Structure of Hormaomycin, a Naturally Occurring Cyclic Octadepsipeptide, in the Crystal

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Z. Naturforsch. **2014**, *69b*, 945 – 949 / DOI: 10.5560/ZNB.2014-4116
Received May 30, 2014

The structure of hormaomycin has been determined in two crystals grown under different conditions, *i. e.* in the absence and in the presence of magnesium chloride. In both crystals, the macrocyclic hexadepsipeptide assumes a rather flat conformation, and the dipeptide side chain resides in the same equatorial plane. This is a significant difference in comparison with the compact bent conformation of hormaomycin in solution, as previously determined by an extensive NMR study.

Key words: Crystal Structure, Hormaomycin, Macrocyclic Depsipeptide, X-Ray Diffraction

Introduction

Hormaomycin **1**, a macrocyclic depsipeptide consisting of eight amino acid residues (Fig. 1), was first isolated and structurally elucidated in 1989 – 1990 [1, 2]. Its absolute configuration was fully assigned some 15 years later [3, 4] and rigorously confirmed by total synthesis [5].

Since hormaomycin **1** features some interesting biological activity [1, 2] including a significant inhibitory effect against *Plasmodium falciparum*, the pathogen causing malaria [6], more than 20 analogs were prepared to enable, among others, the study of structure-activity relationships and the conformational behavior in solution [7 – 9]. Detailed NMR-spectroscopic analysis revealed a rather compact secondary structure of the octapeptide, and it appeared that this compact conformation is essential for the biological activity [9]. It would certainly be interesting to know whether that same conformation prevails in the crystal. Several earlier attempts to crystallize the native hormaomycin were not successful, but finally crystals of the synthetic material **1** could be obtained and subjected to X-ray diffraction.

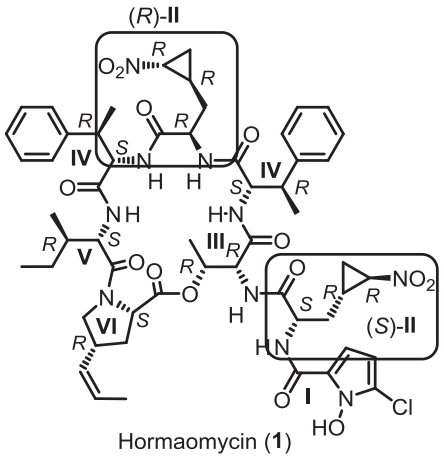

Fig. 1. Structural formula with absolute configuration of hormaomycin (**1**). **I**: Chpca; **II**: (1*R*′,2*R*′)-(3-Ncp)Ala; **III**: (2*R*)-α-Thr; **IV**: (2*S*,3*R*)-(βMe)Phe; **V**: (*S*)-Ile; **VI**: (2*S*,4*R*)-4-(*Z*)-(4-PE)Pro.

Results and Discussion

Hormaomycin crystallized in space groups $P1$ and $P2_1$ from aqueous solutions at weakly basic pH = 8.5,

