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Study on the spatial structure of brachystemin C, a new cyclic peptide from *Brachystemma calycinum*

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Abstract

A new cyclic octapeptide, brachstemin C (the molecular formula: $C_{38}H_{56}N_8O_9$), was isolated from the root of *Brachystemma calycinum*. The types and sequence of the amino acids of the title compound were confirmed by spectral analyses and X-ray diffraction. The stereochemistry of the title cyclic octapeptide was clarified by X-ray crystallographic study. The cyclic octapeptide backbone contains three β-turns. Two of them are type I β-turns and one is type III β-turn (right-handed 3_{10} helix). There are intermolecular hydrogen bonds between the cyclic peptide and the solvent molecules which maintained the steady spatial arrangement in crystal.

Keywords: Cyclic octapeptide; Brachystemin C; X-ray diffraction; Spatial structure

1. Introduction

A number of naturally occurring cyclic peptides have been isolated from various sources. Most of them are from microorganism, marine invertebrates [1] and higher plants [2]. Recently cyclic peptides have attracted attentions worldwide because they exhibit extensively and remarkably biological activities, such as anticancer, antibacterial, antifungal and enzyme inhibition etc [3–6]. And the biological activities and functions of the cyclic peptides are directly relative to their structures, especially the senior structures [7]. So it is important to determine the molecular spatial structure and analyze the characteristics of the spatial arrangement for investigating the structure-activity relationship of the cyclic peptide. X-ray diffraction can precisely describe three-dimensional structures of the molecules and supply the accurate structural information at molecular level in the crystalline state. To our knowledge, very few studies on spatial structures of cyclic peptides using X-ray diffraction method are reported [8–9]. This is because of two factors: one is that the skeleton of the cyclic peptides are too flexible to form the stabile spatial arrangement. The other is that the cyclic peptides usually form hydrogen bonds with the solvent molecules in the period of crystalline, and the crystal would deteriorated rapidly following the solvent volatilizing in the air, so the diffraction experiment cannot be completed successfully.

Brachystemma calycinum D. Don (Caryophyllaceae) is the only species of the genus *Brachystemma* [10]. Among the folks it has been used as a medicine for the treatment of rheumatism, limb numbness, impotence and foot edema [11]. As part of our effort to investigate the cyclic peptide components from natural sources, we have investigated the chemical components of the roots of *Brachystemma calycinum*, and a novel cyclic peptide named brachystemin C was isolated [12]. The amino acid compositions have been determined by the spectral data. We reported the linkage and molecular stereochemistry of the cyclic octapeptide by X-ray crystallographic investigation. The results will provide a valuable basis to further study on the structure-activity relationship of brachystemin C.

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2. Experimental

2.1. Extraction and isolation

The roots of *Brachystemma calycinum* D. Don were collected in March 1999 in Xishuangbanna, Yunnan Province, China. The powdered roots of the plant (13.0 kg) were extracted with 95% ethanol under reflux for three times. After concentration of the combined extracts in vacuum, the residue was suspended in water and partitioned successively with petroleum ether (60–90 °C), EtoAc and *n*-BuOH (presaturated by water). The ethyl acetate portion (50.0 g) was subjected to CC over silica gel (2300 g, 200–300 mesh) eluted with $CHCl_3$–MeOH (17:1 to 8:2) to afford subfraction, which was further purified by RP-18 (gradient MeOH–H_2O 45–70% as eluent) to furnish brachystemin C (8 mg).

2.2. X-ray diffraction experimental

X-ray diffraction qualified crystal of brachystemin C was obtained by slow evaporation from a mixture of methanol and water (1:1, v/v).

The X-ray diffraction data of brachystemin C were collected over a hemisphere of reciprocal space by a combination of 36 images of exposure (ω scan mode, 5° per image) on a Mac DIP-2030K diffractometer equipped with a rotating anode and Mo Kα radiation ($\lambda = 0.71073$ Å). The crystal structure was solved by the direct method and refined using the NOMCSDP software package [14]. In the final structure refinements, non-H atoms were refined with anisotropic temperature factors. H-atoms bonded to carbons were placed geometrically calculated positions, and positions for H-atoms bonded to oxygen and nitrogen were located from different Fourier syntheses and included in the calculation of structure factors with isotropic temperature factors. A summary of crystallographic data and structural refinement parameters of brachystemin C are given in Table 1.

