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Conformation of Cyclic Heptapeptides: Solid and Solution State Conformation of Yunnanin A[1)]

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Abstract : The solid and solution state conformation of yunnanin A, a cyclic heptapeptide, *cyclo*(-Gly-Tyr-Gly-Gly-Pro-Phe-Pro-), isolated from the roots of *Stellaria yunnanensis* were studied. The X-ray diffraction studies showed that the crystal of yunnanin A [orthorhombic form from a ethanol-methanol mixture, a=11.754 (10), b=12.576 (7), c=30.731 (6) Å, space group $P222_1$, z=4] had three intramolecular hydrogen bonds forming one type II, one type II' β-turns, and a classical β-bulge unit with all *trans* amide bonds. The dominant solution conformation analyzed by NMR spectroscopy, Monte Carlo (MC) and restrained molecular dynamics (MD) calculations implemented in MacroModel/Batchmin (Ver. 4.5) was homologous to that in the solid state.

Cyclic peptides are frequently found among natural products and exhibit a wide range of biological activities. The conformation of such cyclic peptides in solid and solution states have been intensively studied, because their biological activities are known to be closely related to their conformational states. We have reported the conformations of a series of cyclic peptides in order to clarify the relationship between their conformations and their biological activities.[2, 3)]

Recently, we studied the conformation of a cyclic heptapeptide, pseudostellarin D, by combination of

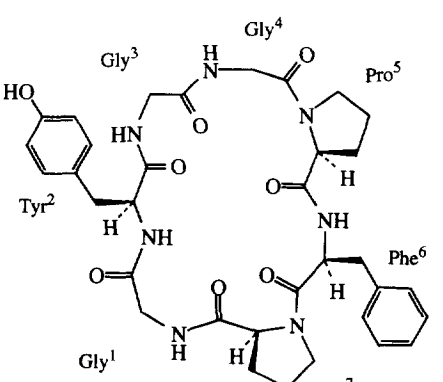

Fig. 1. Structure of yunnanin A. Gly is provisionally numbered as the first amino acid.

X-ray diffraction, high field NMR and computational methods, and found that the conformational feature of pseudostellarin D was characterized by two β-turns (type I and II) and one β-bulge structure.[3] Naturally occurring cyclic heptapeptides are not many, and those whose conformations have been reported are ilamycin B_1,[4] a dolastatin 3 analogue,[5] cycloheptasarcosine,[6] rhizonin A,[7] and evolidine,[8] all being characterized by X-ray analyses, and hymenamide[9] and phakellistatin,[10] both possessing one *cis* amide bond in the backbone. It is not enough to examine the conformational preference of cyclic heptapeptides, compared with those of many cyclic penta, hexa, and octa peptides.

Recently we isolated a cyclic heptapeptide, yunnanin A, *cyclo*(-Gly-Tyr-Gly-Gly-Pro-Phe-Pro-), from the roots of *Stellaria yunnanensis* (Caryophyllaceae), having a cytotoxic activity on P388 cells.[11] In the present paper, we describe the elucidation of solid and solution state conformation of yunnanin A by using X-ray analysis, high field NMR and Monte Carlo/Molecular Dynamics simulations.

Results and Discussion

X-ray diffraction study

To establish the principles governing stable conformation in solid state, a single crystal X ray analysis of yunnanin A was conducted. Yunnanin A was crystallized from MeOH-EtOH mixtures in orthorhombic crystals of space group $P222_1$ (Z=4). The crystallographic data were collected from one single crystal sealed in a thin-walled glass capillary with a small amount of mother liquor. A summary of the final crystallographic data is presented in Table 1. 3853 reflections were collected and the structure was solved by direct methods.[12] Non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included but not refined. The final cycle of full-matrix least-squares refinement was based on 3853 observed reflection with unweighed and weighed agreement factors of R=0.091 and R_w=0.107. Figure 2 shows a ORTEP perspective view of the backbone of yunnanin A. The X-ray crystal structure determination confirmed the amino acid sequence and the absolute configuration of yunnanin A reported in our previous paper.[11] The torsion angles (φ, ψ, ω) are listed in Table 3, which are within the permissible ranges for peptides[13] and suggest that the molecule of this solid state conformation is not under any extra strain.