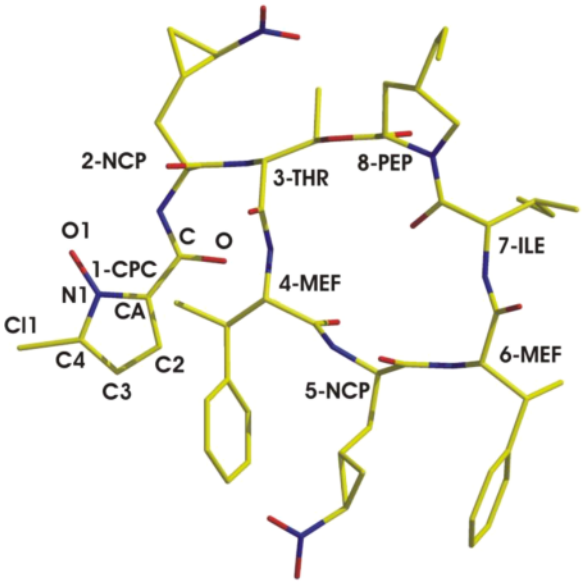

Fig. 2 (color online). Monomer structure of hormaomycin with 3-letter residue labels which correspond to the naming scheme in the deposited CIF files. CPC: **I** (Chpca); 2-NCP: (*S*)-**II** [(2*S*,1*R*′,2*R*′)-(3-Ncp)Ala]; 5-NCP: (*R*)-**II** [(2*R*,1*R*′,2*R*′)-(3-Ncp)Ala]; THR: **III** [(2*R*)-α-Thr]; MEF: **IV** [(2*S*,3*R*)-(βMe)Phe]; ILE: **VI**: [(2*S*,4*R*)-4-(*Z*)-(4-PE)Pro]. Only the atom names for Chpca are given explicitly. For better clarity other atom names are not explicitly given. However, the atom names follow the usual amino acid naming schemes.

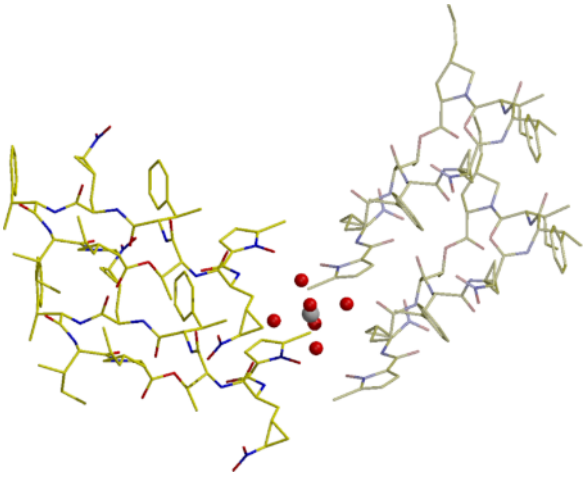

Fig. 3 (color online). Hormaomycin (**1**) in space group *P*1 with a Mg^{2+} ion in six-fold coordination with water molecules surrounded by four hormaomycin molecules.

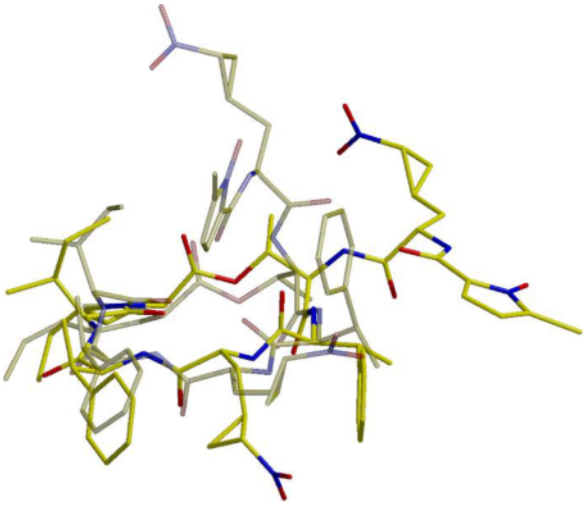

Fig. 4 (color online). Superposition of the *P*1 crystal structure (yellow carbon atoms) with the NMR-derived structure in solution (semi-transparent). The latter assumes a more compact conformation with residues (*S*)-**II** [(2*S*,1*R*′,2*R*′)-(3-Ncp)Ala] and **III** [(2*R*)-α-Thr], and the side chains of **IV** [(2*S*,3*R*)-(βMe)Phe] and **V** [(*S*)-Ile] being bent towards the center of the rather flat main ring.

