Conformational Feature of Aureobasidin E, a New Type of Potent Antifungal Antibiotic

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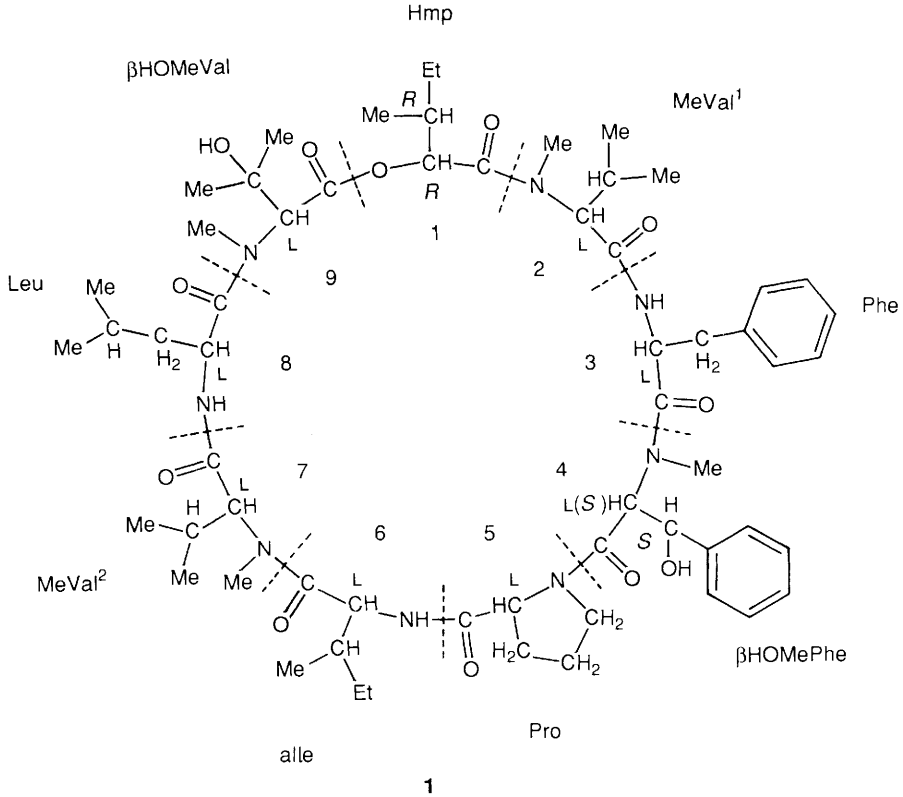
The crystal structure of aureobasidin E, a new type of potent antifungal antibiotic, revealed the molecular conformation stably held by three intramolecular NH···O=C hydrogen bonds, showing a possible feature responsible for its biological activity.

In the search for potent antifungal substances from various microorganisms, a new series of antibiotics named aureobasidins were recently isolated from the fermentation broth of *Aureobasidium pullulans* R106.[1] Among the 18 structurally related aureobasidins isolated,[2,3] some including aureobasidin A and E **1** exhibit potent and broad-spectrum antifungal activity,[1] and their potential for clinical use is being vigorously examined because of their low toxicity. Related cyclic depsipeptides showing antifungal activity have also been isolated from other organisms;[4] most of them characteristically contain fatty acids necessary for their activity. In order to effectively design a clinically usable drug, it is of importance to

Table 1 Conformational torsion angles (°) and their estimated standard deviations in parentheses[a]

	φ	ψ	ω	χ^1	χ^2	χ^3
Hmp	137.7(6)	−174.4(6)	−168.1(6)	−26.6(6)	−58.8(9)	
MeVal[1]	−114.0(6)	110.3(6)	−171.8(6)	−175.8(7)		
				−54.1(6)		
Phe	−129.6(6)	70.8(6)	−175.2(6)	−51.2(5)	100.0(7)	
					−79.7(7)	
βHOMePhe	49.5(5)	−122.9(7)	−170.1(7)	−44.3(5)	−85.5(7)	
					93.9(7)	
Pro	−82.3(6)	4.8(6)	174.7(7)	36.4(4)	−37.7(6)	24.6(6)
alle	−106.4(6)	112.6(6)	175.4(6)	174.4(6)	−173.7(7)	
MeVal[2]	−116.2(5)	91.4(5)	−163.2(6)	−172.9(6)		
				−48.6(6)		
Leu	−148.6(5)	99.5(5)	−173.5(5)	−164.5(6)	174.1(7)	
					−62.4(6)	
βHOMeVal	48.9(5)	42.2(4)	160.6(6)	−169.7(6)		
				−50.3(5)		

[a] The nomenclature of torsion angle follows that of the standard protein. The φ torsion angle of Hmp corresponds to the angle around the –O–C< bond.



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know the molecular conformation of the leading compound, which reflects the biological active form. Thus, the stereostructure of **1** has been elucidated by X-ray crystallography.

After various efforts, plate-like single crystals of **1** were obtained by the slow evaporation of a saturated solution in a mixture of hexane–propan-2-ol–acetonitrile (90:10:5) at room temperature and analysed by X-ray diffraction.† The molecular conformation of **1** is given in Fig. 1, and torsion angles about respective residues are summarized in Table 1.