The turns and hydrogen bonds are crucial determinants of the conformation of cyclic peptides. The distances between O and N involved in the three intramolecular hydrogen bonds between Tyr^2-NH and Phe^6-CO, between Phe^6-NH and Gly^3-CO, and between Gly^3-NH and Phe^6-CO are given in Table 2. In the crystal form, in addition to the three intramolecular hydrogen bonds, two β-turns (type II' and type II) formed by the residues of Gly^4 and Pro^5, and of Pro^7 and Gly^1 restrain the cyclic heptapeptide backbone. The former is denoted as type II' [Gly^4 φ, ψ (60.0, -133.3); Pro^5 φ, ψ (-80.8, 0.0)] and the latter type II [Pro^7 φ, ψ (-65.8, 140.1); Gly^1 φ, ψ (77.0, 8.0)]. These torsion angles are well consistent with their ideal values [type II' ϕ_{i+1}, ψ_{i+1} (60.0, -120.0), ϕ_{i+2}, ψ_{i+2} (-80.0, 0.0); type II ϕ_{i+1}, ψ_{i+1} (-60.0, 120.0), ϕ_{i+2}, ψ_{i+2} (80.0, 0.0)]. All of the amide bonds have *trans* geometry. The two β-turns are stabilized by the hydrogen bonds formed between Tyr^2-NH and Phe^6-CO, between Phe^6-NH and Gly^3-CO. The presence of a β-bulge unit at Tyr^2 - Gly^3 residues

Table 1. Crystal data of yunnanin A.

Empirical Formula	$C_{34}H_{45}N_7O_{10}$(711.77)
Color, Habit	colorless, prismatic
Dimensions	0.50 × 0.40 × 0.20 mm
System	orthorhombic
Dcalc	1.041 g/cm^3
Lattice Parameters (Å)	a=11.754(10)
	b=12.576(7)
	c=30.731(6)
	V=4542(6)Å^3
Space Group	$P222_1$(#17)
Z value	4
Final R value (R_w)	0.091 (0.107)

Table 2. Hydrogen bonds in yunnanin A

From	To	Distance (Å)[a]	Angle (°)[b]
Gly^3-O	Phe^6-N	3.01(0.01)	159
Phe^6-O	Gly^3-N	3.02(0.01)	165
Phe^6-O	Tyr^2-N	3.19(0.01)	141

[a] Distances for N ··· O

[b] Angles for N-H ··· O

Table 3. Backbone dihedral angles in yunnanin A, calculated from vicinal NH-CαH coupling constants (Hz), MD/MM, MC/MM calculations, and X-ray structure

residues		Hz	φ angle(°)[a]	MD/MM[b]	MC/MM[b]	X-ray[c]
Gly^1	φ	4.9, 7.6	**90**, 103	130.1	134..3	77(1)
	ψ			-22.2	-23.0	8(1)
	ω			-179.4	-179.7	-171.0(8)
Tyr^2	φ	10.0	**-134**, -103	-131.0	-131.3	-138.2(8)
	ψ			-60.6	-59.9	-67.5(9)
	ω			180.0	180.0	-176.4(7)
	χ1			168.9	169.6	-176.0(9)
	χ2			-119.7, 60.1	-115.7, 64.1	-99(1), 84(1)
Gly^3	φ	6.8	**150**	164.1	164.4	154.8(7)
	ψ			165.2	164.1	161.2(8)
	ω			174.1	174.1	-178.0(9)
Gly^4	φ	4.3, 6.1	**62**, 2	57.1	57.5	60(1)
	ψ			-120.5	-120.7	-133.3(9)
	ω			-179.4	-179.4	-178.8(8)
Pro^5	φ			-83.5	-83.1	-80.8(10)
	ψ			9.1	8.0	0(1)
	ω			-176.3	-176.3	-169.6(7)
	χ1			35.1	35.0	32(1)
	χ2			-36.5	-36.5	-37(1)
	χ3			25.1	25.1	27(1)
	χ4			-3.0	-3.2	-6(1)
Phe^6	φ	9.0	-144, **-96**	-124.4	-122.8	-106.9(8)
	ψ			86.5	86.2	116.8(8)
	ω			179.6	-178.2	-178.2(7)
	χ1			-53.1	-53.1	-62.7(8)
	χ2			-53.6, 126.5	-46.1, 126.5,	-56(1), 124.3(9)
Pro^7	φ			-65.5	-68.9	-65.8(10)
	ψ			111.0	108.3	140.1(7)
	ω			-169.5	-167.8	-177.6(8)
	χ1			26.6	26.6	21(1)
	χ2			-33.2	-33.2	-27(2)
	χ3			28.1	28.1	21(2)
	χ4			-11.9	-11.9	-7(1)

