186. The Crystal Structure of Enniatin B

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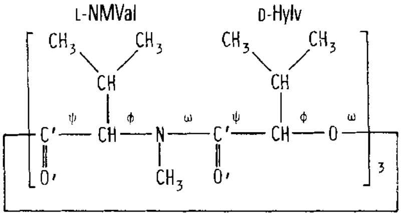
The X-ray crystal structure of enniatin B (trigonal (hexagonal axes), $a = 14.626$ Å, $c = 16.309$ Å, space group $R3$) is described and the conformation compared to results of other investigations.

Introduction. – Enniatin B (**1**) is a natural ion-carrier antibiotic composed of three alternating residues of D-hydroxy-isovaleryl (D-HyIv) and L-*N*-methyl-valyl (L-NMVal) linked into a cyclohexadepsipeptide by amide and ester bonds. The chemical properties [1] as well as the observed ion selectivity have been extensively studied [2–4]. In solution, the existence of two different conformations was suggested by CD and ORD studies [5]. In polar solvents, form P with the threefold symmetry given by the chemical formula was postulated. In this conformation the six carbonyl O-atoms point to the inside, forming a cavity. Form P was also proposed for equimolar cation complexes in all types of solvents [3]. In nonpolar solvents form N having no symmetry was assumed from NMR studies extended to −120°C [5][6]. This conformation is compact, without a cavity. Relaxation measurements [7] indicated the existence of even more than two conformations in solution. Conformations P and N were investigated by empirical energy minimization [8] (using an analoguous compound in which all i-Pr groups were replaced by CH_3 and conformation P also by quantum mechanical calculations [16]. Recently an extended force-field calculation by *Lifson et al.* [9] resulted in a minimum energy conformation with C_3 symmetry similar to form P.



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An early crystal-structure analysis of a KI complex of enniatin B [10] had to make use of poor quality crystals. Therein, the disc-shaped cation complexes are stacked along the hexagonal axis and the anions are statistically distributed in the channels between these stacks. The structure could only be established in projection down the hexagonal axis.

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From this, it was clear that form P is present in the crystal, but it cannot be decided whether the cations sit at the centers of the enniatin B molecules or whether they are situated between them. This latter situation could arise from the observation by spectroscopic measurements [11] that in solution 1:1 and sandwich-type 2:1 complexes exist in equilibrium.

A crystal-structure analysis of a hydrated form containing 1.5 molecules of H_2O per molecule of enniatin B was published by *Tishchenko et al.* [12]. In this the molecule has C_3 crystallographic symmetry with 1 H_2O at its center and another with occupancy 0.5 displaced by 2.6 Å along the threefold axis. Since the H_2O molecules cannot have threefold symmetry, their H-atoms must be disordered.

A crystal-structure investigation of unhydrated enniatin B had been started by us in 1969. Because the crystals were of very poor quality the results were only cited in a review [13]. In unhydrated enniatin B, the amide and ester groups are tilted obliquely to the mean ring plane, in the hydrated form they are practically perpendicular so as to accomodate the H_2O molecule at the center. We were interested to see that the model calculated by *Lifson et al.* [9] resembled the hydrated form rather than the unhydrated. This raised the question which of the two conformations represented the real minimum energy form. It was proposed [14] that crystal packing effects could be responsible for the observed conformation of unhydrated enniatin B and that a form with nearly perpendicular ester and amide groups represented the true energy minimum. In the course of this investigation the crystal structure of unhydrated enniatin B was re-evaluated and refined, and the results are discussed in this contribution.