depending on the presence or absence of $MgCl_2$, respectively. Both crystals contain two independent molecules. The monomer structure of hormaomycin (**1**) is shown in Fig. 2, while Fig. 3 displays the asymmetric unit of the structure in space group *P*1 with a six-fold water-coordinated Mg^{2+} ion. All molecules assume an almost planar conformation and share high local homology. The root-mean-square deviation (rmsd) for the superposition between one molecule from each crystal form is 1.4 Å for 118 atoms. The strongest deviations occur in the flexible side chains, and the rmsd only for the main chain atoms of the flat ring section is 0.26 Å for 24 atoms. In contrast to the crystal structure, the structure in $CDCl_3$ solution, as previously determined in an elaborate NMR-spectroscopic study [9], shows a compactly folded conformation, in which the residues **I** (Chpca) and (*S*)-**II** [(2*S*,1*R*′,2*R*′)-(3-Ncp)Ala] are bent almost perpendicular to the rather flat main ring consisting of residues **III**–**VI**, as illustrated in Fig. 4. This most probably is caused by an energetically favorable π-stacking interaction between the *N*-hydroxy-2-chloropyrrol moiety at the end of the side-chain **I** (Chpca) and one of the phenyl groups of the two β-methylphenylalanine residues **IV** in the macrocycle. In DMSO solution, however, the π-stacking interaction

Table 1. Backbone torsion angles for all four independent crystal structures of hormaomycin in comparison with those obtained by NMR spectroscopy. Torsion angle definitions: ϕ: C_{i-1}–N_i–$C_{\alpha,i}$–C_i; $\psi = N_{i-1}$–$C_{\alpha,i-1}$–C_{i-1}–N_i; $\omega = C_{\alpha,i-1}$–C_{i-1}–N_i–$C_{\alpha,i}$.

Building block	Torsion angle ϕ (deg)			NMR[a]	
	In $P1$ form	In $P2_1$ form	Mean value	DMSO	$CDCl_3$
I (Chpca)	–[b]	–	–	–	–
(*S*)-**II** [(2*S*,1*R*′,2*R*′)-(3-Ncp)Ala]	−71, −70	−57, −65	−65.8	*[c]	−68
(*R*)-**II** [(2*R*,1*R*′,2*R*′)-(3-Ncp)Ala]	140, 137	124, 131	133.0	116	69
III [(2*R*) − α-Thr]	76, 78	63, 60	69.3	*[c]	100
IVa [(2*S*,3*R*)-(β-Me)Phe]	−122, −120	−99, −104	−111.3	−98	−67
IVb [(2*S*,3*R*)-(β-Me)Phe]	−92, −89	−79, −79	−84.8	−83	−90
V (*S*)-Ile	−138, −132	−109, −110	−122.3	−128	−93
VI (2*S*,4*R*)-4-(*Z*)-(4-PE)Pro	−62, −60	−58, −61	−60.3	−66	−61
Building block	Torsion angle ψ (deg)			NMR[a]	
	In $P1$ form	In $P2_1$ form	Mean value	DMSO	$CDCl_3$
I (Chpca)	–[b]	–	–	–	–
(*S*)-**II** [(2*S*,1*R*′,2*R*′)-(3-Ncp)Ala]	123, 122	107, 115	116.8	*[c]	105
(*R*)-**II** [(2*R*,1*R*′,2*R*′)-(3-Ncp)Ala]	−150, −154	−161, −164	−157.3	−133	−135
III [(2*R*)-α-Thr]	36, 33	28, 29	31.5	*[c]	−69
IVa [(2*S*,3*R*)-(β-Me)Phe]	132, 133	135, 133	133.3	126	180
IVb [(2*S*,3*R*)-(β-Me)Phe]	−3, −3	−20, −16	−10.5	−7	−47
V (*S*)-Ile	173, 169	165, 167	168.5	160	152
VI (2*S*,4*R*)-4-(*Z*)-(4-PE)Pro	–[b]	–	–	–	–
Building block	Torsion angle ω (deg)			NMR[a]	
	In $P1$ form	In $P2_1$ form	Mean value	DMSO	$CDCl_3$
I (Chpca)	176, 173	183, 187	179.8	*[c]	182
(*S*)-**II** [(2*S*,1*R*′,2*R*′)-(3-Ncp)Ala]	178, 181	181, 179	179.8	*[c]	178
(*R*)-**II** [(2*R*,1*R*′,2*R*′)-(3-Ncp)Ala]	187, 187	184, 181	184.8	178	192
III [(2*R*)-α-Thr]	185, 184	180, 180	182.3	−173	170
IVa [(2*S*,3*R*)-(β-Me)Phe]	180, 178	171, 173	175.5	170	166
IVb [(2*S*,3*R*)-(β-Me)Phe]	169, 168	173, 171	170.3	180	173
V (*S*)-Ile	181, 188	183, 182	183.5	*[c]	184
VI (2*S*,4*R*)-4-(*Z*)-(4-PE)Pro	–[b]	–	–	–	–

