Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 469–471

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Antimitotic Activity of Moroidin, a Bicyclic Peptide from the Seeds of *Celosia argentea*

Hiroshi Morita, Kazutaka Shimbo, Hideyuki Shigemori and Jun'ichi Kobayashi*

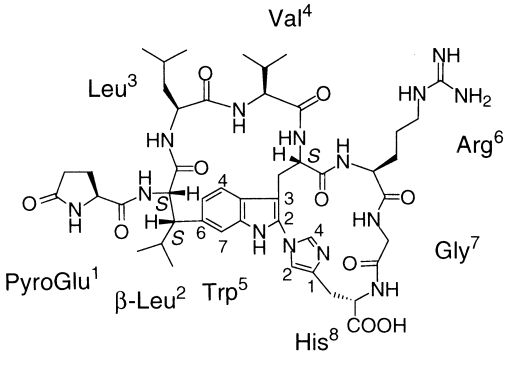
Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo 060-0812, Japan

Received 9 December 1999; accepted 5 January 2000

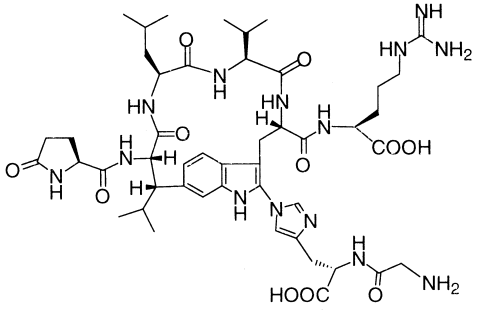
Abstract—A unique bicyclic peptide, moroidin (**1**), from the seeds of *Celosia argentea* (Amaranthaceae) strongly inhibited the polymerization of tubulin. The stereostructure of moroidin (**1**) was reinvestigated by spectroscopic data, chemical degradation, and molecular dynamics simulation.

There are a number of natural compounds which inhibit the microtuble formation and the mitotic arrest of eucaryotic cells.[1] The antimitotic agents have potential applications in drug development. The seeds of *Celosia argentea* are Chinese herbal medicines used as a therapeutic drug for eye and hepatic diseases in China and Japan.[2,3] During our search for bioactive compounds from medicinal plants, we found that moroidin (**1**), a unique bicyclic peptide isolated from the seeds of *C. argentea*, remarkably inhibited the tubulin polymerization. Furthermore, the stereostructure of moroidin (**1**) was reinvestigated by spectroscopic data, chemical degradation, and a computational approach, although that of moroidin (**1**) obtained from *Laportea moroides* (Labiatae) have been elucidated by NMR data and molecular modeling.[4,5] This paper describes the potent inhibitory activity of moroidin (**1**) on tubulin assembly, and the assignments of the NMR data and stereostructure 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. *n*-BuOH-soluble materials were subjected to a Diaion HP-20 column (MeOH: H_2O, 0:1→1:0) followed by a C_{18} column (CH_3CN: 0.05% TFA, 4:1) to afford moroidin (**1**, 0.02% yield) as colorless powder. FABMS data of **1** {$[\alpha]_D^{24}$ −55° (*c* 0.3, 50% MeOH)} showed the pseudomolecular ion at *m/z* 987 $(M+H)^+$, and the molecular formula, $C_{47}H_{66}N_{14}O_{10}$, was established by HRFABMS (*m/z* 987.3421, $(M+H)^+$, Δ −0.6 mmu). IR absorptions implied the presence of an amide carbonyl group (1675 cm^{-1}). The assignments of ^1H and ^{13}C signals of moroidin (**1**) were made by the combination of DQF-COSY, TOSCY, HMQC, and HMBC data in DMSO-d_6. The 2-D NMR spectra of moroidin (**1**) led to the complete assignments of ^1H and ^{13}C signals of individual amino acid residue as shown in Table 1.