The backbone conformation of **1** can be grouped into three characteristics, *i.e.*, an antiparallel β-sheet structure, and β- and γ-turns. Three intramolecular hydrogen bonds of [Leu] NH···O=C [Hmp] [2.960(7) Å], [Phe] NH···O=C [aIle] [2.892(7) Å] and [aIle] NH···O=C [Phe] [3.104(7) Å] form an antiparallel β-sheet structure between the Hmp-MeVal¹-Phe and aIle-MeVal²-Leu sequences, although torsion angles (φ, ψ) of respective residues deviate somewhat from the standard values (φ = ~ −139, ψ = ~135°).[5] Two kinds of turn conformations are observed. One is the usual type II′ β-turn structure[6] with a βHOMePhe-Pro sequence at the corner of the bend, which is stabilized by an intramolecular hydrogen bond of [aIle] NH···O=C [Phe] and by an electrostatic interaction of [Pro] N···O=C [Phe] [3.041(7) Å]. The other is a kind of γ-turn structure, where the βHOMeVal residue locates at the corner; the (φ,ψ) torsion angles of this residue take the values of the left-handed α-helix found frequently in peptides containing the uncommon amino acid Aib (φ = ~ 57, ψ = ~ 47°).[7] It appears important to note that the OH group of βHOMeVal is bifurcatedly hydrogen-bonded to two carbonyl atoms of Leu [= 2.740(6) Å] and βHOMeVal [= 3.075(7) Å]. This, in addition to an electrostatic interaction of [βHOMe-

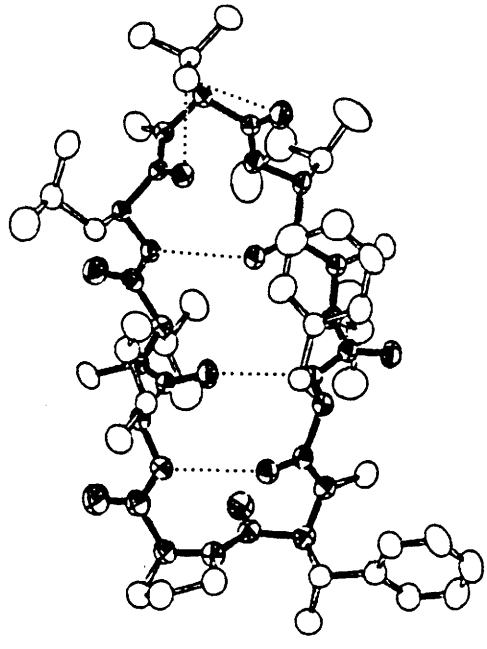

Fig. 1 A perspective view of molecular conformation of **1**. The backbone chains are shown by the detailed thermal ellipsoids and thick bonds. Possible intramolecular hydrogen bonds are shown by dotted lines.

† *Crystal data*: $C_{60}H_{92}N_8O_{12}$, M_r = 1117.43, monoclinic, space group $P2_1$, a = 16.458(3), b = 10.638(3), c = 18.133(6) Å, β = 103.51(2)°, V = 3087(1) Å^3, Z = 2, D_c = 1.202, D_o = 1.198(3) g cm^{-3} (flotation method using H_2O–KI saturated H_2O mixture), λ(Cu-Kα) = 1.5418 Å, μ(Cu-Kα) = 6.45 cm^{-1}, F(000) = 1208. The crystals were sealed in glass capillaries containing some mother liquor. A single crystal of dimensions 0.2 × 0.3 × 0.5 mm was used for X-ray diffraction data collection on a Rigaku AFC-5 diffractometer employing graphite-monochromated Cu-Kα radiation. A total of 5808 independent reflections within 2θ = 130° were collected in an ω–2θ scan mode and were corrected for the Lorentz and polarization factors. Among them, 5553 reflections of $|F_o|>\sigma(F_o)$ were used for the structure determination and refinement. The structure was finally solved by direct methods and refined by least-squares analysis with use of the anisotropic temperature factors for non-H atoms and isotropic factors for H atoms, where H atoms, except some methyl protons, were observed on a final difference Fourier map. The present discrepancy indexes R and R_w are 0.067 and 0.089, respectively. The atomic coordinates, anisotropic thermal parameters, and bond lengths and angles have been deposited at the Cambridge Crystallographic Data Centre. See Notice to Authors, Issue No. 1.

Val] N···O [Hmp ester] [2.661(6) Å], stabilizes the γ-turn conformation of βHOMeVal residue.

The present X-ray results suggest consideration of the following questions which are closely related to the emergence of activity. A possible reason why the amide NH groups of MeVal,[1] βHOMePhe, MeVal[2] and βHOMeVal residues are methylated is the formation of the only pentagonal-like conformation shown in Fig. 1, which is stabilized by three intramolecular hydrogen bonds. Otherwise, many conformations, depending on different combinations of intramolecular hydrogen bonds, would be possible for **1**. The βHOMePhe and Pro residues function to form a type II′ β-turn structure. This turn structure, together with the β-sheet structure between Hmp-MeVal[1]-Phe and aIle-MeVal[2]-Leu sequences, makes the βHOMeVal residue located at the tip of **1** an entire conformation. Since the OH absence in βHOMeVal leads to a significant decrease in activity,[1] it may be reasonable to consider that the βHOMeVal γ-turn conformation, which is stably fixed by two intramolecular OH···O hydrogen bonds, is necessary for binding with the receptor or for revealing its activity, in addition to the participation of its OH group in binding to the receptor.

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