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A pair of cyclopeptide epimers from the seeds of *Celosia argentea*

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[ABSTRACT] Two cyclopeptides, celogentin L (**1**) and its epimer lyciumin A (**2**) were firstly isolated from *Celosia argentea* L.. The planar structures of the two compounds were fully determined by spectroscopic data, including 1D-, 2D-NMR, and HR-ESI/MS. The absolute configurations of amino acid components were assigned *via* chiral-phase HPLC analyses after acid hydrolysis. Furthermore, the configuration of C-N linkage at the glycine Cα was elucidated by extensive analyses of 2D-NMR and comparison of the experimental and calculated electronic circular dichroism (ECD) spectra. Cytotoxicity of the two compounds against human alveolar epithelial A549, hepatocellular carcinoma HepG2, and cervical cancer Hela cell lines was assayed. Although both of them were inactive in these cells, the present findings add new facets for the chemistry of *Celosia argentea*.

[KEY WORDS] *Celosia argentea*; Cyclopeptides; Lyciumin A; Celogentin L; Absolute configuration

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Introduction

Plant cyclopeptides represent a large array of structurally complex natural products, which are defined as cyclic compounds formed with the peptide bonds of 2−37 protein or non-protein amino acids, mainly L-amino acids [1]. Since cyclolinopeptide A was isolated and determined from *Linum usitatissimum* in 1959 by Kaufmann and Tobschirbel [2-5], over 450 cyclopeptides with intriguing skeletons have been discovered from high plants [6]. These cyclopeptides showed a broad range of bioactivities, such as antimitotic [7], antihypertensive [8], antileishmanial [9], anti-HIV [10-11], cytotoxic [12], and immunosuppressive [4] effects. The fantastic structures and the demonstrated bioactivities of naturally occurring cyclopeptides have attracted sustaining attentions from phytochemists in recent years [12-15].

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The seeds of *Celosia argentea* L. (Amaranthaceae) have been used for a long time in China and Japan as a therapeutic drug for eye and hepatic diseases [16-17], from which many cyclopeptides such as bicyclic peptides celogentins A−C [7], D−H, J [18], K and moroidin [19] and monocyclic peptides celogenamide A, lyciumins A and C methylates [20] have been isolated. Continuing our interest in cyclopeptides in *Celosia argentea* L, we found a pair of epimers, celogentin L (**1**) and lyciumin A (**2**) (Fig. 1) and tested their cytotoxitity on human alveolar epithelial A549, hepatocellular carcinoma HepG2, and cervical cancer Hela cell lines. In this paper, we report the isolation, structure elucidation, and cytotoxicity assay of the two compounds.

Results and Discussion

Compound **1** was negative to ninhydrin but positive after hydrolysis with concentrated HCl [21]. The molecular formula determined to be $C_{42}H_{51}N_9O_{12}$ by HR-ESI/MS ($[M + H]^+$ at *m/z* 874.375 0, Calcd. for $C_{42}H_{51}N_9O_{12}$, 874.373 0), indicating 22 degrees of unsaturation. The ^{1}H NMR spectrum revealed some characteristic amide and phenyl signals ($\delta_H > 6.55$) and several Hα singlets (δ_H 3.34−4.55). The ^{13}C NMR spectrum showed obviously nine amide carbonyl carbons in the δ_C 165.87−177.31 regions and 12 signals corresponding to 14 phenyl carbons in the δ_C 111.30−155.79 regions. These data, combined with those of HSQC, HMBC, COSY, and TOCSY, suggested that compound **1** was a cyclopeptide [22] and the

amino acid residues were identified as one proline, tyrosine, valine, serine, tryptophan, pyroglutamic acid, and two glycines. The residues were also confirmed by chiral HPCL after acid hydrolysis and assigned as L-configurations.

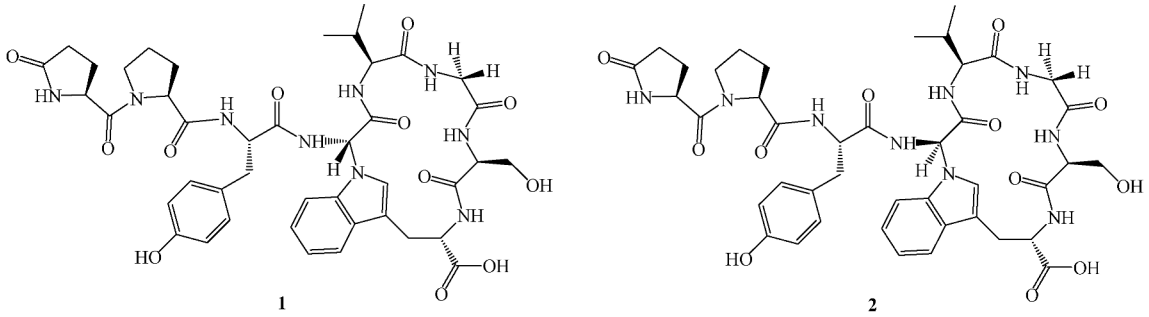

Fig. 1 Structures of celogentin L (1) and its epimer lyciumin A (2)