[a] As reported for the NMR models in DMSO [10] and $CDCl_3$ [9]; [b] empty entries mark undefined angles in the respective residue; *[c] not reported in ref. [10].

apparently does not play a significant role, and the conformation of the side chain is less well defined [10]. Table 1 compares the mean backbone angles of the two crystallographic structures with those reported by Blackledge and Griesinger [10] for the NMR models in DMSO and $CDCl_3$. As was assumed for the NMR-derived structures, in the crystals all peptide bonds have an *s-trans* orientation.

Conclusion

Two different crystal structures of hormaomycin (**1**) with two independent molecules each assume an open, flat conformation and show no *s-cis*-peptide bonds. The overall similarity of all four molecules is very high with small variations in the flexible side chains. This structure in the crystal significantly differs from the compact folded structure in $CDCl_3$ solution [9], but in DMSO the side chain is more or less in a similar orientation as in the crystal. Blackledge, Griesinger *et al.* reported rms deviations between the superimposed macrocyclic rings of 2.3 Å between the solution and crystal structures, but only of 0.66 Å between the structures in the two different solvents [10]. They attributed these differences to the different hydrogen-bonding properties of the solvents involved. In view of the much larger differences between the crystal and the NMR-derived structures than between the latter two, we suggest that intermolecular interactions in the crystal may have even more influence on the confor-

mations than the solvents. Since hormaomycin in this study crystallized under aqueous conditions, we suggest that the crystal structures represent the conformation of hormaomycin *in vivo*. Whereas at least one of the NMR studies [10] appears to have used natural hormaomycin, the crystal structure analyses were performed on the synthetic product. Since these atomic resolution structures were solved by *ab initio* Direct Methods without making any assumptions about the chemical structures involved, they provide a convincing confirmation that the natural and synthetic compounds are indeed identical.

Experimental Section

Crystals were grown by the hanging drop vapor diffusion method. A drop (1 μL) of a solution of approx. 56 g L^{-1} (*w*/*v*) hormaomycin (**1**) in EtOH mixed with 1 μL of an aqueous buffer solution was hung over 300 μL of the reservoir solution. The reservoir solution for the crystal in space group $P2_1$ contained 0.1 M Tris-HCl (2-amino-2-hydroxymethylpropane-1,3-diol adjusted to pH = 8.5 by addition of aq. HCl solution), 50% MPD (2-methylpentane-2,4-diol) and 5% dioxane, while the reservoir solution for the crystal in space group *P*1 contained 0.1 M Tris-HCl (pH = 8.5), 50% isopropanol and 20 mM $MgCl_2$. The crystal form $P2_1$ was improved by micro-seeding. X-ray data were collected at the synchrotrons DESY in Hamburg, Germany ($P2_1$), and at the Paul Scherrer Institute (Swiss Light Source) in Switzerland (*P*1). The crystals diffracted poorly even with synchrotron radiation, so although the structures were unambiguously determined, their precision is not as high as usual for small molecule crystal structures. Data were processed and scaled with XDS [11]. The structures were solved and refined with SHELX-2013 [12]. The models were built with COOT [13] and SHELXLE [14]. Restraints for solvent molecules were generated from the grade-server (http://grade.globalphasing.org). Figures were created with MOLSCRIPT [15] and RASTER3D [16].