1



2

*Corresponding author. Tel.: +81-11-706-4985; fax: +81-11-706-4985; e-mail: jkobay@pharm.hokudai.ac.jp

0960-894X/00/$ - see front matter
PII: S0960-894X(00)00029-9

Table 1. ^{1}H and ^{13}C NMR data and NOE correlations of moroidin (**1**) in DMSO-d_6

	δ_H [int. mult, J (Hz)]		δ_C	NOE relationship
PyroGlu[1]				
α	4.12 (1H, dd, 3.7, 8.7)	α	55.1	PyroGlu[1]: Hβ, NH; β[s]-Leu[2]: NH, Hβ
β	2.26 (2H, m)	β	28.9	PyroGlu[1]: Hγ
γ	1.77 and 2.05 (2H, m)	γ	25.3	
NH	7.70 (1H, s)	δ	177.4	
		C=O	172.2	
β[s]-Leu[2]				
α	4.86 (1H, t, 11.1)	α	54.9	β[s]-Leu[2]: NH, Hβ, Hδ; Trp[5]: H4, H5
β	3.05 (1H, dd, 3.5, 11.1)	β	51.4	β[s]-Leu[2]: NH, Hδ; Trp[5]: H5, H7
γ	2.15 (1H, m)	γ	26.3	β[s]-Leu[2]: NH, Hδ; Trp[5]: H5, H7
δ	0.78 (3H, d, 6.8)	δ	18.4	β[s]-Leu[2]: NH; Trp[5]: H7
	0.86 (3H, d, 6.8)		22.9	
NH	8.30 (1H, d, 11.1)	C=O	172.0	
Leu[3]				
α	4.01 (1H, dt, 4.2, 10.5)	α	51.4	Leu[3]: NH, Hγ, Hδ
β	1.24 and 1.45 (each 1H, m)	β	41.9	Leu[3]: NH
γ	1.46 (1H, m)	γ	26.3	Leu[3]: NH, Hδ
δ	0.71 (3H, d, 6.8)	δ	20.9	Leu[3]: NH, Hδ
	0.80 (3H, d, 6.8)		23.9	
NH	8.25 (1H, d, 10.5)	C=O	171.3	β[s]-Leu[2]: Hα
Val[4]				
α	3.69 (1H, t, 7.7)	α	57.5	Val[4]: Hβ, Hγ; Trp[5]: NH
β	1.86 (1H, m)	β	31.1	Val[4]: Hγ
γ	0.76 (3H, d, 7.0)	γ	18.2	Trp[5]: NH
	0.79 (3H, d, 7.0)		21.6	
NH	6.88 (1H, d, 7.7)	C=O	169.5	
Trp[5]				
α	5.49 (1H, brs)	α	41.9	Trp[5]: NH, H4, H5, Hβ; Arg[6]: NH
β	2.70 (1H, dd, 11.8, 14.8)	β	26.6	Arg[6]: NH, Hα; His[8]: H4
	3.38 (1H, dd, 4.8, 14.8)	C2	124.8	
NH1	11.20 (1H, s)	C3	101.0	Trp[5]: H7; His[8]: H2, H4
H4	7.53 (1H, d, 8.5)	C4	112.7	
H5	6.97 (1H, d, 8.5)	C5	117.8	
H7	6.87 (1H, s)	C6	119.3	β[s]-Leu[2]: Hα
NH	7.94 (1H, d, 8.1)	C7	118.6	Arg[6]: Hα, Hβ
		C8	137.3	
		C9	128.7	
		C=O	171.7	
Arg[6]				
α	4.13 (1H, m)	α	52.1	Arg[6]: Hβ; Gly[7]: NH
β	1.73 (1H, m)	β	28.3	Arg[6]: Hγ
	1.87 (1H, m)	γ	25.0	
γ	1.51 (2H, m)	δ	39.9	
δ	3.12 (2H, m)	ϵ	157.2	
ϵ(NH)	7.40 (1H, br s)	C=O	172.7	
NH	8.62 (1H, br s)			Arg[6]: Hα
Gly[7]				
α	3.67 (1H, br d, 16.8)	α	42.1	His[8]: Hβ, H4
	3.80 (1H, br d, 16.8)	C=O	165.8	
NH	7.22 (1H, br s)			
His[8]				
α	4.45 (1H, br t, 10.0)	α	51.7	His[8]: H4
β	2.75 (1H, dd, 10.0, 15.8)	β	29.8	His[8]: H4, H2
	3.15 (1H, m)	C1	137.3	
H2	7.73 (1H, s)	C2	117.8	Trp[5]: NH
H4	7.33 (1H, s)	C4	130.7	Trp[5]: Hα; Arg[6]: NH
NH	7.98 (1H, d, 10.0)	C=O	172.2	His[8]: Hα, Hβ, H4