The sequence of these amino acid residues were determined using ^{1}H-^{1}H COSY, HMBC and ROESY correlations (Fig. 2) and verified by MS/MS fragmentation. The ROESY correlations between the amine proton and each adjacent amino acid residue (Hα of PyroGlu^{1}/NH of Pro^{2}, Hα of Pro^{2}/NH of Tyr^{3}, Hα of Tyr^{3}/NH of Gly^{4}, Hα of Gly^{4}/NH of Val^{5}) suggested the sequence of PyroGlu^{1}-Pro^{2}-Tyr^{3}-Gly^{4}-Val^{5}. ^{1}H-^{1}H COSY correlations such as Hα of PyroGlu^{1}/Hδ of Pro^{2}, Hα of Pro^{2}/NH of Tyr^{3} and HMBC correlation between NH of Val^{5} and C=O of Gly^{4} also allowed the above sequence. The C=O of Val^{5} was correlated to Hα and NH of Gly^{6}, as well as Hα of Val^{5} was correlated to NH of Gly^{6} to form the PyroGlu^{1}-Pro^{2}-Tyr^{3}-Gly^{4}-Val^{5}-Gly^{6} structure partially. HMBC correlations of Hα of Ser^{7}/C=O of Gly^{6} and NH of Trp^{8}/C=O of Ser^{7} indicated Ser^{7} bonded with Gly^{6} and Trp^{8}. In addition, the ROESY correlations between H2 and H7 of Trp^{8} and Hα of Gly^{4} as well as HMBC correlation between H8 of Trp^{8} and Hα of Gly^{4} indicated that Gly^{4}-Val^{5}-Gly^{6}-Ser^{7}-Trp^{8} was a ring structure. Furthermore, ^{1}H NMR data showed the signal of Gly^{4} Hα (δ_H 6.86, 1H, d) was obviously shifted downfield compared to that of Gly^{6} Hα (δ_H 3.34, 2H, m), indicating that Cα of Gly^{4} was attached to electron-withdrawing group. Taken together, those DEPT, HMBC and ROESY data indicated that Cα of Gly^{4} was bonded to N1 of Trp^{8}.

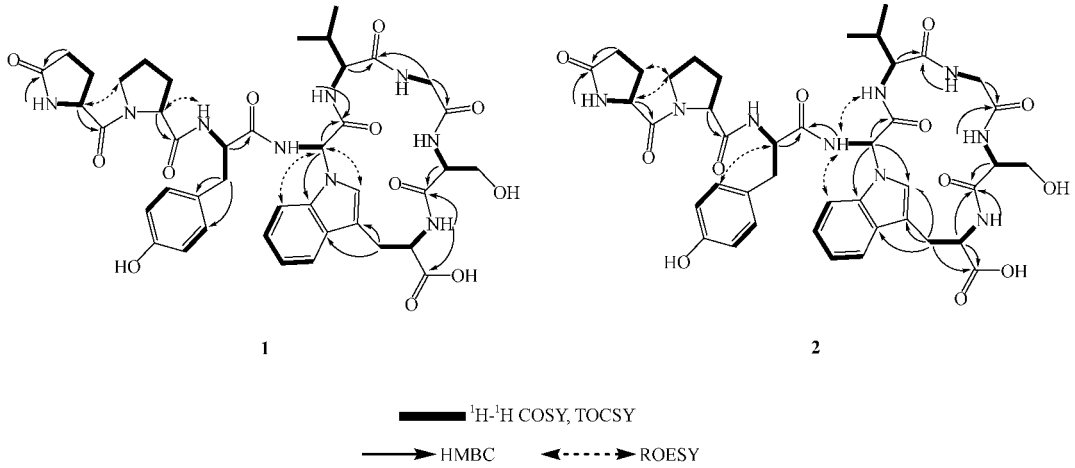

Fig. 2 Key 2D-NMR correlations of celogentin L (1) and its epimer lyciumin A (2)

