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Dichotomin A, A New Cyclic Hexapeptide from *Stellaria dichotoma* L. var. *lanceolata* Bge. [1)]

Hiroshi Morita, Takashi Kayashita, Akira Shishido, Koichi Takeya, Hideji Itokawa* and Motoo Shiro†

Department of Pharmacognosy, School of Pharmacy, Tokyo University of Pharmacy and Life Science, Horinouchi 1432-1, Hachioji, Tokyo 192-03, Japan and †Rigaku Corporation, 3-9-12 Matsubara, Akishima, Tokyo 196, Japan

Abstract: A new cyclic hexapeptide, dichotomin A, showing cell growth inhibitory activity, has been isolated from the roots of *Stellaria dichotoma* L. var. *lanceolata* Bge. and the structure and solid state conformation were elucidated by extensive 2D NMR, chemical degradation and X-ray analysis.

On the basis of the existence of many naturally occurring cyclic peptides with unique structures and biological activities, we have focused our attention on various cyclic peptides, with various biological activities, from higher plants.[2)] Despite their importance, surprisingly few studies of higher plants occurring cyclic peptides exist in the literature.[3)] As a part of our continuing studies in search of new bioactive cyclic peptides from higher plants, we have isolated a novel cyclic peptides, named dichotomin A, showing cell growth inhibitory activity, from the roots of *Stellaria dichotoma* L. var. *lanceolata* Bge. (Caryophyllaceae), which was used as folk medicines for antifebrile and so on. In this communication, we report the structure elucidation and solid state conformation of dichotomin A (**1**) by extensive 2D NMR, chemical degradation and X-ray crystallographic analysis.

The methanolic extract of the roots of *S. dichotoma* L. var. *lanceolata* Bge. was partitioned between n-BuOH and H_2O. The n-BuOH soluble material was subjected to Diaion HP-20 column (H_2O - MeOH), and 80% and 100% MeOH eluted fractions were chromatographed on a silica gel column, followed by HPLC on ODS to yield a peptidic compound as colourless needles, named dichotomin A (**1**: 0.007 %), which showed cell growth inhibitory activity against p-388 lymphocytic leukemia cells (IC_{50} 2.5 μg/ml).

Dichotomin A (**1**),[4)] colourless needles, mp. 179 - 180 °C, $[\alpha]_D$ +14.0° (c 0.10, MeOH), showed a high-resolution FAB-MS spectral quasimolecular ion peak at *m/z* 681.3652 (M^++H, Δ -4.0 mmu), corresponding to

molecular formula, $C_{35}H_{48}N_6O_8$. The IR absorptions at 3309 and 1649 cm^{-1} were attributed to amino and amide carbonyl groups, respectively. The hexapeptide nature of **1** was evident from its ^1H and ^{13}C NMR spectra, showing six amide NH and six amide carbonyl groups, as shown in Table 1. Further, the relatively high intensity of the molecular ion (base peak) and the lack of terminal amino group protons in the ^1H NMR suggested that **1** might be cyclic peptide. In order to elucidate the amino acid composition, **1** was subjected to complete hydrolysis with 6N HCl by heating at 110 °C for 24 h in a sealed tube. The hydrolysate was then analyzed by HPLC and the amino acid composition was shown to be one glycine (Gly), one threonine (Thr), one valine (Val), one leucine (Leu), one phenylalanine (Phe) and one tyrosine (Tyr) per molecule of **1**. These six amino acid units accounted for the observed mass molecular weight and 15 degrees of unsaturation. The absolute stereochemistry of the component amino acids in **1** was determined to be L-configuration by derivatization of the acid hydrolysate with Marfey's reagent,[5] followed by HPLC analysis.