[a] Calculated by using the Karplus-Bystrov equation: $^3J_{HN\alpha}=9.4\cos^2|60-\phi|-1.1\cos|60-\phi|+0.4\sin^2|60-\phi|$ for non-Gly residues and and $\Sigma^3J_{HN\alpha}2=6.0\cos^2\phi-1.5\cos\phi+12.5\sin^2\phi$ for Gly residue. The calculated φ angles shown by bold letters are close to those calculated by X-ray structure

[b] Averaged dihedral angles of the 10 lowest energy conformers from MD/MM and MC/MM calculations. Standard deviation was less than 8.7° except for side chains.

[c] Dihedral angles calculated by X-ray structure of yunnanin A

is caused by the hydrogen bond between Gly^3-NH and Phe^6-CO. The β-bulge was originally defined by Richardson,[14] and it displays a pair of convergent hydrogen bonds from N-H's of adjacent residues on one strand to C=O of the residue opposite. The type of this β-bulge is of classic type according to Richardson from φ, ψ angles of Tyr^2 and Gly^3 (Tyr^2: φ -138.2, ψ -67.5; Gly^3: φ 154.8, ψ 161.2).

The side chain conformations of Tyr^2 and Phe^6 were indicated to be *trans* and *gauche*$^-$, respectively. The aromatic ring of Phe^6 was situated over the pyrolidine ring of Pro^5. The conformation of pyrolidine rings of Pro^5 and Pro^7 were both C_2-Cγ-*endo*.[15]

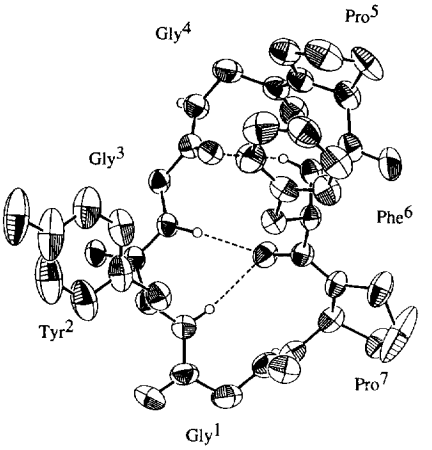

Fig. 2. ORTEP perspective view of the crystal structure of yunnanin A. Broken lines represent intramolecular H-bonds.

NMR study

A close inspection of the NMR spectra by ^1H-^1H COSY, HMQC and HMBC experiments led to complete ^1H and ^{13}C assignments of individual amino acid, as shown in our previous paper.[11] The solution conformational analysis of yunnanin A was carried out by using the NMR data such as interatomic distances from a phase sensitive ROESY experiment, temperature effects on NH protons, and torsion angles calculated from $^3J_{NH\text{-}C\alpha H}$ coupling constants.

The ROE relationships measured by its phase sensitive ROESY spectrum in DMSO-d_6 are shown in Fig. 3. The following ROE enhancements is related to the two *trans* X-Pro bonds. The strong ROE correlations between Phe^6-Hα and Pro^7-Hδ, and between Gly^4-Hα and Pro^5-Hδ indicated a *trans* geometry for the two proline amide bonds. The chemical shifts of Cβ and Cγ of Pro^7 (Cβ δ29.04, Cγ δ24.72) also supported the *trans* geometry of the Pro^7 amide bond.[16] However, the chemical shift of Cγ of Pro^5 was δ22.54, indicating a *cis* geometry of Pro^5 amide bond.[17] This unusual high field resonance was considered to be due to the ring current effect of the aromatic ring in Phe^6.

The presence of type II β-turn was implied also by the strong ROE correlations between Gly^1-NH and Tyr^2-NH, and between Pro^7-Hα and Gly^1-NH. The weak ROE correlations between Pro^5-Hβ and Phe^6-NH, and between Pro^5-Hδ and Phe^6-NH were indicative of the presence of β-turn structure around Pro^5. Furthermore, the characteristic bond-through ROE correlations were observed between Phe^6-Hβ and Tyr^2-Hδ, and between Phe^6-Hδ and Tyr^2-Hδ. The other ROEs indicated by the arrows in Fig. 3 were also noted in the crystal conformation.

