7.14 (1H, s)	C-3	111.58	
	H4	7.50 (1H, d, 8.4)	C-4	118.14	Lys[9]: H-α, NH
	H5	7.05 (1H, t, 7.2)	C-5	119.05	
	H6	7.16 (1H, m)	C-6	121.37	
	H7	7.41 (1H, d, 8.3)	C-7	109.47	
	NH	8.38 (1H, d, 8.3)	C-8	135.86	
			C-9	127.67	
			C=O	170.63	
Lys[9]					
	α	4.18 (1H, m)	α	51.54	Lys[9]: H-β
	β	1.72, 1.59 (each 1H, m)	β	30.24	
	γ	1.34 (2H, m)	γ	22.04	
	δ	1.53 (2H, m)	δ	26.31	
	ϵ	2.76 (2H, m)	ϵ	38.46	
	NH	7.92 (1H, m)	C=O	171.50	

respectively). IR absorptions of **2** and **3** implied the presence of ester (1730 cm^{-1}) and amide carbonyl groups (1675 cm^{-1}). The detailed analysis of the ^{1}H and ^{13}C NMR data of **2** and **3** including their ^{1}H−^{1}H COSY, TOSCY, HMQC, and HMBC spectra revealed that **2** and **3** were lyciumins A[6] and C[6] methyl esters.

Celogenamide A (**1**) is a new 17-membered cyclic peptide containing a unique connection between the C-α carbon of Gly[4] and a nitrogen of the indole ring of Trp[8]. Celogenamide A (**1**) and lyciumins A and C methylates (**2** and **3**, respectively) did not inhibit polymerization of tubulin at 100 μM (IC_{50} >100 μg/M).[2,3]

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO P-1030 polarimeter. UV spectra were recorded on a Shimadzu UV1600PC spectrophotometer and IR spectra on a JASCO FTIR-230 spectrometer. ^{1}H and 2D NMR spectra were recorded on a 600 MHz spectrometer at 300 K, while ^{13}C NMR spectra were measured on a 150 MHz

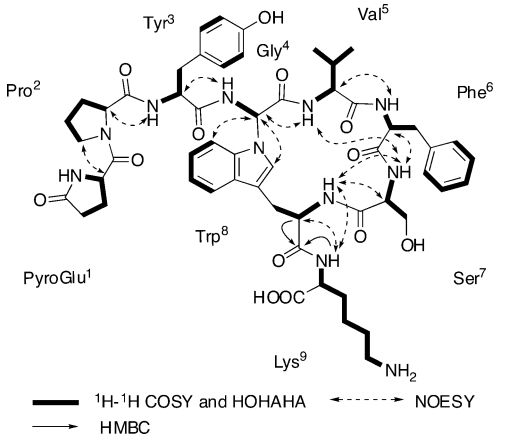

Figure 1. Selected 2D NMR correlations of celogenamide A (**1**).

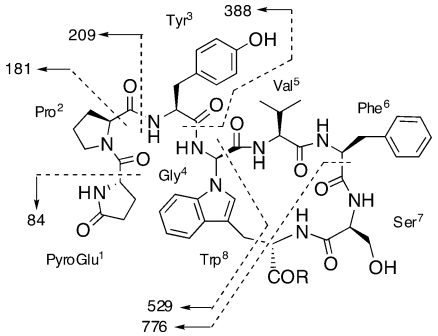

Figure 2. Fragmentation patterns observed in the negative ion FABMS-MS spectrum of celogenamide A (**1**) (precursor ion *m*/*z* 1092, R = L-Lys).

spectrometer. Compounds **1**−**3** were prepared by dissolving 1.0 mg in 30 μL of DMSO-d_6 in 2.5 mm micro cells (Shigemi Co. Ltd., Tokyo, Japan), and chemical shifts were reported using residual DMSO (δ_H 2.50 and δ_C 39.5) as internal standard. Standard pulse sequences were employed for the 2D NMR experiments. ^{1}H−^{1}H COSY, HOHAHA, and NOESY spectra were measured with spectral widths of both dimensions of 4800 Hz, and 32 scans with two dummy scans were accumulated into 1K data points for each of 256 t_1 increments. NOESY and HOHAHA spectra in the phase-sensitive mode were measured with a mixing time of 800 and 30 ms, respectively. For HMQC spectra in the phase-sensitive mode and HMBC spectra, a total of 256 increments of 1K data points were collected. For HMBC spectra with the *Z*-axis PFG, a 50 ms delay time was used for long-range C−H coupling. Zero-filling to 1K for F_1 and multiplication with squared cosine-bell windows shifted in both dimensions were performed prior to 2D Fourier transformation.