Refinement of **1** × $[Mg(H_2O)_6]X_2$ (space group *P*1): Due to the poor quality of the intensity data set the Flack parameter could not be determined reliably. The chirality was determined relative to the chirality of the standard L-amino acids.

Table 2. Crystal structure data for **1** × $[Mg(H_2O)_6]X_2$ and **1**.

	1 × $[Mg(H_2O)_6]X_2$	**1**
Formula	$(C_{55}H_{68}ClN_{10}O_{14})_2$, $(C_3H_7O)_2$, Mg, O_{18} $C_{116}H_{150}Cl_2MgN_{20}O_{48}$	$(C_{55}H_{68}N_{10}O_{14}Cl)_2$, $(C_6H_{12}O_2)_4$, $(C_4H_8O_2)_2$, Na, O_{10} $C_{142}H_{200}Cl_2N_{20}NaO_{50}$
M_r	2687.76	3081.10
Cryst. size (max/mid/min), mm	0.10/0.20/0.20	0.07/0.07/0.05
Crystal system	triclinic	monoclinic
Space group	*P*1 (no. 1)	$P2_1$ (no. 4)
Wavelength, Å	0.77492	0.8430
a, Å	7.370(2)	14.080(3)
b, Å	15.969(4)	32.760(7)
c, Å	30.280(5)	18.660(4)
α, deg	92.086(10)	90
β, deg	91.01(2)	101.73(3)
γ, deg	102.693(10)	90
V, Å^3	3473.1(13)	8427(3)
Z	1	2
T, K	100(2)	100(2)
$D_{calcd.}$, g cm^{-3}	1.29	1.21
$\mu(MoK_\alpha)$, cm^{-1}	0.2	0.2
F(000), *e*	1416	3274
hkl range	±7, ±17, ±31	−12/13, ±31, −16/18
θ(min/max), deg	1.63/27.99	1.48/24.40
Refl. measured	24 167	28 028
Refl. unique/R_{int}	16 630/0.0943	14 773/0.0844
Refl with $I > 2\,\sigma(I)$	14 734	10 235
Data/restraints/parameters	16 630/2717/1589	14 773/3784/1921
$R(F)/wR(F^2)$[a] (all refls.)	0.1313/0.3267	0.1632/0.3527
GoF (F^2)[b]	1.312	1.216
$\Delta\rho_{fin}$ (max/min), *e* Å^{-3}	1.05/−0.75	0.68/−0.47

Only the two antibiotics molecules and the Mg with its six coordinating oxygen atoms were refined anisotropically, and no attempt was made to place hydrogens on the water oxygens.

Refinement of **1** (space group $P2_1$): As above, the chirality was determined relative to the chirality of the standard L-amino acids. Only the two antibiotics molecules were refined anisotropically, and no attempt was made to place hydrogens on the water oxygens. Despite the low density and high U values for MPD (2-methyl-2,4-pentanediol) and DOX (dioxane), they show up as difference density when the molecules are removed. The large shift/error and the short H···H contacts reported by PLATON refer to one of the dioxane molecules. The data resolution does not allow the detailed identification of ions nor a discussion of hydrogen bonds.

Table 2 contains the crystal data and numbers pertinent to data collection and structure refinement of both structure determinations.

CCDC 1003606 contains the supplementary crystallographic data for space group $P1$. CCDC 1003607 contains the supplementary crystallographic data for space group $P2_1$. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre *via* www.ccdc.cam.ac.uk/data_request/cif.

Acknowledgement

This work was supported by the Deutsche Forschungsgemeinschaft (SFB-416, Project A3). T. G. was supported by the Volkswagen Stiftung *via* the Niedersachsenprofessur awarded to G. M. S.

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