The proposed structure was also in agreement with the MS/MS data. According to literature, three common fragmentation patterns in peptide slitting process are b_x-y_z, $b_x \rightarrow b_{x-1}$ and $b_x \rightarrow a_x$, in which b_x-y_z plays important role in linear peptide sequence, supported by the loss of neutral molecules like H_2O, NH_3 and CO, while $b_x \rightarrow b_{x-1}$ is the most powerful evidence for cycle peptide sequence [23]. In our MS/MS spectrum, a series of crucial signals in accord with the above fragmentation rules were found. Firstly, the most abundant ion had an *m/z* value of 468, which was consistent with the loss of side chain S (PyroGlu^{1}-Pro^{2}-Tyr^{3}, S^{+} 372) together with neutral molecule NH_3. Three pairs of complementary ions including 666/209, 503/372, and 486/389 were assigned to the fragments of B6/S2, B5/S3, and B5'/S3', respectively (Fig. 3). And the losses of 208 Da and 163 Da also confirmed the side chain S (PyroGlu^{1}-Pro^{2}-Tyr^{3}, S^{+} 372). Secondly, the cycle peptide residue B dissociated into fragments B5', B4', B3', and B2', elucidating the sequence of

Gly[4]-Val[5]-Gly[6]-Ser[7]-Trp[8]. Therefore, the structure of **1** was established as (Gly[4] Cα, Trp[8] indole N1)-cyclo-L-PyroGlu[1]-L-Pro[2]-L-Tyr[3]-L-Gly[4]-L-Val[5]-L-Gly[6]-L-Ser[7]-L-Trp[8].

Compound **2** was negative to ninhydrin but positive after hydrolysis with concentrated HCl [21]. The molecular formula determined to be $C_{42}H_{51}N_9O_{12}$ by HR-ESI/MS ([M + H]$^+$ at *m/z* 874.375 0, Calcd. for $C_{42}H_{51}N_9O_{12}$, 874.373 0). The ^{1}H and ^{13}C NMR data were very similar to those of compound **1**, indicating the present of the same amino acids. The planar structure was confirmed to be identical with **1** according to ^{1}H-^{1}H COSY, HMBC, and ROESY correlations and all amino acid residues were assigned as L-configurations.

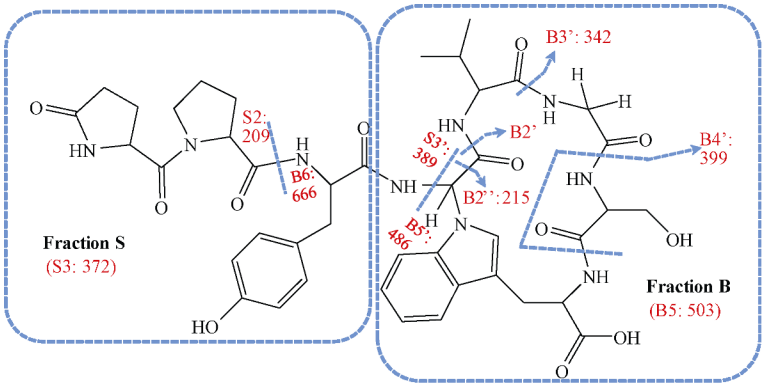

Fig. 3 Fragmentation patterns observed in the positive ion HR-ESI/MS spectrum of compound 1

^{13}C NMR spectrum showed that major differences were the signals of C=O of PyroGlu[1], Cα of Tyr[3], $\delta_{C2,6}$ of Tyr[3], C=O of Tyr[3], Cα of Gly[4], C=O of Gly[4], Cα of Trp[8], C2 of Trp[8] and Cα of Val[5] (δ_C 171.39, 54.75, 129.12, 171.52, 61.5, 166.21, 52.05, 124.26, 109.63, 59.39 in compound **2** while δ_C 169.57, 53.36, 130.21, 170.54, 59.8, 165.87, 53.05, 123.88, 111.3, 58.41 in compound **1**). The ROESY correlations including NH of Trp[8]/H2 of Trp[8], H2 of Trp[8]/NH of Gly[4], H2 of Trp[8]/Hα of Gly[4] and NH of Val[5]/Hα of Gly[4] in compound **2** implied that the configuration of Gly[4] Cα was *R* [24], while no such correlations were observed in compound **1**, elucidating the configuration of Gly[4] Cα compound **1** was different. The absolute configuration of Gly[4] Cα was finally determined by comparing the experimental ECD and calculated ECD. The theoretically calculated ECD spectra were in good accordance with the experimental ECD spectra, as shown in Fig. 4, revealing that the absolute configurations of Gly[4] Cα were *S* in compound **1** and *R* in compound **2**.