The temperature dependent studies distinguish intra- and intermolecular hydrogen bonding constituting the β-turns and β-bulge, implied by the X-ray crystallographic analysis. The temperature dependence of the amide hydrogen chemical shift[18] was recorded in ten-deg intervals over the range 300-330 K in DMSO-d_6 (Table 4). The strong temperature dependence of the signals of the amide protons in Gly^1 and Gly^4 indicates that these amide groups are exposed to the solvent and may not be

involved in intramolecular hydrogen bonding. In contrast, the Tyr^2, Gly^3 and Phe^6 NH protons exhibited low temperature dependence, suggesting that they were involved in the formation of intramolecular hydrogen bonding. These three intramolecular hydrogen bonds implied by the NMR study correspond to those observed in the crystal conformation by X-ray study.

The side chain conformations of Tyr^2 and Phe^6 were determined by the ROE relationship and vicinal coupling constant between Hα and Hβ. The coupling constants (6.6 and 9.3 Hz) of Hα-Hβ in Tyr^2 indicated that the aromatic ring in Tyr^2 rotated freely. In addition, the vicinal coupling constants (3.0 and 8.2 Hz) between Hα and Hβ in Phe^6 and the presence of ROE correlations between Pro^5 and Phe^6 residues, such as Pro^5-Hβ - Phe^6-NH, Pro^5-Hδ - Phe^6-NH, and between aromatic protons in Phe^6 and Pro^5-Hβ, Hγ, suggested that the rotamer in Phe^6 was *gauche*$^-$, which causes the ring current effect described above.

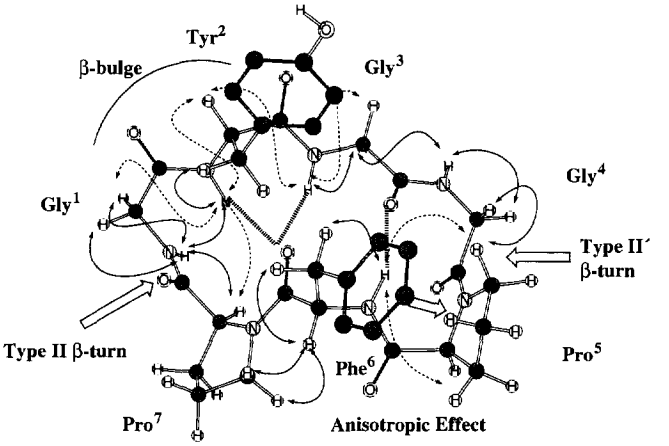

Fig. 3 Proposed conformation of yunnanin A in solution. Arrows show strong ROE relationship and broken arrows show medium or weak ROE relationship. Three thick broken lines represent intramolecular hydrogen bonds.

Table 4 Temperature gradients Δδ/ΔT (ppb/K) of the NH signals of yunnanin A in DMSO-d_6

residue	Gly^1	Tyr^2	Gly^3	Gly^4	Phe^6
Δδ/ΔT	5.3	2.3	3.0	6.7	2.3

ROE restraint MD simulation

Reliable structural information of yunnanin A in DMSO-d_6 was obtained by MD simulations using the ROE relationships, which were performed from the coordinates of X-ray structure of yunnanin A. The interproton distances calculated from the volume of the cross peaks in a phase sensitive ROESY spectrum in DMSO-d_6 were classified into three ranges, 1.8-2.5 Å, 1.8-3.3 Å and 3.0-5.0Å, corresponding to strong, medium and weak ROEs, respectively, of 32 constraints; 10 strong, 14 medium, and 8 weak ROEs. The rMD simulation was equilibrated for a duration of 100 psec in a thermal bath set at 500K, *in vacuo*. The 1000 sampled conformers were finally minimized by the molecular mechanics (MM) calculation of AMBER* force field *in vacuo*.[19] Comparison of the structures deprived of hydrogens was performed to eliminated possible duplicate conformations again

and the maximum atomic deviation allowed for identical conformation was set to 0.25 Å. The 1000 conformers obtained by rMD simulation was reduced to 37 conformers by MM calculation. The total energy of the global minimum structure was 8.42 kcal/mol. Of the 37 conformers by the MM calculation, 22 conformers (659 times) were within a range of 1 kcal/mol from 8.42 kcal/mol of the global minimum structure.