Discussion. – The structure of enniatin B is depicted in *Fig. 1*. The molecule has crystallographic C_3 symmetry. The conformations of the ester and the amide groups are given in *Table 1*, where they are compared to other determinations. Both groups are tilted with respect to the hexagonal axis, the ester group by 23°, the amide group by 17°. The amide group shows substantial deviation from planarity as witnessed by the out-of-plane torsion angles $\theta_N = 4.7°$ and $\theta_{C'} = 5.6°$ (for the definition of out-of-plane torsion angles see *Table 1*). The octahedron formed by the 6 carbonyl O-atoms has approximately equal

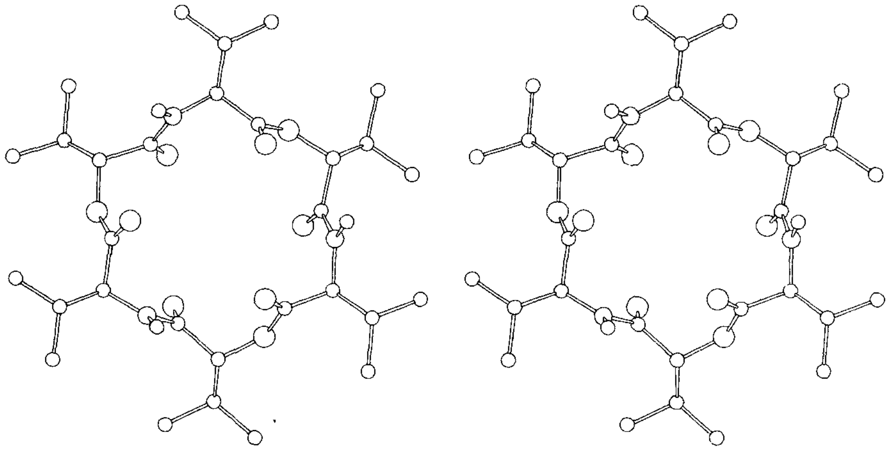

Fig. 1. *Stereoview of Enniatin B*

Table 1. *Conformations of Enniatin B*[a])

	D-HyIv					L-NMVal					
	ϕ	ψ	ω	$\theta_{C'}$[c])	α[b])	ϕ	ψ	ω	$\theta_{C'}$	θ_N[c])	α[b])
(1)	79.3	−125.4	−165.1	5.6	+ 23	−95.0	134.3	177.6	3.0	4.7	+17
(2)	118.7	−109.9	−172.2	0.7	−3	−119.7	107.0	175.7	0.1	−0.7	−4
(3)	112	−98	178	0	−4	−127	112	−178	+3	−1	−10
(4)	46	−108				−88	122				
(5)	74	−136				−103	171				

[a]) First column: (1) Enniatin B unhydrated, this work; (2) Enniatin B·1.5 H_2O [12]; (3) extended force-field calculation [9]; (4) PCILO calculation [16]; (5) empirical calculation, P form [8].

[b]) Tilt angle between hexagonal axis and least-squares plane through ester or amide group. α is positive if the carbonyl-O-atom is closer to the center than N- or O-atoms, negative otherwise.

[c]) Out-of-plane torsion angles defined for ester group: $\theta_C' = \omega_1 - \omega_2 + \pi$, with $\omega_1(C^\alpha{-}C'{-}O{-}C^\alpha)$ and $\omega_2(O'{-}C'{-}O{-}C^{\alpha'})$; for amide group: $\theta_N = \omega_2 - \omega_3 + \pi = -\omega_1 + \omega_4 + \pi$, $\theta_{C'} = \omega_1 - \omega_3 + \pi = -\omega_2 + \omega_4 + \pi$, with $\omega_1(C^\alpha{-}C'{-}N{-}C^{\alpha'})$, $\omega_2(O'{-}C'{-}N{-}R)$, $\omega_3(O'{-}C'{-}N{-}C^{\alpha'})$ and $\omega_4(C^\alpha{-}C'{-}N{-}R)$.

sides (NMVal O′:4.39 Å, HyIv O′:4.30 Å, NMVal O′..HyIv O′:3.89, 4.01 Å). The i-Pr groups are staggered, with torsion angles close to 60° (NMVal $C^{\gamma 1}$, 59.3°; $C^{\gamma 2}$, −62.1°; HyIv $C^{\gamma 1}$, −56.8°; $C^{\gamma 2}$, 59.5°). Thus, the C^γ-atoms lie in planes 0.83 Å above and below the mean molecular plane.