Conclusively, compound **2** was structurally identical with lyciumin A, which was firstly isolated from *Lycium chinense* (Solanaceae) and the absolute configuration of Gly[4] Cα in the proposed molecule was determined to be *R* by a computer-simulated experiment using the Monte Carlo and ROE restrained molecular dynamics calculation [24]. On this basis, we identified a new cyclopeptide (compound **1**) from the seeds of *Celosia argentea* by 2D-NMR, chiral HPLC and ECD calculation analyses and named celogentin L.

The cytotoxicity of the two compounds against three human cancer cell lines, including A-549, HepG2, and Hela, was evaluated using MTT method, with 5-FU as the positive control. Unfortunately, neither of them showed significant inhibitory effects on these tested cancer cell lines (IC_{50} > 100 μmol·L^{-1}).

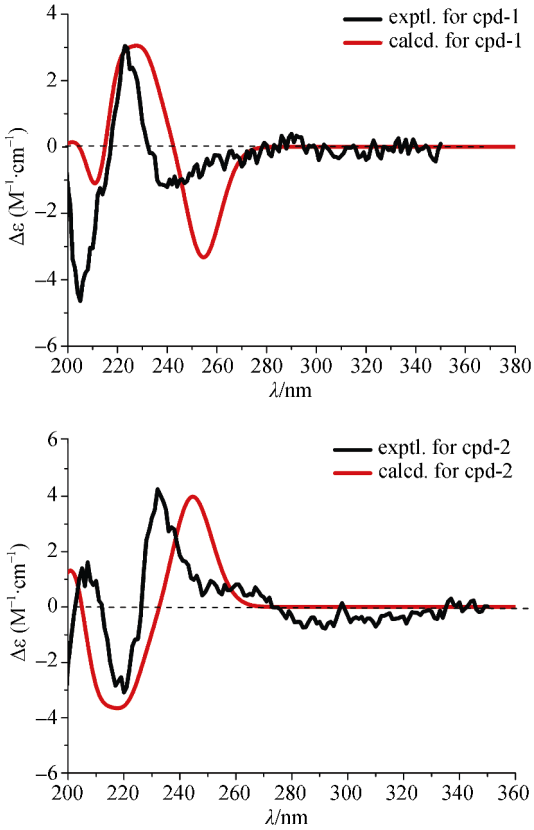

Fig. 4 Experimental ECD and calculated ECD spectra of compounds 1 and 2

Experimental

General experimental procedures

Optical rotations were recorded on an AutoplIV-T automatic polarimeter (Rudolph Research Analytical, USA).

HR-ESI/MS data were acquired in the positive ion mode on an Agilent Technologies liquid chromatograph connected to Q-TOF mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). Electronic circular dichroism (ECD) data were acquired on a JASCO J-810 spectropolarimeter (JASCO Co., Tokyo, Japan). All 1D- and 2D-NMR spectra were obtained on a Bruker Avance III 500 NMR (Bruker, Zurich, Switzerland) spectrometer with TMS as internal standard. Chemical shifts (δ) were expressed in ppm with reference to the solvent signals.

Column chromatography (CC) was performed on D101 macroporous resin (Anhui Sanxing Resin Technology Co., Ltd., Anhui, China), silica gel (200–300 mesh; Qingdao Marine Chemical Inc., China), and RP-18 silica gel (50 μm, YMC Co., Ltd., Tokyo, Japan). Thin layer chromatography (TLC) was carried out with glass precoated with silica gel GF254 (Yantai Wish chemical products Co., Ltd, Yantai, China). Semi-preparative HPLC separation was performed with an Agilent 1100 HPLC (Agilent Technologies, Santa Clara, CA, USA) system using a Shim-Pack C_{18} column (10 mm × 250 mm, 5 μm, Shimadzu Co., Tokyo, Japan) at a flow rate of 3 mL·min^{-1}, with monitoring at 203 nm.

Plant materials

The seeds of *Celosia argentea* were purchased from Bozhou, Anhui Province, China. The botanical identification was made by Professor LI Hui-Jun, China Pharmaceutical University. A voucher specimen (No. 20140903) was deposited in the State Key Laboratory of Natural Medicines, Nanjing, China.