Table 1
Crystal data and structure refinement for brachystemin C

Compound	Brachystemin C
Color/shape	Colorless/block
Cryst dimens (mm^3)	$0.1 \times 0.2 \times 0.3$
Chemical formula	$(C_{38}H_{56}N_8O_9)_1 \cdot (CH_3OH)_1 \cdot (H_2O)_2$
Formula weight	768.91
Temperature (K)	296(2)
Crystal system	Orthorhombic
Space group	$P2_12_12_1$ (No. 19)
Unit cell dimens	$a = 10.477(1)$ Å
	$b = 14.556(1)$ Å
	$c = 28.264(1)$ Å
Volume (Å^3)	4310.4(4)
Z	4
Density (mg/m^3)	1.271
Abs coeff (mm^{-1})	0.09
Diffractometer/Scan	Mac DIP-2030K
θ range (deg)	1.50–25.00
Indepnt reflns	5224
Obsd reflns [$\lvert F\rvert^2 = 8\sigma\lvert F\rvert^2$]	4119
R_1[$\lvert F\rvert^2 = 8\sigma\lvert F\rvert^2$]	0.071
wR_2 (all data)	0.070

$R_1 = \Sigma||F_0| - |F_c||/\Sigma|F_0|$, $wR_2 = [\Sigma[w(F_0^2 - F_c^2)^2]/\Sigma[w(F_0^2)^2]]^{1/2}$.

3. Results and discussion

Brachystemin C, white solid, mp > 250 °C, $[\alpha]_D^{25}$ −21.0° (*c* 0.25, methanol). Brachystmin C showed negative reaction to ninhydrin reagent but positive after hydrolysis by 6N HCl at 110 °C for 1 h. Positive FABMS *m/z*: 769$[M + H]^+$(99), 662, 606, 481, 436, 382, 365, 308, 268, 86, 70(100). The ^{1}H and ^{13}C-NMR spectra (see Table 2) exhibited the presence of seven C_α methines, one C_α methane, six amide NH signals and eight amide carbonyl signals, respectively. These data suggested that the title compound was a cyclic octapeptide. Its molecular formula $C_{38}H_{56}N_8O_9$ possessing 15 degrees of unsaturation was derived from the combination of positive FABMS at *m/z* 769 $[M + H]^+$, ^{13}C-NMR and DEPT spectra. Amino acid residues were identified as one tyrosine, two prolines, one isoleucine, one glycine, one valine and two alanines by the analysis of ^{1}H–^{1}H COSY spectrum and positive FABMS fragment pattern. Two peptide fragments –N–Val–Ala–Ala–Tyr–CO– and –NH–Ile–Gly–CO– were readily rationalized by the correlations between δ 7.25 and δ 170.0 (Ala^7_{NH}–Ala^8_{CO}), δ 7.82 and δ 173.6 (Tyr^1_{NH}–Ala^8_{CO}), δ 8.95 and δ 172.9 (Gly^5_{NH} − Ile^6_{CO}) in its spectrum.

The sequence of the cyclic octapeptide is *cyclo* (–Tyr^1–Pro^2–Pro^3–Ile^4–Gly^5– Val^6–Ala^7–Ala^8–) (see Fig. 1). Except for glycine, the relative configurations of all other seven amino acid residues are *L*. The configurations of all peptide bonds are *trans*.

Range of backbone bond distances (N_{i1}–C^α_{i2} 1.444–1.494, C_{i1}–C^α_{i2} 1.511–1.573, O_{i1}–C_{i1} 1.206–1.244 and C_{i1}–$N_{(i+1)1}$ 1.330–1.357 Å) and angles ($C_{(i-1)1}$–N_{i1}–C^α_{i2} 118.0–122.6°, N_{i1}–C^α_{i2}–C_{i1} 106.9–117.0°, O_{i1}–C_{i1}–C^α_{i2} 117.0–122.7°, C^α_{i2}–C_{i1}–$N_{(i+1)1}$ 114.3–122.3° and O^α_{i1}–C_{i1}–$N_{(i+1)1}$ 120.7–124.9°), are within the corresponding

