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Study on the spatial structure of annomuricatin A, a cyclohexapeptide from the seeds of *Annona muricata*

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Abstract

A cyclic hexapeptide, annomuricatin A (the molecular formula: $C_{27}H_{38}N_6O_7$), was isolated from the seeds of *Annona muricata*. The types and sequence of the amino acids were confirmed by X-ray diffraction analysis. The stereochemistry of the title cyclopeptide was clarified by X-ray crystallographic study. The backbone contains two β-turns, one is type I β-turn and the other is type II, which are stabilized by two transannular 4 → 1 backbone hydrogen bonds between Ala and Phe. There are intermolecular hydrogen bonds between the cyclopeptide and the solvent molecules which maintained the steady spatial arrangement in crystal.

Keywords: Cyclohexapeptide; Annomuricatin A; X-ray diffraction; Crystal structure

1. Introduction

The study on cyclopeptide comes from the research on natural products. Since the first cyclopeptide gramicidin S was discovered in the 1940s of the 20th century [1], a lot of naturally occurring cyclopeptides have been isolated from various sources such as marine invertebrates and higher plants [2,3]. The characteristic structure of cyclopeptides and their stability to enzyme offer them extensively and remarkably biological activities, such as anti-tumor, antifungal, antivirus, enzyme inhibition, etc. [4–7], which are closely related to their molecular conformation. X-ray diffraction analysis can precisely describe the three-dimensional molecular conformation of such structures in solid state, and several studies on crystal structures of cyclopeptide have been reported [8–10]. However, most of the structures of cyclopeptides are clarified by spectrum method, and sometimes the amino acid sequence is not determined properly [11,12].

Annona muricata L.(*Annonaceae*), which is from the family of *dicotyledon*, *Magnolia* subclass, is widely spread in torrid and semi-torrid zones. It has recently come under intense scrutiny as potential sources of Annonaceous acetogenin, one kind of anti-tumor natural product. In the past few years, cyclopeptides from *Annonaceae* have also been reported. As part of our effort to investigate the cyclopeptide components from natural sources, we have investigated the chemical components of the seeds of *Annona muricata*, and a new cyclohexapeptide named annomuricatin A was isolated. The amino acid compositions have been determined by spectral method [13]. Now we report its spatial and stereo structure by X-ray crystallographic investigation, which revised the structure. The result is homologous to that of annomuricatin C [14].

2. Experiment

2.1. Extraction and isolation

Crushed air-dried seeds of *Annona muricata* (2 kg, cultivated in Xishuangbanna, Yunnan province in China)

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were repeatedly percolated with 95% EtOH and the extracts concd in vacuo. The EtOH extract was partitioned with $CHCl_3$ to yield the $CHCl_3$ soluble fraction which was then partitioned between petroleum ether and 90% aqueous MeOH (1:1) to yield the 90% aqueous MeOH soluble fraction (200 g). The 90% aqueous MeOH fraction (160 g) was subjected to column chromatography on silica gel using petroleum ether:EtOAc:-MeOH gradient elution, affording annomuricatin A (105 mg).

2.2. X-ray diffraction experiment

A qualified crystal of annomuricatin A for X-ray diffraction was obtained by slow evaporation from MeOH under 10 °C after one month.

The X-ray diffraction data of annomuricatin A 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 MoKα radiation ($\lambda = 0.71073$ Å). The crystal structure was solved by the direct method and refined using SHELX-97 [15], In the final structure refinements, non-H atoms were refined anisotropically. H-atoms bonded to carbons were placed in geometrically calculated positions, and positions for H-atoms bonded to oxygen and nitrogen were located at 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 annomuricatin A is given in Table 1.

Table 1
Crystal data and structure refinement for annomuricatin A

Compound	Annomuricatin A
Color/shape	Colorless/needle
Cryst dimens (mm^3)	$0.08 \times 0.10 \times 0.50$
Chemical formula	$C_{27}H_{38}N_6O_7{\cdot}CH_3OH$
Formula weight	590.68
Temperature (K)	296 (2)
Crystal system	Orthorhombic
Space group	$P2_12_12_1$ (No. 19)
Unit cell dimension	$a = 8.566(2)$ Å
	$b = 14.955(3)$ Å
	$c = 24.038(5)$ Å
Volume (Å^3)	3079.4 (11)
Z	4
Density (mg/m^3)	1.274
Abs. coeff. (mm^{-1})	0.094
Diffractometer/Scan	Mac DIP-2030K/ω
θ range (deg)	1.60–27.27
Indepnt. reflns.	3631
Obsd. reflns. [$F^2 > 2\sigma(F^2)$]	2615
$R_1[F^2 > 2\sigma(F^2)]$	0.0675
$wR_2(w = 1/[\sigma^2(F_o^2) + (0.1139P)^2 + 1.2130P]$, where $P = (F_o^2 + 2F_c^2)/3$)	0.1686