The absolute configurations of the PyroGlu[1], Leu[3], Val[4], Arg[6], and His[8] residues in moroidin (**1**) were determined as all L-configurations by chiral HPLC analysis of the hydrolysate of **1**. The Trp[5] residue was transformed into Asp by treatment of **1** with O_3/AcOH and then H_2O_2 followed by acid hydrolysis.[6] Chiral HPLC analysis of Asp in the degradation products revealed it to be L-form, indicating *S*-configuration at Cα of the Trp[5] residue. The absolute configurations at Cα and Cβ of the β-substituted Leu[2] (β-Leu[2]) residue were elucidated by the floating chirality method as follows. The floating chirality method[7] allows the distance constraints to guide the molecule into configurations consistent with its NOE data. For molecules possessing complex ring systems, high-temperature dynamics alone may fail to invert certain chiral centers with sufficient frequency. This chiral inversion is caused by the application of NOE constraints (Table 1).[8] Simulation starting from the possible four isomers possessing different two pair of chirality at Cα and Cβ of the β-Leu[2] residue

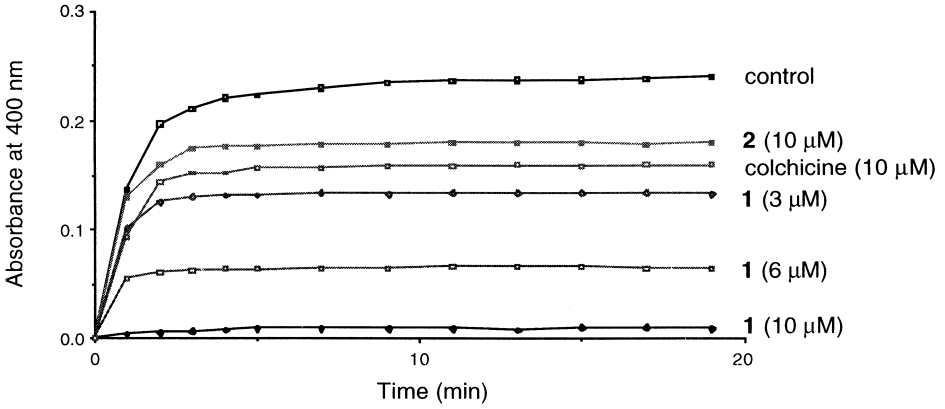


Figure 1. Effect of moroidin (**1**), moroidin hydrolysate (**2**) and colchicine on the polymerization of tubulin. Moroidin (**1**) at various concentrations was mixed with tubulin (1.5 mg/mL) at 0 °C and incubated at 37 °C. The absorbance at 400 nm was measured.

was concentrated to be both *S*-configurations.[9] These configurations were identical with those proposed for moroidin (**1**) obtained from *L. moroides*.[4,5]

Generally antimitotic agents bind to either the colchicine binding site or the vinca alkaloid binding site. In this study it was found that moroidin (**1**) remarkably inhibited the polymerization of tubulin[10] (Fig. 1). The inhibitory activity (IC_{50}, 3.0 μM) of the tubulin polymerization by moroidin (**1**) was more potent than that (IC_{50}, 10 μM) of colchicine. On the other hand, the hydrolysate (**2**) of **1** by α-chymotrypsin,[11] which possessed the structure cleavaged between the Arg and Gly residues of **1**, showed less activity than moroidin (**1**) but comparable activity to colchicine. These results suggest that moroidin (**1**) is a new class of microtuble inhibitor and its bicyclic ring system may be important for the activity.

Acknowledgements

The authors thank Professor S. Iwasaki and Dr. Y. Koiso, Institute of Molecular and Cellular Biosciences, University of Tokyo, for useful advice in tubulin assay, and Professor K. Takeya, Tokyo University of Pharmacy and Life Sciences, for helpful discussion. This work was partly supported by a Grant-in-Aid from the Ministry of Education, Science, Sports and Culture of Japan.