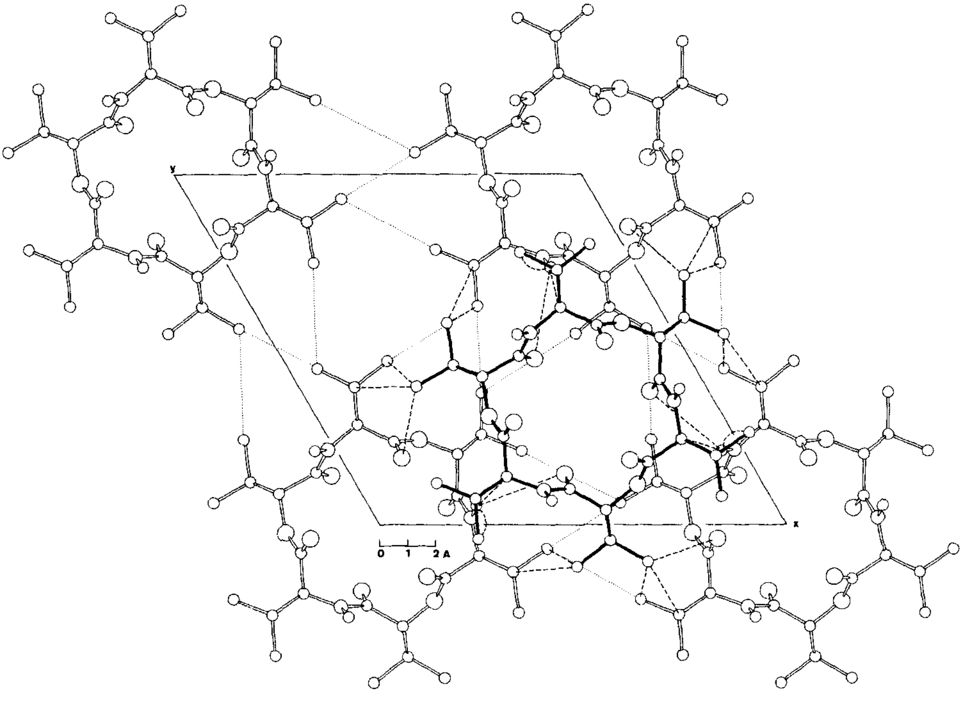

Fig. 2. *Packing of enniatin B molecules in the unit cell.* Molecules with empty bonds are in the layer z = O, the molecule with full bonds is at (2/3, 1/3, 1/3). Interatomic distances less than 4.2 Å indicated. Dotted: between molecules in the same layer; dashed: to molecules in different layers.

The packing is shown in *Fig. 2*. The molecules are arranged in hexagonal layers with the centers of molecules at (0,0,0); (2/3, 1/3, 1/3) and (1/3, 2/3, 2/3). There are 3 intermolecular distances < 4.2 Å between neighbouring molecules within a hexagonal layer (shortest distance NMVal C$^{\gamma2}$..HyIv C$^{\gamma2}$, 3.53 Å), and 8 to molecules above or below (shortest distance NMVal O′..HyIv C$^{\gamma2}$, 3.79 Å). Three contacts involve the *N*-CH$_3$ group of NMVal residues, the others are mainly to atoms of the i-Pr groups. An assumed solvent molecule, SOLV, has its closest contacts to atoms of the HyIv i-Pr group (3.48..3.82 Å) and the *N*-CH$_3$ group (3.74 Å).

Table 2. *Atomic Coordinates and Isotropic Temperature Factors* (U_{iso} [Å^2]) *with Estimated Standard Deviations in Parentheses*