3. Results and discussion

The physicochemical data and the NMR data of annomuricatin A have been previously published[12], and amino acid analysis of the compound after hydrolysis with 6 mol/L HCl at 110 °C gave the composition: Ser (le q), Gly (le q), Ala (le q), Val (le q), Phe (le q), and Pro (le q). The cyclopeptide sequence *cyclo* (-Val[1]-Ser[2]-Ala[3] -Pro[4]-Gly[5]-Phe[6]-) (see Fig. 1) was deduced from X-ray diffraction analysis. Except for glycine, the relative configurations of all other five amino acid residues are *L*, while the absolute configuration of the cyclopeptide was not determined. The configurations of all peptide bonds are *trans*.

Range of backbone distances (C_{i1}–$N_{(i+1)1}$: 1.299–1.359, C_{i1}–C^{α}_{i2}: 1.510–1.554, C_{i1}–O_{i1}: 1.214–1.245, N_{i1}–C^{α}_{i2}: 1.415–1.483 Å) and angles ($C_{(i-1)1}$–N_{i1}–C^{α}_{i2}: 120.1–125.1, N_{i1}–C^{α}_{i2}–C_{i1}: 108.3–116.5, O_{i1}–C_{i1}–C^{α}_{i2}: 118.0–122.2, $N_{(i+1)1}$–C_{i1}–C^{α}_{i2}: 114.2–120.3, O_{i1}–C_{i1}–$N_{(i+1)1}$: 120.9–124.3°) are within the corresponding acceptable ranges reported for cyclopeptide, which suggests that the molecule of this solid state conformation does not have any unusual strain [16].

The turns and hydrogen bonds are crucial determinants of the conformation of cyclopeptide, which have been implicated in the bioactivity of several naturally occurring peptides. There are two intramolecular $4 \rightarrow 1$ hydrogen bonds in annomuricatin A, which stabilized the 18-membered macrocyclic backbone formed by the six amino acids. The distances between O and N involved in the two intramolecular hydrogen bonds are between Phe[6]–NH...Ala[3]–CO (N...O: 2.836 Å, N–H...O: 154.3°) and between Ala[3]–NH...Phe[6]–CO (N...O: 4.054 Å, N–H...O: 163.4°). In the crystal form, besides the two intramolecular hydrogen bonds, two β-turns formed by the residues Val[1] and Ser[2] (type I), and of Pro[4] and Gly[5] (type II) restrained the cyclic hexapeptide backbone. These secondary structural elements exhibit ϕ and Ψ values close to the canonical structures [17] (see Table 2). The two β-turns are connected and stabilized by the intramolecular hydrogen bonds, and formed a short antiparallel β-sheet arrangement.

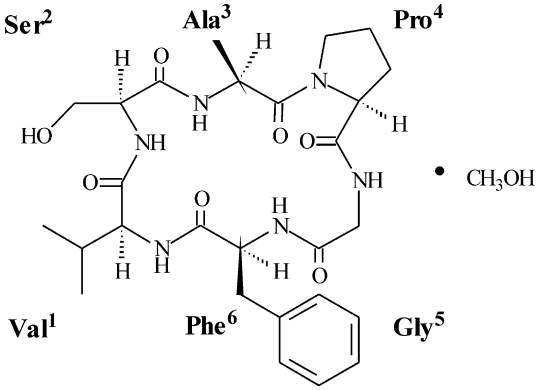

Fig. 1. Structural formula of annomuricatin A.

Table 2
The dihedral angles of ϕ and Ψ for the secondary structure

Type of turn	ϕ_2°	Ψ_2°	ϕ_3°	Ψ_3°
Type I β-turn	−60	−30	−90	0
Type II β-turn	−60	+120	+80	0
Val[1]-Ser[2]	−69	−36	−113	8
Pro[4]-Gly[5]	−58	+122	+93	−6