The NMR spectra of **1** were taken at 500 MHz in pyridine-d_5 which gave well-resolved sharp signals. The ^1H NMR spectrum showed five doublet methyl signals (δ 0.78, 0.78, 1.09, 1.13 and 1.49) ascribable to Leu, Val and Thr residues. The ^1H-^1H COSY spectrum allowed the coupling sequence of each amino acid resonance and the corresponding carbon resonances were elucidated on the basis of HMQC spectra[6] as shown in Table 1. The gross structure including the sequence of the amino acids for **1** was assembled by connecting the individual amino acids on the basis of connectivities observed in the HMBC experiment (Fig. 1).[7] From the HMBC experimental results representing long range $^2J_{H\text{-}C}$ couplings from α protons and adjacent amide protons, the sequence was deduced to be cyclo[Gly-Thr-Phe-Leu-Tyr-Val]. As the amide carbonyl signals of Phe^3 and Val^6 showed the same chemical shift at δ 172.72, the proposed structure for dichotomin A (**1**) was unequivocally verified by single-crystal X-ray crystallography, as follows.

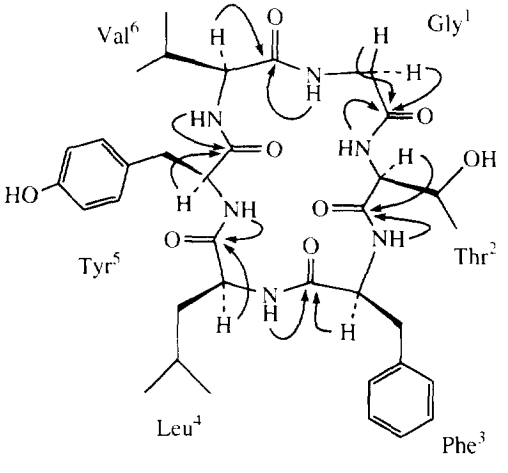

Fig. 1 Structure of dichotomin A (**1**); Arrows show HMBC correlations representing long range $^2J_{H\text{-}C}$ couplings from α protons and adjacent amide protons.

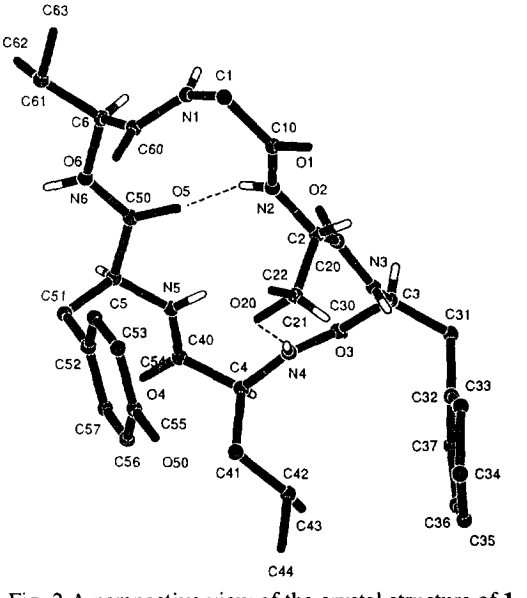

Fig. 2 A perspective view of the crystal structure of **1** The dotted lines indicate two intramolecular H bonds

Table 1. ^1H and ^{13}C NMR Signal Assignments of Dichotomin A (**1**) in pyridine-d_5.

	^1H NMR	^{13}C NMR			
assignment	δ_H (int. mult. J(Hz))	δ_C		δ_H	δ_C
Gly[1]			Leu[4]		
α	4.83 (1H, dd, 5.5, 15.6)	44.24	α	4.40 (1H, dt, 7.5)	55.08
	3.88 (1H, dd, 5.5, 15.6)		β	1.97 (2H, t, 7.5)	40.04
NH	9.97 (1H, t, 5.5)		γ	1.51 (1H, m)	24.86
C=O		170.69	δ	0.78 (3H, d, 6.5)	21.48
				0.78 (3H, d, 6.6)	23.08
Thr[2]			NH	9.07 (1H, d, 7.5)	
α	4.99 (1H, dd, 3.2, 7.9)	59.49	C=O		172.39
β	4.91 (1H, dq, 3.2, 6.4)	66.97	Tyr[5]		
γ	1.49 (3H, d, 6.4)	20.18	α	4.82 (1H, m)	56.34
NH	9.21 (1H, d, 7.9)		β	3.56 (1H, dd, 6.7, 14.0)	37.12
C=O		171.29		3.51 (1H, dd, 6.8, 14.0)	
			γ		128.57
Phe[3]			δ	7.42 (2H, d, 8.4)	131.21
α	5.19 (1H, ddd,7.5,8.2,8.6)	56.34	ε	7.14 (2H, d, 8.4)	116.29
β	3.57 (1H, dd, 8.2, 13.9)	38.06	ζ		157.73
	3.37 (1H, dd, 8.6, 13.9)		NH	8.60 (1H, d, 6.9)	
γ		138.16	C=O		172.33
δ	7.20 (2H, d, 7.4)	128.73	Val[6]		
ε	7.35 (2H, t, 7.4)	129.56	α	4.53 (1H, t, 6.0)	61.25
ζ	7.17 (1H, m)	126.84	β	2.46 (1H, m)	30.22
NH	8.79 (1H, d, 7.5)		γ	1.13 (3H, d, 6.7)	19.39[a]
C=O		172.72		1.09 (3H, d, 6.8)	19.23[a]
			NH	8.35 (1H, d, 6.0)	
			C=O		172.72