Extraction and isolation

The seeds (50 kg) of *Celosia argentea* were crushed and extracted with 70% EtOH thrice (50 L, 1 h for each). The solvent was concentrated to obtain a residue (50 L). The extract was suspended in H_2O and chromatographed over D101 macroporous resin column using EtOH/H_2O (0 : 100, 30 : 70, 40 : 60 and 95 : 5, *V/V*) as eluent to give four fractions (A−D). The Fr. B (320 g) was purified by silica gel column stepwise eluting with CH_2Cl_2−MeOH (9 : 1−0 : 10, *V/V*) and then MeOH−H_2O (10 : 0−3 : 7, *V/V*) to obtain four subfractions (B_1−B_4). Fr. B_3 (50 g) was further submitted to a RP-18 silica gel column eluting with MeOH−H_2O (1 : 9−7 : 3, *V/V*) to obtain Fr. B_{3a}−B_{3d}. Fr. B_{3c} (400 mg) was then purified by semi-preparative HPLC using CH_3CN−0.1% HCOOH/ H_2O (22 : 78, *V/V*) solvent system to afford Compounds **1** (15 mg) and **2** (11.5 mg).

Table 1 1D and 2D NMR spectroscopic data (δ, *J* in Hz) of compound 1 (in DMSO-d_6)

		δ_H		δ_C	HMBC H→C	ROESY
PyroGlu[1]						
	α	4.32 or 4.55 (1H, m)	*α*	53.66	PyroGlu[1]: C*β*, C*γ*	PyroGlu[1]: NH; Pro[2]: H*δ*
	β	2.10 and 2.25 (2H, m)	*β*	23.97		
	γ	1.86 and 2.09 (2H, m)	*γ*	29.02		
	NH	7.72 (1H, s)	*δ*	177.31		
			C=O	170.61		
Pro[2]						
	α	4.28 (1H, m)	*α*	59.33	Pro[2]: C*β*, C*γ*, C=O	Pro[2]: H*β*, H*γ*; Tyr[3]: NH
	β	1.85, 1.77 (2H, m)	*β*	28.52		
	γ	1.87, 1.67 (2H, m)	*γ*	24.15		
	δ	3.36 and 3.52 (2H, m)	*δ*	46.05		
			C=O	170.94		
Tyr[3]						
	α	4.42 (1H, m)	*α*	53.36	Tyr[3]: C*β*, C*γ*, C=O	Tyr[3]: H*β*, Hδ_2, NH; Gly[4]: NH
	β	2.70 and 2.86 (2H, m)	*β*	36.31	Tyr[3]: C*γ*, Cδ_2, Cδ_6	Tyr[3]: Hδ_2, Hδ_6; Gly[4]: NH
	γ	-	*γ*	126.93		
	δ_2	6.91 (1H, d, 8.5)	*δ*2	130.21	Tyr[3]: Cδ_6, *ζ*	Tyr[3]: $\varepsilon_{3,5}$, NH
	δ_6	6.97 (1H, d, 8.5)	*δ*6	129.93	Tyr[3]: Cδ_2, *ζ*	Tyr[3]: $\varepsilon_{3,5}$
	$\varepsilon_{3,5}$	6.55 (2H, d, 8.5)	*ε*3,5	114.66	Tyr[3]: C*γ*, Cδ_2, Cδ_6, *ζ*	
	ζ	-	*ζ*	155.79		
	NH	7.61 (1H, d, 8.0)	C=O	170.54	Tyr[3]: C*α*	Tyr[3]: H*α*; Gly[4]: H*α*; Trp[8]: H6
Gly[4]						
	α	6.86 (1H, d, 9.0)	*α*	59.80	Gly[4]: C=O	Gly[4]: NH; Trp[8]: H2, H7; Val[5]: NH
	NH	8.94 (1H, m)	C=O	165.87	Val[5]: C*α*	Gly[4]: H*α*; Trp[8]: H2, H7