Of these 22 conformers, 15 conformers sufficiently comply with the constraints [average of the sum of the restraint violations was 0.26 (0.02) Å]. The comparison of the backbone atoms of the 15 conformers gave an rms deviation (RMSD) of 0.131 Å. The geometry of all of the peptide bonds including two X-Pro amide bonds in these molecules was *trans*. These conformers contain two β-turn structures between Gly^4 and Pro^5 (type II') and between Pro^7 and Gly^1 (type II), and the β-bulge structure at Tyr^2-Gly^3 as in the crystal structure. Three relatively strong intramolecular hydrogen bonds were observed for these 15 conformers like in the solid state [Tyr^2-NH...Phe^6-C=O: 2.805 (0.02)Å, 160.16 (1.92)°; Gly^3-NH...Phe^6-C=O: 2.740 (0.01)Å, 151.51 (2.09)°; Phe^6-NH...Gly^3-C=O: 2.804 (0.01)Å, 154.96 (0.98)°]. A little shortness of these intramolecular hydrogen bonds compared with those in the crystal state is considered to be due to the calculation under the vacuums[20] and the three intramolecular hydrogen bonds are consistent with the low temperature gradient of the Tyr^2-, Gly^3- and Phe^6-NH protons.

The side chain conformation of Tyr^2 and Phe^6 were shown to be *trans* and *gauche*$^-$, respectively. The pyrolidine of Pro^5 takes C_2-Cγ *endo* conformation as in the crystal, whereas, that of Pro^7 either of the two conformations, C_2-Cγ *endo* and C_2-Cγ *exo*.[15] The large mobility of the pyrolidine ring of Pro^7 was corresponding to the large T1 relaxation time (NT1 value: N=number of attached protons, T1=longitudinal relaxation time) of Pro^7-Cγ and Cδ than those of Pro^5 (Pro^5: Cγ 367 msec., Cδ 299 msec.; Pro^7: Cγ 507 msec., Cδ 321 msec.) in DMSO-d_6.

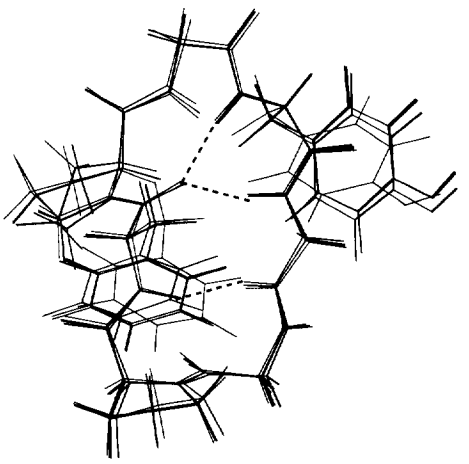

Fig. 4. Superposition of 10 stable conformers of yunnanin A from MD/MM simulation. Broken lines show intramolecular hydrogen bonds.

MC simulation

In the next step, to avoid dependence to the initial X-ray structure, exhaustive Monte Carlo conformational search was conducted by using the systematic pseudo Monte Carlo (MC) search[21] in MacroModel/Batchmin[19] (Ver. 4.5). The pseudosystematic MC procedure of Still and Goodman[21] was performed, in a pseudosystematic way, by changing the torsion angles of 15 ϕ, ψ, χ bonds in the backbone ring in the range of 0 - 180°. A total of 10,000 MC steps were performed to produce 950 conformers. Those conformers whose energy level was less than 25 kJ/mol above that of the global minimum-energy conformer were chosen. To eliminate possible duplicate

conformations, a comparison of the heavy atoms-only structures was performed. After the MC conformational search, each of the resulting conformations was subjected to the energy-minimization calculation using AMBER* force field with the same constraints as in the MD simulation. Of the resulting 472 conformers, 11 conformers appeared to have total energy within a range of 1 kcal/mol above the lowest energy. 10 conformers of these 11 conformers sufficiently comply with the constraints [average of the sum of the restraint violations was 0.28 (0.02) Å]. The comparison of the locations of backbone atoms of the 10 conformers gave an rms deviation (RMSD) of 0.141 Å. The geometry of all of the peptide bonds, including two X-Pro amide bonds, was *trans*. The presence of two β-turns, one β-bulge, and three intramolecular hydrogen bonds [Tyr^2-NH...Phe^6-C=O: 2.796 (0.01)Å, 161.25 (0.90)°; Gly^3-NH...Phe^6-C=O: 2.736 (0.01)Å, 151.47 (1.87)°; Phe^6-NH...Gly^3-C=O: 2.809 (0.01)Å, 155.19 (0.51)°] complied with the conformation obtained by MD simulation. The conformation of side chains and pyrolidine ring by MC were also identical with the results from MD calculation.