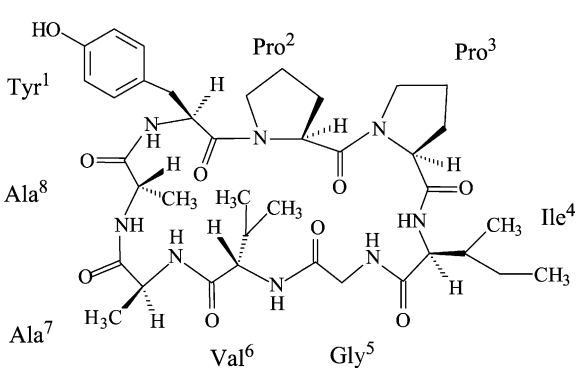

Fig. 1. Structural formula of brachystemin C.

Table 2
^{1}H and ^{13}C-NMR spectral data of brachystemin C in pyridine-d_5

	C–O	C_α	Cβ	Cγ	Cδ	H_N	H_α	Hβ	Hγ	Hδ
Tyr[1]	169.4	55.1	36.5	129.9	131.1 157.1 116.1	7.82 d (5.8)	5.22 m	3.29 d (6.28)		7.54 d (8.2) 7.18 d (8.2)
Pro[2]	172.1	64.5	29.7	25.5	47.3		4.81 m	2.39 m 1.80 m	1.70 m	3.40 m 2.20 m
Pro[3]	174.7	64.3	28.4	26.3	47.3		4.68 m	2.32 m	1.60 m	4.27 m 3.26 m
Ile[4]	172.9	57.9	37.1	25.1	12.3	8.11 d (10.4)	5.42 dd (10.4, 3.5)	2.72 m	2.12 m 1.27 d (7.5)	1.06 t (7.4)
Gly[5]	172.8	44.1				8.95 t (6.1)	4.75 m 3.82 d (11.6)			
Val[6]	170.7	51.6	33.1	17.1 19.9		8.40 d (4.5)	4.70 m	2.40 m	0.88 d (6.7) 1.18 d (6.8)	
Ala[7]	175.6	55.7	17.9			7.25 d (9.8)	5.34 m	1.50 d (7.40)		
Ala[8]	173.6	54.0	16.6			9.96 brs	4.20 m	1.55 d (6.6)		

acceptable ranges reported for cyclic peptides and suggest that the crystal conformation does not have unusual strain [15].

In the crystal structure of brachystemin C, the amino acids form a 24-membered macrocyclic backbone which is stabilized by the intramolecular hydrogen bonds. The protons of three NH groups in Tyr[1], Ile[4] and Gly[5] are located inward of the macrocyclic ring and the significant intramolecular N–H···O hydrogen bonds exist, which contact the type 4 → 1 between Tyr[1]–HN11 and Val[6]–O61 [N···O of 2.941 (5) Å and N–H···O of 162.5 (5)°], Ile[4]–HN41 and Tyr[1]–O11 [N···O of 2.953 (5) Å and N–H···O of 170.8 (5)°] and Gly[5]–HN51 and Pro[2]–O21 [N···O of 2.931 (5) Å and N–H···O of 158.6 (5)°] (see Fig. 3). These hydrogen bonds make three 10-membered rings and form three β-turns inside the covalent 24-membered ring. The Val[6]– Tyr[1] and Tyr[1]– Ile[4] turns are denoted as type III β-turns (right-handed 3_{10} helix), while the Pro[2]–Gly[5] turn adopt the type I β-turn. These secondary structural elements exhibit Φ and Ψ values close to the canonical structures (see Table 3).

Cyclic peptides are constrained as they contain turns in the backbones, which have been implicated in the bioactivity of several of these naturally occurred peptides, and these turns are often stabilized by intramolecular hydrogen bonds.