Continued

		δ_H		δ_C	HMBC H→C	ROESY
Val[5]						
	α	4.21 (1H, m)	α	58.41	Val[5]: C=O, Cβ	Val[5]: Hβ, Hγ_1, NH
	β	1.96 (1H, m)	β	29.68	Val[5]: Cα, Cγ_1, Cγ_2	Val[5]: Hα, Hγ_1, Hγ_2
	γ_1	0.92 (3H, d, 5.5)	γ1	18.52	Val[5]: Cβ, Cγ_2	
	γ_2	0,89 (3H, d, 7.0)	γ2	19.22	Val[5]: Cβ, Cγ_1	
	NH	8.94	C=O	171.27	Val[5]: Cα, Cβ; Gly[4]: C=O	Gly[4]: Hα
Gly[6]						
	α	3.34 (2H, m)	α	43.13	Gly[6]: C=O, Val[5]: C=O	Gly[6]: NH
	NH	8.44	C=O	169.30	Gly[6]: C=O,Val[5]: C=O	Gly[6]: Hα, Val[5]: Hα
Ser[7]						
	α	4.19 (1H, m)	α	55.17	Ser[7]: Cβ, Gly[6]: C=O, Ser[7]: C=O	Ser[7]: Hβ, NH; Trp[8]: NH
	β	3.48 (2H, m)	β	60.71	Ser[7]: Cα	Trp[8]: NH; Ser[7]: Hα
	NH	8.97	C=O	169.57		Ser[7]: Hα
Trp[8]						
	α	4.31 (1H, m)	α	53.05 53.37	Trp[8]: Cβ, C3	Trp[8]: Hβ, H4; Trp[8]: NH
	β	2.99 and 3.21 (2H, m)	β	26.68	Trp[8]: C2, C3, C9	Trp[8]: H2, H4, Hα, NH
	2	7,12 (1H, s)	2	123.88	Trp[8]: C3, C8, C9	Trp[8]: Hβ
	3	-	3	111.67		
	4	7.52 (1H, d, 8.0)	4	117.97	Trp[8]: C3, C6, C8, C9	Trp[8]: Hα, Hβ, H5
	5	7.08 (1H, t, 7.5)	5	119.45	Trp[8]: C7, C8, C9; Tyr[3]: Cδ_2	Trp[8]: H4,
	6	7.15 (1H, m)	6	121.35	Trp[8]: C4, C8, C7	Gly[4]: Hα; Trp[8]: H7
	7	7.60 (1H, d, 7.5)	7	111.30	Trp[8]: C5, C8, C9	Gly[4]: Hα; Trp[8]: H6,
	8	-	8	135.91		
	9	-	9	128.01		
	NH	7.91 (1H)	C=O	173.13	Trp[8]: C=O; Ser[7]: Cα, C=O	Trp[8]: Hα; Ser[7]: Hα

Celogentin L (**1**), White, amorphous power; $[\alpha]_D^{25}$ +5.28º (*c* 0.068, DMSO); HR-ESI/MS *m/z* 874.375 0 $[M + H]^+$ (Calcd. for $C_{42}H_{51}N_9O_{12}$, 874.373 0); ^1H and ^{13}C NMR data are shown in Table 1.

Lyciumin A (**2**), Offwhite, amorphous power; $[\alpha]_D^{25}$ −27.03º (*c* 0.056, DMSO); positive HR-ESI/MS *m/z* $[M + H]^+$ 874.375 2 (Calcd. for $C_{42}H_{51}N_9O_{12}$, 874.373 0).

Absolute configuration of amino acids

The absolute configurations of PyroGlu[1], Pro[2], Tyr[3], Val[6] and Ser[7] were determined by chiral HPLC after acid hydrolysis according to literature [7] with slight modification. Briefly, each solution of **1** and **2** (0.2 mg) in 6 N HCl (0.4 mL) was heated at 110 °C for 24 h and then concentrated to dryness. The residue was dissolved in H_2O (200 μL) to obtain the test solution, 10 μL of which was injected into chiral HPLC system with a SUMICHIRAL OA-5000 column (4.6 mm × 150 mm, 5 μm, Sumitomo Chemical Industry, Japan) maintained at 40 °C and detected at 254 nm. MeOH−H_2O (15 : 85, *V/V*) containing 2 mmol·L^{-1} $CuSO_4$ was used as the mobile phase at a flow rate of 1 mL·min^{-1}. Retention times (min) of authentic amino acids were as follows: L-Glu (23.3), D-Glu (25.1), L-Pro (3.7), D-Pro (8.2), L-Tyr (12.1), D-Tyr (17.3), L-Val (6.0), D-Val (9.2), L-Ser (3.1), D-Ser (3.3). Retention times (min) of the hydrolysates of **1** and **2** were as follows: **1**, L-Glu (23.0), L-Pro (3.7), L-Ser (3.1), L-Val (5.9), L-Tyr (12.1); **2**, L-Glu (23.4), L-Pro (3.7), L-Ser (3.1), L-Val (5.9), L-Tyr (12.2).