The backbone dihedral angles of conformers obtained from the above MD and MC calculations and from ^3J coupling constants calculated by using Bystrov equation[22] were compared with those from the X-ray structure of yunnanin A (Table 3). The backbone conformation in solution is quite similar to that in crystals.

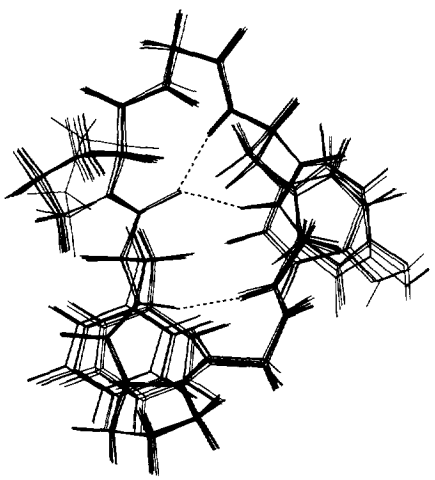

Fig. 5 Superposition of 10 stable conformers of yunnanin A from MC/MM simulation. Broken lines show intramolecular hydrogen bonds.

Conclusion

The solid and solution state conformational analysis of yunnanin A, a cyclic heptapeptide isolated from the roots of *Stellaria yunnanensis* was carried out by X-ray diffraction studies, NMR spectroscopy including studies of temperature dependence of amide protons, ROE correlations, and vicinal coupling constants, and computational methods using MD/MM and MC/MM simulations. The results showed that the molecule had two β-turns (one type II and one type II') incorporating a classical β-bulge motif and all *trans* amide bonds. The dominant solution conformation was homologous to that observed in the solid state.

These conformational characteristics of yunnanin A were also observed in pseudostellarin D[3]: these may be favorable and common features for all cyclic heptapeptides consisting of all L amino acids, whether or not the peptide contains a *cis* amide bond, as in evolidine and hymenamide. Cyclic heptapeptides, such as yunnanin A, pseudostellarin D, evolidine and hymenamide, have different types of β-turns, but they have almost the same pattern of three intramolecular hydrogen bonds constituting a β-bulge motif. Such conformational preference may be observed generally to the conformations of cyclic heptapeptides and may be related to be biological activity.

Experimental

Proton and carbon spectra were recorded on Bruker spectrometers (AM400 and AM500) processed on a Bruker data station with an Aspect 3000 computer and Varian Unity 400 spectrometer. 15 mg of yunnanin A in a 5mm tube (0.5ml DMSO-d_6, degassed) was used for the homonuclear and heteronuclear measurements. The temperature effects on NH chemical shifts to assess the solvent accessibilities to the amide protons were measured at 10°C intervals, over the range of 300 - 330 K, by using a linear regression analysis.

Extraction and Isolation

Yunnanin A used in this experiment was purified as described in our previous paper.[11]

X-ray analysis of yunnanin A

A colorless prismatic crystal of $C_{34}H_{45}N_7O_{10}$ dihydrate having approximate dimensions of 0.50 × 0.40 × 0.20 mm was sealed in a glass capillary. All measurements were made on a Rigaku AFC7R diffractometer with graphite monochromated Cu-Kα radiation and a 12kW rotating anode generator. Cell constants and an orientation matrix for data collection, obtained from a least-squares refinement using the setting angles of 16 carefully centered reflections in the range $57.96<2\theta<59.75°$ corresponded to a primitive orthorhombic cell with dimensions: a=11.754(10), b=12.576(7), c=30.731(6) Å, V=4542(6) Å^3. For Z=4 and F.W.=711.77, the calculated density is 1.04 g/cm^3. Based on the systematic absences of: 00l: $l \neq 2n$, the space group was uniquely determined to be: $P222_1$(#17). The data were collected at a temperature of 23 ± 1°C using the ω-2θ scan technique to a maximum 2θ value of 120.3°. Omega scans of several intense reflections, made prior to data collection, had an average width at half-height of 0.25° with a take-off angle of 6.0°. Scans of (1.63 + 0.30 tan θ)° were made at a speed of 16.0° / min (in omega). The weak reflections ($I < 10.0\sigma(I)$) were rescanned (maximum of 7 scans) and the counts were accumulated to ensure good counting statistics. Stationary background counts were recorded on each side of the reflection. The ratio of peak counting time to background counting time was 2:1. The diameter of the incident beam collimator was 1.0 mm, the crystal to detector distance was 235 mm, and the computer controlled detector aperture was set to 3.0 × 4.5 mm (horizontal × vertical).