References and Notes

1. Iwasaki, S. *Med. Res. Rev.* **1993**, *13*, 183.
2. Hase, K.; Kadota, S.; Basnet, P.; Takahashi, T.; Namba, T. *Biol. Pharm. Bull.* **1996**, *19*, 567.
3. Hayakawa, Y.; Fujii, H.; Hase, K.; Ohnishi, Y.; Sakukawa, R.; Kadota, S.; Namba, T.; Saiki, I. *Biol. Pharm. Bull.* **1998**, *21*, 1154.
4. Leung, T.-W. C.; Williams, D. H.; Barna, J. C. J.; Foti, S. *Tetrahedron* **1986**, *42*, 3333.
5. Leung, T.-W. C.; Williams, D. H.; Barna, J. C. J.; Foti, S. *J. Org. Chem.* **1989**, *54*, 1901.
6. Uemoto, H.; Yahiro, Y.; Shigemori, H.; Tsuda, T.; Takao, T.; Shimonishi, Y.; Kobayashi, J. *Tetrahedron* **1998**, *54*, 6719.
7. Falk, M.; Spierenburg, P. F.; Walter, J. A. *J. Comp. Chem.* **1996**, *17*, 409.
8. Interatomic distances were classified into five ranges, ≤2.5, ≤3.0, ≤3.5, ≤4.0, and ≤5.0 Å, corresponding to the integrated volumes of the NOESY cross peaks. Because of the lack of stereospecific assignments in some methylene protons, the upper distances of these methylene and methyl protons were further relaxed by means of the pseudoatoms corrections.
9. Initial structures satisfying the experimental restraints were embedded by distance geometry calculations. Structural calculations were carried out using simulated annealing protocol. Calculations were carried out with the program SYBYL, and the produced conformers were then subjected to restrained energy minimization with TRIPOS force field.
10. Kobayashi, J.; Ogiwara, A.; Hosoyama, H.; Shigemori, H.; Yoshida, N.; Sasaki, T.; Li, Y.; Iwasaki, S.; Naito, M.; Tsuruo, T. *Tetrahedron* **1994**, *50*, 7401.
11. α-Chymotrypsin (0.25 mg dissolved in 50 μL of 0.001% HCl, Merck) was added to NH_4HCO_3 solution (1%, 0.45 mL) of **1** (0.5 mg) and the digestion was performed at 37 °C with the pH maintained at 8.0 by addition of 0.1 N HCl. After 2 days, the reaction was stopped by adjusting the solution to pH 2.2 with 1 N HCl, and the digestion mixture was lyophilized to dryness and subjected to HPLC (Develosil ODS-HG-5 column, 10 mm i.d.×250 mm, Nomura Chemical, eluted with 17% CH_3CN/0.05%TFA, flow rate 2 mL/min) to give the hydrolysate (**2**) as amorphous powder: FABMS *m/z* 1005 $(M+H)^+$, ^1H NMR (DMSO-d_6) δ_H: PyroGlu[1]: α, 4.14 (1H, dd, 3.8, 8.0); β, 1.77 and 2.25 (each 1H, m); γ, 2.10 (2H, m); NH, 7.80 (1H, s); β^s-Leu[2]: α, 4.82 (1H, t, 10.0); β, 3.04 (1H, dd, 3.4, 10.0); γ, 2.15 (1H, m); δ, 0.85 (3H, d, 6.5), 0.77 (3H, d, 6.5); NH, 8.39 (1H, d, 10.0); Leu[3]; α, 4.02 (1H, m); β, 1.21, 1.50 (each 1H, m); γ, 1.43 (1H, m); δ, 0.70 (3H, d, 6.5), 0.76 (3H, d, 6.5), NH, 8.29 (1H, d, 9.0); Val[4]: α, 3.75 (1H, t, 7.5); β, 1.87 (1H, m); γ, 0.79 (3H, d, 6.6), 0.82 (3H, d, 6.6); NH, 7.11 (1H, d, 7.5); Trp[5]: α, 5.48 (1H, brs); β, 2.94 (1H, m),3.25 (1H, m); NH1, 11.50 (1H, s); H4, 7.56 (1H, d, 8.4); H5, 6.96 (1H, d, 8.4); H7, 6.91 (1H, s); NH, 8.17 (1H, d, 8.6); Arg[6]: α, 4.20 (1H, m); β, 1.65 (1H, m), 1.77 (1H, m); γ, 1.51 (2H, m); δ, 3.13 (2H, m); ϵ(NH), 8.10 (1H, br s); NH, 7.99 (1H, d, 6.8); Gly[7]: α, 3.62 (1H, d, 11.3), NH, 7.42 (1H, d, 7.5); His[8]: α, 4.60 (1H, m); β, 3.03 (1H, m), 3.08 (1H, m); H2, 7.90 (1H, s); H4, 7.27 (1H, s); NH, 8.65 (1H, d, 7.0).