Absolute configuration of Trp

Trp[8] was completely destructed during acid hydrolysis. Herein, it was transformed into Asp by treatment with O_3/AcOH and then H_2O_2 before acid hydrolysis. Chiral HPLC analysis of Asp in the degradation products revealed the configuration of Trp[8] [7]. Each solution of **1** and **2** (0.2 mg) in 0.4 mL of AcOH was treated with O_3 at −78 °C for 1 min. After removal of excess ozone by a stream of nitrogen, the mixture was treated with H_2O_2 at room temperature for 3 h. Then the mixture was concentrated to dryness and the residue dissolved with 6 N HCl was heated at 110 °C for 6 h. The remaining procedures were same as above mentioned. The retention times of authentic L- and D-Asp were found to be 13.5 and 17.4 min, respectively. The retention times of Asp in

the degradation products of **1** and **2** were found to be 13.6 min (L-Asp).

ECD calculations

All calculations were performed with the Gaussian 09 program package (Gaussian, Inc., Wallingford, CT, USA). The relative configurations were submitted to random conformational analysis with the MMFF94s force field and using the Sybyl-X 1.1 software package. The conformers were further optimized using the TDDFT method at the B3LYP/6-31G (d) level and the frequency was calculated at the same level of theory. And finally, four stable conformers of **1** and five stable conformers of **2** were obtained. These stable conformers without imaginary frequencies were subjected to ECD calculation by the TDDFT method at the B3LYP/6-31+G (d) level in MeOH as solvent. ECD spectra of different conformers were simulated using SpecDis (http:/specdis-software.jimdo.com) with a half-band width of 0.16–0.3 eV, and the final ECD spectra were obtained according to the Boltzmann-calculated contribution of each conformer [25-26].

Cytotoxic activity assay

Three human tumor cell lines including A-549, HepG2, and Hela were cultured in DMEM supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 U·mL^{-1} penicillin and 100 μg·mL^{-1} streptomycin at 37 °C in an atmosphere containing 5% CO_2 and 95% air.

The cytotoxic effect against all the cells was determined using the MTT assay. Briefly, 100 μL of adherent cells was seeded into each well of 96-well cell culture plates and allowed to adhere for 24 h before drug addition, while suspended cells were seeded just before drug addition to an initial density of 5 × 10^4 cells/mL. Each tumor cell line was exposed to the tested compounds dissolved in DMSO at various concentrations in triplicate for 72 h, with 5-fluoro-2, 4-(1H, 3H)-pyrimidinedione (5-FU) as a positive control and cells without treatment were used as a control [27]. Formazan crystals were solubilized by addition of DMSO, and optical density (OD) value was measured at 490 nm. The percentage of cell growth inhibition was calculated based on a comparison with untreated cells.

$$\text{Cell growth inhibition rate} = \frac{OD_{control} - OD_{sample}}{OD_{control}} \times 100\%$$

All the experiments were run in triplicate. The data were expressed as means ± SEM (standard errors). Statistical analysis was performed by unpaired Student's *t*-test for two comparisons. $P < 0.05$ was considered statistically significant.

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References

[1] Tan NH, Zhou J, Plant cyclopeptides [J]. *Chem Rev*, 2006, **106**(3): 840-895.

[2] Blasio BD, Rossi F, Benedetti E, *et al*. Bioactive peptides: solid-state and solution conformation of cyclolinopeptide A [J]. *J Am Chem Soc*, 1989, **111**(25): 9089-9098.

[3] Matsumoto T, Shishido A, Morita H, *et al*. Conformational analysis of cyclolinopeptides A and B [J]. *Tetrahedron*, 2002, **58**(25): 5135-5140.

[4] Morita H, Shishido A, Matsumoto T, *et al*. Cyclolinopeptides B−E, new cyclic peptides from *Linum usitatissimum* [J]. *Tetrahedron*, 1999, **55**(4): 967-976.

[5] Matsumoto T, Shishido A, Morita H, *et al*. Cyclolinopeptides F–I, cyclic peptides from linseed [J]. *Phytochemistry*, 2001, **57**(2): 251-260.

[6] Tang J, Wang CK, Pan XL, *et al*. Isolation and characterization of bioactive cyclotides from *Viola labridorica* [J]. *Helv Chim Acta*, 2010, **93**(11): 2287-2295.

[7] Kobayashi J, Suzuki H, Shimbo K, *et al*. Celogentins A−C, new antimitotic bicyclic peptides from the seeds of *Celosia argentea* [J]. *J Org Chem*, 2001, **66**(20): 6626-6633.

[8] Yahara S, Shigeyama C, Nohara T, *et al*. Structures of anti-ace and -renin peptides from *Lycii radicis* cortex [J]. *Tetrahedron Lett*, 1989, **30**(44): 6041-6042.