The two five-membered rings of the prolines 2 and 3 adopt the envelop conformation with C-24 and C-34 displaced by 0.579 (14) and 0.550 (15) Å from the corresponding least-squares planes of the remaining four atoms, respectively. In the backbone of the cyclic octapeptide, the Tyr[1], Pro[2] and Pro[3] are connected. For obtaining the less restraint in space, the phenyl ring of tyrosine and two pyrrolidine rings of prolines are outside the peptide backbone and almost perpendicular to one another which are adjoined. The dihedral angles are 89.0 (3)° and 69.8 (4)°, respectively. The phenyl ring of Tyr[1] and the pyrrolidine ring of Pro[3] is almost parallel and the dihedral angle is 30.0 (3)° (see Fig. 2).

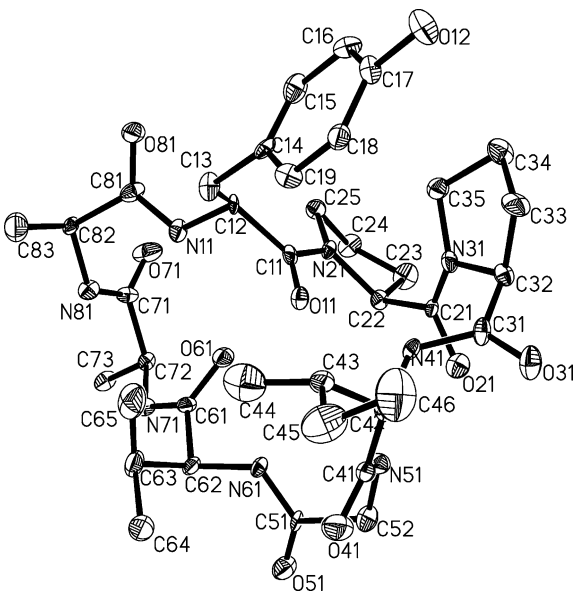

Fig. 2. The molecular structure and atomic numbering scheme for brachystemin C. Displacement ellipsoids are drawn at the 30% probability level. The hydrogen atoms are omitted for clarity.

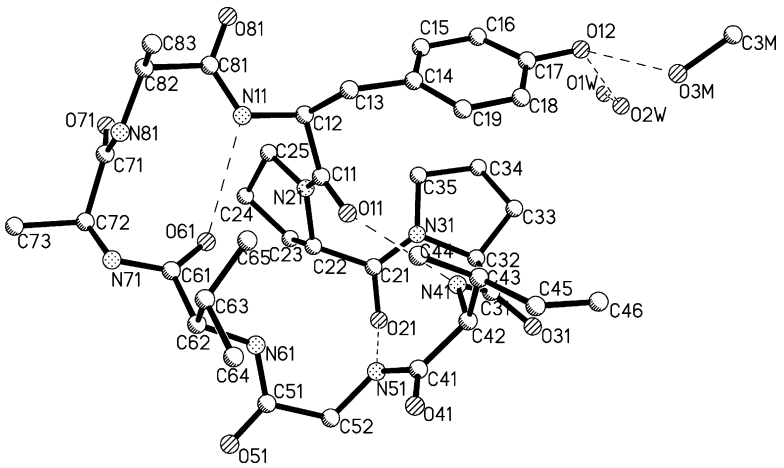

Fig. 3. The intramolecular and intermolecular hydrogen bonds in brachystemin C (dashed lines indicate hydrogen bonds).

Table 3
The dihedral angles of Φ and Ψ [13] for the secondary structure

Type of turn	Φ_2	Ψ_2	Φ_3	Ψ_3
Type I β-turn	−60°	−30°	−90°	0°
Type III β-turn	−60°	−30°	−60°	−30°
Pro^2–Gly^5	−58°	−18°	−71°	5°
Val^6–Tyr^1	−62°	−26°	−73°	−24°
Tyr^1–Ile^4	−52°	−33°	−58°	−18°

Besides one cyclic octapeptide, there are two water molecules and one methanol molecule in the asymmetric unit. The solvent molecules are distributed outside of the cyclic octapeptide chain and formed hydrogen bonds with the cyclic octapeptide (see Table 4 and Fig. 3). As the solvent molecules had action of the strengthen crystal lattice, the crystal of the cyclic octapeptide can exist in stable state.