a) Assignment may be interchanged.

Dichotomin A was crystallized from MeOH-H_2O mixtures in monoclinic crystals of space group $P2_1$ (Z=2).[8] Because the crystals deteriorated rapidly upon drying, they were sealed in a thin-walled glass capillary containing the mother liquor. The X-ray crystal structure determination gave exactly the same amino acid sequence and absolute configuration originally assigned dichotomin A. Figure 2 shows the backbone of compound **1** with intramolecular hydrogen bonds, as illustrated by the dotted lines.

It is known that the conformational feature of cyclic peptides can be relevant for their biological activity. To examine the correlation between the conformations and pharmacological properties of **1**, we are interested in the conformation of dichotomin A. It appeared desirable to explore the structural role of hydrogen bonding in a cyclic peptide of moderate size for which X-ray data are available. In the crystal form of **1**, the end of the molecule is constrained to two β-turns formed by the residues from Val^6 to Gly^1, and from Phe^3 to Leu^4. The former is denoted as type II with the intramolecular hydrogen bond between Thr^2-NH and Tyr^5-CO [N2---O5 of 2.904(8) Å, HN2---O5 of 1.95 Å, and <O5---H-N2 of 162°], and the other type I without a transannular intramolecular hydrogen bond. It is interesting that the side chain of Thr^2 directs toward the interior and a

significant intramolecular NH···O hydrogen bonding contact between Thr^2-O and Leu^4-NH exists [N4---O20 of 3.059(11) Å, HN4---O20 of 2.11 Å, and ,O20---H-N4 of 164°].

Studies on the structure and conformational analyses, and biological evaluations of a series of dichotomins are in progress.

References and Notes

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4. Dichotomin A (**1**): Colourless needles, mp. 179-180°C, $[\alpha]_D$ +14.0° (c 0.10, MeOH); *m/z* 681 (Found: $(M+H)^+$, 681.3652. $C_{35}H_{49}N_6O_8$ requires, 681.3612); ν_{max} (KBr)/cm^{-1} 3309 (NH) and 1649 (amide C=O); λ_{max} (MeOH) / nm 276 (ε 1800).
5. P. Marfey, *Carlsberg Res. Commun.*, **1984**, *49*, 591.
6. A. Bax and S. Subramanian, *J. Magn. Reson.*, **1986**, *67*, 565.
7. A. Bax and D. G. Davis, *J. Magn. Reson.*, **1985**, *65*, 355.
8. A colorless prismatic crystal was sealed in a glass capillary. All measurements were made on a Rigaku AFC7R diffractometer with graphite monochromated Cu-Kα radiation using the ω-2θ scan technique to a maximum 2θ value of 110.2°. Cell constants are a=13.176(3), b=11.450(3), c=15.166(3) Å, V=2152.3(8) $Å^3$, β=109.83(1)°, Z=2. All calculations were performed using the teXsan[9] for 3377 unique reflections and final R factor is 0.070 (R_w=0.105). The refined fractional atomic coordinates, the bond lengths, the bond angles, the hydrogen-atom coordinates and the thermal parameters have been deposited at the Cambridge Crystallographic Data Centre (CCDC).
9. teXsan: Crystal Structure Analysis Package, Molecular Structure Corporation (1985 & 1992).

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