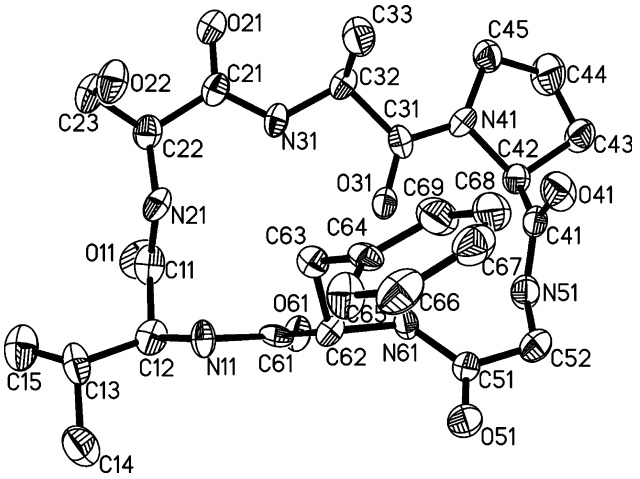

Fig. 2. The molecular structure and atomic numbering scheme for annomuricatin A. Displacement ellipsoids are drawn at the 30% probability level. The hydrogen atoms are omitted for clarity.

In the solid state of annomuricatin A, the Pro[4] exhibits *trans* configuration, and its pyrrolidine ring adopts the envelop conformation with C-44 displaced by 0.490 (12) Å from the corresponding least-squares plane of the remaining four atoms. For obtaining less restraint in space, the phenyl ring of Phe[6] extends outside the main peptide backbone, and the dihedral angle is 125.8 (3)° (see Fig. 2), which makes the Phe[6]–CO run inside the peptide ring and this may be the reason why the hydrogen bond Ala[3]–NH ... Phe[6]–CO is weak (4.054 Å).

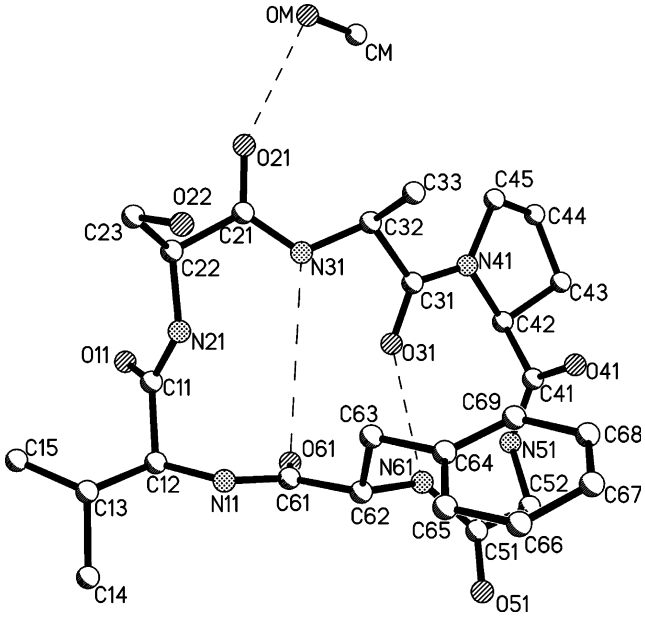

Fig. 3. The intramolecular and intermolecular hydrogen bonds in annomuricatin A (dashed lines indicate hydrogen bonds).

Table 3
Hydrogen bonds of anno muricatin A

Hydrogen bonds	Distance (Å)	Symmetry code
N–61 ··· O–31	2.836	*x*,*y*,*z*
N–31 ··· O–61	4.054	*x*,*y*,*z*
O–M ··· O–21	2.770	1−*x*,−1/2+*y*,1/2−*z*

Besides one cyclopeptide molecule, there is one methanol molecule in the asymmetric unit. The solvent molecule lies outside the main peptide chain and forms hydrogen bonds with the cyclopeptide (see Fig. 3 and Table 3), which stabilized the crystal lattice further more, so the crystal of the cyclopeptide can exist stably.

4. Conclusion

Cyclopeptides are generally difficult to crystallize, and their backbones are considered to be quite flexible. β-Turns play an important role in restraining such structures, and it is significant to highlight the structure role of the hydrogen bonds for which X-ray data are available. Here we report the accurate and complete stereo structure data for the cyclohexapeptide annomuricatin A by X-ray diffraction analysis. The backbone contains two β-turns, one is type I β-turn and the other is type II, which are stabilized by the two intramolecular $4 \rightarrow 1$ hydrogen bonds. There are intermolecular hydrogen bonds between the cyclopeptide and the solvent molecule, which stabilize the crystal lattice. The crystal structure of annomuricatin A is consistent with solution state of annomuricatin C [14], which indicates that these two cyclopeptides are the same compound in fact. The result based on detailed X-ray diffraction analysis not only highlights the important role of hydrogen bonds in forming the secondary structure of annomuricatin A, but also clarifies the spatial structure of the cyclopeptide which may be related to its bioactivity.

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