The alkyls which exist in the terminals of isoleucine and valine have less strain and freely rotate on the single bonds. It induce the position disorders and the thermal factors higher than usual for the atoms C-44, C-45, C-46, C-64 and C-65.

Table 4
Hydrogen bonds of brachystemin C

Hydrogen bonds	Distance (Å)	Symmetry code
N-11···O-61	2.941	x, y, z
N-41···O-11	2.953	x, y, z
N-51···O-21	2.931	x, y, z
O-12···O-3m	2.675	x, y, z
N-71···O-2w	2.896	$1+x, y, z$
N-81···O-51	3.243	$2-x, -1/2+y, 3/2-z$
O-1w···O-21	2.982	$-1/2+x, 3/2-y, 1-z$
O-1w···O-2w	2.789	$1/2-x, 1-y, -1/2+z$
O-2w···O-12	2.896	$1/2-x, 2-y, -1/2+z$
O-3m···O-81	2.730	$1/2+x, 3/2-y, 2-z$

4. Conclusions

The cyclic peptide backbone is generally considered to be quite flexible. To find the conformation of the cyclic peptide, it is significant to explore the structural role of hydrogen bond for which X-ray data are available. The accurate and complete stereochemical data for brachystemin C were obtained and analyzed by X-ray diffraction. The backbone of brachystemin C had three β-turns, two type I β-turns and one type III β-turn (right-handed 3_{10} helix), which are stabilized by the intramolecular hydrogen bonds. There are intermolecular hydrogen bonds between the cyclic octapeptide and the solvent molecules in stabilizing the cyclopeptide lattice. These results highlight the important role of the hydrogen bonds not only in stabilizing the cyclic peptide lattice and becoming crystalline, but also in forming the secondary structures of brachystemin C.

References

[1] Y.H. Wang, S.J. Yan, J.Y. Su, L.M. Zeng, H. Li, Chin. J. Org. Chem. 21 (2001) 16–24.
[2] N.H. Tan, J. Zhou, S.X. Zhao, Acta Pharm. Sinica 32 (1997) 388–399.
[3] N.K. Gulavita, S.P. Gunasekera, S.A. Pomponi, E.V. Robinson, J. Org. Chem. 57 (1992) 1767–1772.
[4] S. Matsunaga, N. Fusetani, S. Konosu, Tetrahedron Lett. 25 (1984) 5165–5168.
[5] J. Kobayashi, M. Tsuda, T. Nakamura, Y. Mikami, H. Shigemori, Tetrahedron 49 (1993) 2391–2402.
[6] N. Fusetani, S. Matsunaga, J. Am. Chem. Soc. 112 (1990) 7053–7054.
[7] (a) R.H. Mattern, T.A. Tran, M. Goodman, J. Med. Chem. 41 (1998) 2686–2692.
(b) A. Lombardi, G.D. Auria, O. Maglio, F. Nastri, L. Quartara, C. Pedone, V. Pavone, J. Am. Chem. Soc. 120 (1998) 5879–5886.
[8] R.W. Jiang, Y. Lu, Z.D. Min, Q.T. Zheng, J. Mol. Struct. 655 (2003) 157–162.
[9] H. Morita, A. Gonda, K. Takeya, H. Itokawa, Y. Iitaka, Tetrahedron 53 (1997) 1616–1626.

[10] C.L. Tang, P. Ke, D.Q. Lu, L.H. Zhou, C.Y. Wu, Flora Reipublicae Popularis Sinicae, Tomus 26, Science Press, Beijing, 1996, p. 253 (in Chinese).
[11] C.Y. Wu, T.Y. Zhou, P.G. Xiao, A Compendium of New China Herbal Medicine, vol. 3, Science and Technology Press, Shanghai, 1990, p. 43 (in Chinese).
[12] Y.X. Cheng, J. Zhou, N.H. Tan, Acta Botanica Sinica 43 (2001) 760–765.
[13] T. Shen, J.Y. Wang, Biochemistry, Second ed., Higher Educational Press, Beijing, 1998, pp. 140–150 (in Chinese).
[14] B.M. Wu, Y. Lu, Chin. Chem. Lett. 3 (1992) 637–640.
[15] M. Levitt, J. Mol. Biol. 47 (1974) 1129.