MS Experiments. FABMS were measured on a JEOL JMS-HX110 by using glycerol as the matrix. FABMS-MS spectra were recorded on a JEOL JMS-700TZ tandem mass spectrometer equipped with a CCD array detector using the magic bullet as a matrix. The mass spectrometer was operated at an accelerating voltage of 10 kV xenon beam and in the negative mode.

Plant Material. The seeds of *Celosia argentea* were purchased from Uchida Wakannyaku Co., Tokyo, Japan, in 1996. The botanical identification was made by Mr. N. Yoshida, Health Sciences University of Hokkaido. A voucher specimen (No. 960002) has been deposited in the herbarium of Hokkaido University.

Extraction and Isolation. The seeds (13.5 kg) of *C. argentea* (Lot. 1) were crushed and extracted with MeOH (18 L × 3), and the MeOH extract was in turn partitioned with hexane, EtOAc, and *n*-BuOH. The *n*-BuOH-soluble materials were subjected to a Diaion HP-20 column (MeOH−H_2O, 0:1 → 1:0), in which a fraction eluted with 80% MeOH was purified by an amino silica gel column ($CHCl_3$−MeOH−H_2O, 7:3:0.5 → 6:4:1) followed by C_{18} HPLC (CH_3CN−0.1% CF_3CO_2H, 3:7) to afford celogenamide A (**1**, 0.0002%) and lyciumins A and C^6 methylates (**2**, 0.00001%; **3**, 0.00003%).

Celogenamide A (1): colorless solid; $[\alpha]_D^{22}$ +3° (*c* 0.3, DMSO); UV (MeOH) λ_{max} (log ϵ) 283 (3.7) and 226 (4.2) nm; IR (KBr) ν_{max} 3280, 3090, 2960, 1680, 1530, and 1200 cm^{-1}; ^{1}H and ^{13}C NMR (Table 1); FABMS *m*/*z* 1092 $[M + H]^+$; HRFABMS *m*/*z* 1092.5190 [M + H; calcd for $C_{55}H_{70}N_{11}O_{13}$, 1092.5154].

Amino Acid Analysis of 1. Each solution of **1** (0.1 mg each) in 6 N HCl was heated at 110 °C for 24 h in a sealed tube. After cooling, each solution was concentrated to dryness. The hydrolysates were dissolved in 0.02 N HCl and were subjected to evaluation in an amino acid analyzer (nihydrin method).

Absolute Configuration of Amino Acids. Each solution of **1** (each 0.1 mg) in 6 N HCl (0.2 mL) was heated at 110 °C for 24 h. The solution was concentrated to dryness. The residue was dissolved in H_2O (50 μL), and chiral HPLC analyses were carried out using a SUMICHIRAL OA-5000 column (Sumitomo Chemical Industry, Osaka, Japan; 150 mm; 25 °C, detection at 254 nm). Retention times (min) of authentic amino acids were as follows: L-Glu (19.2), D-Glu (24.2), L-Tyr (7.4), D-Tyr (10.8), L-Val (6.1), D-Val (9.0), L-Phe (19.3), and D-Phe (27.3) [eluent: MeOH−H_2O (15:85) containing 2.0 mM $CuSO_4$, flow rate 1.0 mL/min], L-Pro (7.1), D-Pro (14.8), L-Ser (2.5), and D-Ser (2.8) [eluent: 1.0 mM $CuSO_4$, flow rate 1.0 mL/min], L-Lys (12.0) and D-Lys (13.6) [eluent: 1.0 mM $CuSO_4$, flow rate 0.2 mL/min]. Retention times of the hydrolysates of **1** were as follows: **1**, L-Glu (19.1), L-Pro (7.1), L-Tyr (7.4), L-Val (6.1), L-Phe (19.3), L-Ser (2.5), and L-Lys (12.0).

Microtubule Assembly Assay.[2,3] Microtubule assembly was monitored spectroscopically by using a spectrophotometer equipped with a thermostatically regulated liquid circulator. The temperature was held at 37 °C, and changes in turbidity were monitored at 400 nm. The turbidity changes were monitored throughout the incubation time.

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