		x	*y*	*z*	*U*
NMVal	N	0.2350(11)	0.0209(11)	0.0332(–)	0.041(4)
	C′	0.0820(15)	–0.1540(15)	0.0329(13)	0.056(5)
	O′	0.0409(13)	–0.1491(12)	0.0949(13)	0.091(5)
	C(N)	0.2647(15)	0.0549(15)	0.1166(11)	0.074(6)
	C$^{\alpha}$	0.1974(12)	–0.0897(13)	0.0088(12)	0.039(5)
	C$^{\beta}$	0.2584(9)	–0.1361(10)	0.0529(12)	0.051(5)
	C$^{\gamma1}$	0.2144(15)	–0.2526(11)	0.0292(13)	0.071(6)
	C$^{\gamma2}$	0.3749(10)	–0.0701(14)	0.0270(13)	0.066(5)
HyIv	O	0.0241(10)	–0.2189(10)	–0.0284(8)	0.052(4)
	C′	0.2312(14)	0.0827(14)	–0.0278(12)	0.043(5)
	O′	0.1889(10)	0.0487(10)	–0.0920(11)	0.064(4)
	C$^{\alpha}$	–0.0897(15)	–0.2918(13)	–0.0172(12)	0.050(5)
	C$^{\beta}$	–0.1226(11)	–0.3795(11)	–0.0817(13)	0.057(5)
	C$^{\gamma1}$	–0.2429(11)	–0.4536(14)	–0.0759(13)	0.073(6)
	C$^{\gamma2}$	–0.0622(15)	–0.4400(15)	–0.0728(15)	0.085(6)
SOLV		0.1167(21)	0.1433(20)	–0.2105(17)	0.117(8)

Table 3. *Bond Lengths* (Å). Estimated standard deviations range from 0.013..0.023 Å.

NMVal N –NMVal C$^{\alpha}$	1.48	NMVal C′ –HyIv O	1.35	HyIv O –HyIv C$^{\alpha}$	1.47
–HyIv C′	1.36	–NMVal O′	1.20	HyIv C′ –HyIv O′	1.19
–NMVal C(N)	1.44	NMVal C$^{\beta}$–NMVal C$^{\gamma1}$	1.54	HyIv C$^{\alpha}$–HyIv C′	1.52
NMVal C$^{\alpha}$–NMVal C′	1.52	–NMVal C$^{\gamma2}$	1.54	–HyIv C$^{\beta}$	1.54
–NMVal C$^{\beta}$	1.54			HyIv C$^{\beta}$ –HyIv C$^{\gamma1}$	1.54
				–HyIv C$^{\gamma2}$	1.54

Table 4. *Bond Angles* (°). Estimated standard deviations range from 1.0..1.7°.

NMVal C$^{\alpha}$–NMVal N–HyIv C′	114	NMVal C$^{\gamma1}$–NMVal C$^{\beta}$–NMVal C$^{\gamma2}$	110
NMVal C$^{\alpha}$–NMVal N–NMVal C(N)	121	NMVal C′–HyIv O–HyIv C$^{\alpha}$	120
HyIv C′–NMVal N–NMVal C(N)	125	NMVal N–HyIv C′–HyIv O′	123
NMVal N–NMVal C$^{\alpha}$–NMVal C′	107	NMVal N–HyIv C′–HyIv C$^{\alpha}$	119
NMVal N–NMVal C$^{\alpha}$–NMVal C$^{\beta}$	112	HyIv O′–HyIv C′–HyIv C$^{\alpha}$	118
NMVal C′–NMVal C$^{\alpha}$–NMVal C$^{\beta}$	108	HyIv O–HyIv C$^{\alpha}$–HyIv C$^{\beta}$	107
NMVal C$^{\alpha}$–NMVal C′–HyIv O	111	HyIv O–HyIv C$^{\alpha}$–HyIv C′	109
NMVal C$^{\alpha}$–NMVal C′–NMVal O′	128	HyIv C$^{\beta}$–HyIv C$^{\alpha}$–HyIv C′	112
HyIv O–NMVal C′–NMVal O′	121	HyIv C$^{\alpha}$–HyIv C$^{\beta}$–HyIv C$^{\gamma1}$	108
NMVal C$^{\alpha}$–NMVal C$^{\beta}$–NMVal C$^{\gamma1}$	111	HyIv C$^{\alpha}$–HyIv C$^{\beta}$–HyIv C$^{\gamma2}$	113
NMVal C$^{\alpha}$–NMVal C$^{\beta}$–NMVal C$^{\gamma2}$	107	HyIv C$^{\gamma1}$–HyIv C$^{\beta}$–HyIv C$^{\gamma2}$	112

Table 5. *Selected Torsion Angles* (°). Estimated standard deviations for ϕ, 4 and ω in parentheses.