A total of 3853 reflections was collected. The intensities of three representative reflection were measured after every 100 reflections. No decay correction was applied. The linear absorption coefficient, μ, for Cu-Kα radiation is 6.5 cm^{-1}. Azimuthal scans of several reflections indicated no need for an absorption correction. The data were corrected for Lorentz and polarization effects. A correction for secondary extinction was applied (coefficient = 4.83620e-06).

The structure was solved by direct methods[12] and expanded by using Fourier techniques.[23] An asymmetric unit contains one heptapeptide molecule and two water molecules which are disordered at the locations represented by the numbers of 80 level. Non-hydrogen atoms, except those of water molecules were refined anisotropically, while the rest were refined isotropically. Hydrogen atoms, excluding those of the OH of tyrosine and waters, were included but not refined. The final cycle of full-matrix least-squares refinement was based on 2340 observed reflections ($I>3.00\sigma(I)$) and 463 variable parameters and converged (largest parameter was 0.01 times its esd) with unweighted and weighted agreement factors of : R=0.091, R_w=0.107. Rather high value of R factor seemed to be caused by disordered solvent molecules. The standard deviation of an observation of unit weight was 1.99. The weighting scheme was based on counting statistics and included a factor (p=0.100) to downweight the intense reflections. Plots of $\Sigma\omega(|Fo|-|Fc|)^2$ versus $|Fo|$, reflection order in data collection, sin θ/λ and various classes of indices showed no unusual trends. The maximum and minimum peaks on the final difference Fourier map corresponded to 0.63 and -0.31 e$^-$/Å^3, respectively. Neutral atom scattering factors were taken from Cromer and Waber.[24] Anomalous dispersion effects were included in Fcalc[25]; the values for Δf' and Δf" were those of Creagh and

McAuley.[26] The values for the mass attenuation coefficients are those of Creagh and Hubbel.[27] All calculations were performed using the teXsan[28] crystallographic software package of Molecular Structure Corporation.

The refined fractional atomic coordinates, bond lengths, bond angles, hydrogen-atom coordinates and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre (CCDC).

Computational Methods - Computer modeling experiments were carried out by using the MACROMODEL program (version 4.5) on an IRIS 4D computer. Molecular mechanics and dynamics calculations were performed with the Amber force field*. The dielectric constant (ε) was assumed to be proportional to the interatomic distances (r) as ε=r. Solvent molecules were not included in the calculations. The extended cutoff distances employed were 8 Å for van der Waals, 20 Å for charge/electrostatics and 10 Å for charge/multipole electrostatics. The ROE relationships shown in Fig. 3 were taken into account in the calculations of the constraint minimizations and dynamics with an extra harmonic term of the form $k(\theta-\theta_0)$ added to the force field (k=100 kJ/Å^2). The obtained structures were energy minimized by the use of the derivative convergence criteria at a value of 0.001 kJ/Å-mol.

[Molecular Dynamics calculation]

Initial calculation was derived from the coordinates of the crystal structure of yunnanin A analyzed by X-ray crystallography. After an initial equilibration period of 15 ps at 500 K, MD at 500 K with 1.0-fs time steps for a total of 100 ps were calculated with $\varepsilon=R_{ij}$. Structures were sampled by time at 0.1-ps intervals. The sampled structures derived from the dynamics trajectories were then energy minimized with the AMBER* all-atom force field.

[Pseudo Monte Carlo calculation]

MC search was carried out by using the Pseudo Monte Carlo routine in MACROMODEL. The starting structure was chosen from the coordinates of the crystal structure of yunnanin A. The closure bond was chosen at C1-C2 with a closure limit of 1 - 3 Å. 10000 Monte Calro step were performed and the produced 950 conformers, which were obtained within 25 kJ/mol of the lowest energy conformer, were minimized by the use of molecular mechanics calculation of AMBER* all-atom force field with an extra harmonic term as in MD simulation.

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