[9] Medina RP, Schuquel ITA, Pomini AM, *et al*. Ixorine, a new cyclopeptide alkaloid from the branches of *Ixora brevifolia* [J]. *J Brazil Chem Soc*, 2016, **27**(4): 753-758.

[10] Daly NL, Gustafson KR, Craik DJ. The role of the cyclic peptide backbone in the anti-HIV activity of the cyclotide kalata B1 [J]. *FEBS Lett*, 2004, **574**(1): 69-72.

[11] Gustafson KR, Mckee TC, Bokesch HR. Anti-HIV cyclotides [J]. *Curr Protein Pept Sci*, 2004, **5**(5): 331-340.

[12] Figueiredo PO, Matos MFC, Perdomo RT, *et al*. Rubiaceae-type cyclopeptides from *Galianthe thalictroides* [J]. *J Nat Prod*, 2016, **79**(4): 1165-1169.

[13] Vanyolos A, Dekany M, Kobacs B, *et al*. Gymnopeptides A and B, cyclic octadecapeptides from the mushroom *Gymnopus fusipes* [J]. *Org Lett*, 2016, **18**(11): 2688-2691.

[14] Nguyen KNT, Nguyen GKT, Nguyen PQT, *et al*. Immunostimulating and Gram-negative-specific antibacterial cyclotides from the butterfly pea (*Clitoria ternatea*) [J]. *FEBS J*, 2016, **283**(11): 2067-2090.

[15] Chen XQ, Zhao SM, Wang Z, *et al*. Rubicordins A−C, new cyclopeptides from *Rubia cordifolia* with cytotoxicity and inhibiting NF-*κ*B signaling pathway [J]. *Tetrahedron*, 2015, **71**(52): 9673-9678.

[16] Hase K, Kadota S, Basnet P, *et al*. Protective effect of celosian, an acidic polysaccharide, on chemically and immunologically induced liver injuries [J]. *Biol Pharm Bull*, 1996, **19**(4): 567-572.

[17] Hayakawa Y, Fujii H, Hase K, *et al*. Anti-metastatic and immunomodulating properties of the water extract from *Celosia argentea* seeds [J]. *Biol Pharm Bull*, 1998, **21**(11): 1154-1159.

[18] Suzuki H, Morita H, Iwasaki S, *et al*. New antimitotic bicyclic peptides, celogentins D−H, and J, from the seeds of *Celosia argentea* [J]. *Tetrahedron*, 2003, **59**(28): 5307-5315.

[19] Suzuki H, Morita H, Shiro M, *et al*. Celogentin K, a new cyclic peptide from the seeds of *Celosia argentea* and X-ray structure of moroidin [J]. *Tetrahedron*, 2004, **60**(11): 2489-2495.

[20] Morita H, Suzuki H, Kobayashi J. Celogenamide A, a new cyclic peptide from the seeds of *Celosia argentea* [J]. *J Nat Prod*, 2004, **67**(9): 1628-1630.

[21] Zhou T, Tan NH. Application of a new TLC chemical method for detection of cyclopeptides in plants [J]. *Chin Sci Bull*, 2000, **45**(20): 1825-1831.

[22] Jia AQ, Tan NH, Zhou J. Two new minor cyclopeptides froom *Sagina japonica* (Caryophyllaceae) [J]. *Fitoterapia*, 2009, **80**(3): 192-195.

[23] Paizs B, Suhai S. Fragmentation pathways of protonated peptides [J]. *Mass Spectrom Rev*, 2005, **24**(4): 508-548.

[24] Morita H, Yoshida N, Takeya K, *et al*. Configurational and conformational analyses of a cyclic octapeptide, lyciumin A, from *Lycium chinense* Mill [J]. *Tetrahedron*, 1996, **52**(8): 2795-2802.

[25] Huang RY, Liang LF, Guo YW. Time-dependent density functional theory electronic circular dichroism (TDDFT-ECD) calculation as a promising tool to determine the absolute configuration of natural products [J]. *J Int Pharm Res*, 2015, **42**(6): 686-698.

[26] Liu Y, Attila M, Li XM, *et al.* Peniciadametizine A, a dithiodiketopiperazine with a unique spiro [furan-2, 7'-pyrazino[1, 2-b][1, 2]oxazine] skeleton, and a related analogue, peniciadametizine B, from the Marine sponge-derived fungus *Penicillium adametzioides* [J]. *Mar Drug*, 2015, **13**(6): 3640-3652.

[27] Wang XL, Xu KP, Long HP, *et al*. New isoindolinones from the fruiting bodies of *Hericium erinaceum* [J]. *Fitoterapia*, 2016, **111**: 58-65.

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