HyIv C′–NMVal N–NMVal C^{α}–NMVal C′	– 95 (2.0)	ϕ NMVal
HyIv C′–NMVal N–NMVal C^{α}–NMVal C^{β}	146	
NMVal C^{α}–NMVal N–HyIv C′–HyIv C^{α}	–165 (1.7)	ω HyIv
NMVal C^{α}–NMVal N–HyIv C′–HyIv O′	9	
NMVal C(N)–NMVal N–HyIv C′–HyIv O′	–166	
NMVal C(N)–NMVal N–HyIv C′–HyIv C^{α}	20	
NMVal N–NMVal C^{α}–NMVal C′–HyIv O	134 (1.6)	4 NMVal
NMVal C^{β}–NMVal C^{α}–NMVal C′–HyIv O	–105	
NMVal N–NMVal C^{α}–NMVal C^{β}–NMVal $C^{\gamma 1}$	178	
NMVal N–NMVal C^{α}–NMVal C^{β}–NMVal $C^{\gamma 2}$	– 62	
NMVal C^{α}–NMVal C′–HyIv O–HyIv C^{α}	178 (1.7)	ω NMVal
NMVal O–NMVal C′–HyIv O–HyIv C^{α}	– 5	
NMVal C′–HyIv O–HyIv C^{α}–HyIv C^{β}	–159	
NMVal C′–HyIv O–HyIv C^{α}–HyIv C′	79 (2.2)	ϕ HyIv
HyIv O–HyIv C^{α}–HyIv C^{β}–HyIv $C^{\gamma 1}$	–176	
HyIv O–HyIv C^{α}–HyIv C^{β}–HyIv $C^{\gamma 2}$	60	
HyIv O–HyIv C^{α}–HyIv C′–NMVal N	–125 (1.7)	4 HyIv
HyIv C–HyIv C^{α}–HyIv C′–NMVal N	117	

Experimental. – This analysis is based on data collected in 1969. The crystals of enniatin B were grown from synthetic material²) in a mixture of MeOH and H_2O. Various crystal forms were obtained under different crystallization conditions. All crystals were of poor quality and showed a rapid fall-off of intensities with increasing scattering angle.

Crystallographic Data. Enniatin B, $C_{33}H_{57}O_9N_3$, $M = 639.8$, trigonal (hexagonal axes), $a = b = 14.626(16)$, $c = 16.309(18)$ Å, $V = 3021.4$ Å³, $Z = 3$, space group *R3* (C_3^4), $d_m = 1.13$, $d_x = 1.06$ g/cm³ (with MeOH solvent molecule: 1.11 g/cm³). The reflection intensities were measured with a four-circle diffractometer (*Hilger & Watts Y290*) using graphite-monochromatized MoK_{α} radiation. In the range $\theta < 18°$ all reflections were measured, in the range $\theta = 18...22°$ only strong reflections were included. 612 unique reflections were obtained, of which 575 with $F > \sigma(F)$ were used in the structure determination. The reflections had high background values. Symmetry equivalent reflections were carefully checked and reflections showing large discrepancies or uneven background were excluded.

Structure Analysis and Refinement. The structure was solved by direct methods using a preliminary version of SHELX84 [15]. All non-H-atoms could be assigned. Least-squares refinement with isotropic temperature factors lead to a model in which positions for most of the H-atoms could be seen in a subsequent difference *Fourier* map. In further stages calculated H positions were used. A peak at about (0.12, 0.14, 0.37) was interpreted as O-atom of an included solvent molecule, either H_2O or MeOH, and refined. In the final difference *Fourier* map the highest unaccounted residual electron density peaks were 0.35 e/Å³, located mainly close to O-atoms. They may stem from deficiencies in the refinement model using only isotropic temperature factors. The final R factor is 0.113, $R_w = 0.126$ (for weights $1/\sigma^2(F) + 0.005\ F^2$). The atomic coordinates are given in *Table 2* and details of the molecular topography in *Tables 3, 4*, and *5*.

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