CRYSTAL STRUCTURE OF CYCLOSPORIN E

Michal HUSAK[a1], Bohumil KRATOCHVIL[a2], Martin BUCHTA[b], Ladislav CVAK[b] and Alexandr JEGOROV[c,*]

[a] *Department of Solid State Chemistry, Prague Institute of Chemical Technology, 166 28 Prague 6, Czech Republic; e-mail: [1] husakm@vscht.cz, [2] kratochb@vscht.cz*

[b] *Galena Co., R.&D., 747 70 Opava-Komarov, Czech Republic*

[c] *Galena Co., Research Unit, Branisovska 31, 370 05 Ceske Budejovice, Czech Republic; e-mail: husakm@marvin.jcu.cz*

Received August 26, 1997
Accepted October 15, 1997

The structure of cyclosporin E was determined by X-ray diffraction methods and compared with the structure of related cyclosporins. In contrast to cyclosporin A, which crystallizes from acetone as tetragonal dihydrate, cyclosporin E acetone solvate monohydrate ($C_{61}H_{109}N_{11}O_{12}\cdot C_3H_6O\cdot H_2O$) crystallizes under the same conditions in the monoclinic space group $P2_1$ with a = 15.698(2) Å, b = 21.333(3) Å, c = 13.224(2) Å, β = 103.74(1)°, Z = 2, and V = 4 302(1) Å^3.

Key words: Cyclosporin E; Clathrate; Conformation; Crystal structure determination.

Cyclosporins are natural undecapeptides derived from cyclosporin A (CsA), cyclo-(-MeBmt[1]-Abu[2]-Sar[3]-MeLeu[4]-Val[5]-MeLeu[6]-Ala[7]-D-Ala[8]-MeLeu[9]-MeLeu[10]-MeVal[11]-), where MeBmt = (4*R*)-4-[(*E*)-2-butenyl]-4,*N*-dimethyl-L-threonine [(2*S*,3*R*,4*R*,6*E*)-3-hydroxy-4-methyl-2-(*N*-methylamino)-6-octenoic acid)] (Fig. 1), by formal substitution of one or two amino acids. Cyclosporin A is nowadays widely used as immunosuppresant for organ transplantations and treatment of autoimmune diseases (*Consupren*®, Galena; *Sandimmun*®, Novartis). Although cyclosporins are structurally almost identical, they strongly differ in their pharmacological activity[1]. Several crystal structures of cyclosporins were reported including: cyclosporin A dihydrate[2] ($P4_1$), cyclosporin A monohydrate[3] ($P2_12_12_1$), cyclosporin A dimethylisosorbide solvate[4] ($P2_1$), isocyclosporin A (ref.[5]) ($C222_1$), iodocyclosporin A (ref.[6]) ($P2_1$), thiocyclosporin[7] ($P2_1$), cyclosporin H (ref.[8]) ($I2$), and [3,*O*-didehydro-MeBmt[1],Val[2]]cyclosporin[9] ($P3_221$). The aim of this paper is to evaluate the role of MeVal[11] demethylation of cyclosporin A on the conformation and hydrogen bonding in the solid state.

* The author to whom correspondence should be addressed.

EXPERIMENTAL

Crystal Structure Determination of Cyclosporin E

Cyclosporin E (440 mg, Galena Co.) was dissolved in acetone (10 ml), and heptane (50 ml) was added under vigorous stirring. The solution was allowed to stand in an open flask to slowly evaporate. Since the crystals were desolvatation-prone, a suitable single crystal was mounted in a capillary with a residual amount of a mother liquor. Cyclosporin E acetone solvate monohydrate ($C_{61}H_{109}N_{11}O_{12}\cdot C_3H_6O\cdot H_2O$, M_r = 1 264.7, monoclinic system, space group $P2_1$ (No. 4), a = 15.698(2) Å, b = 21.333(3) Å, c = 13.224(2) Å, β = 103.74(1)°, Z = 2, V = 4 302(1) Å^3, D_{calc} = 0.976 g cm^{-3}, μ(CuKα) = 0.53 mm^{-1}, F(000) = 1 380. Data collection conditions are given in Table I.

The coordinates of atoms from the known structure of cyclosporin A dimethylisosorbide solvate[4] were used as starting model and refined. The position of one water and two acetone molecules were found from subsequent Fourier series. All non-H atoms of cyclosporin and water oxygen were refined anisotropically by full-matrix least-squares based on F values. Positions of acetone atoms were refined with restrained geometry and fixed during the final refinement. The occupancy factor for both two acetone molecules was fixed on the value 0.5 because the NMR analysis indicated 1 : 1 cyclosporin E/acetone ratio. Positions of C and N hydrogens were found from expected geometry and

TABLE I
Data collection and refinement parameters

Crystal dimensions	0.8 × 0.8 × 0.6 mm
Diffractometer and radiation used	Enraf–Nonius CAD4, CuKα, λ = 1.54056 Å
Scan technique	ω/2θ
Temperature	293 K
No. and θ range of reflections for lattice parameter refinement	22; 21.9–55.0°
Range of h, k and l	0→16, –22→22, –14→14
Standard reflections monitored in interval; intensity fluctuation	120 min; 13.8%
Total number of reflections measured	10 944
2θ range	1–110°
No. of observed independent reflections	4 979
Criterion for observed reflections	$I \geq 1.96\sigma(I)$
Function minimized	$\sum w (F_o - F_c)^2$
Weighting scheme	Chebychev weighting (ref.[10])
Parameters refined	766
Value of R, wR, and S	8.3, 8.4, 1.0
Ratio of max. least-squares shift to e.s.d. in the last cycle	0.17
Max. and min. heights in final Δρ map	–0.30, 0.50 e Å^{-3}
Source of atomic scattering factors	International Tables for X-Ray Crystallography (ref.[11])
Programs used	CRYSTALS (ref.[12]), PARST (ref.[13]), SIR92 (ref.[14])

constrained during refinement. The –OH and H_2O hydrogens were not found from difference Fourier maps. The same numbering scheme as for cyclosporin A (ref.[4]) was used for cyclosporin E in order to enable easy correlation of related parameters. Since cyclosporin E is the demethylated cyclosporin A analogue, the corresponding number of the C57 atom was omitted. Tables of observed and calculated structure factors, final positional and thermal parameters of all atoms can be obtained from the first author upon request.

RESULTS AND DISCUSSION

Cyclosporin E is a natural analogue of cyclosporin A with the 11th amino acid, MeVal demethylated ([Val[11]]cyclosporin) (Fig. 1). Since additional N–H groups in its structure

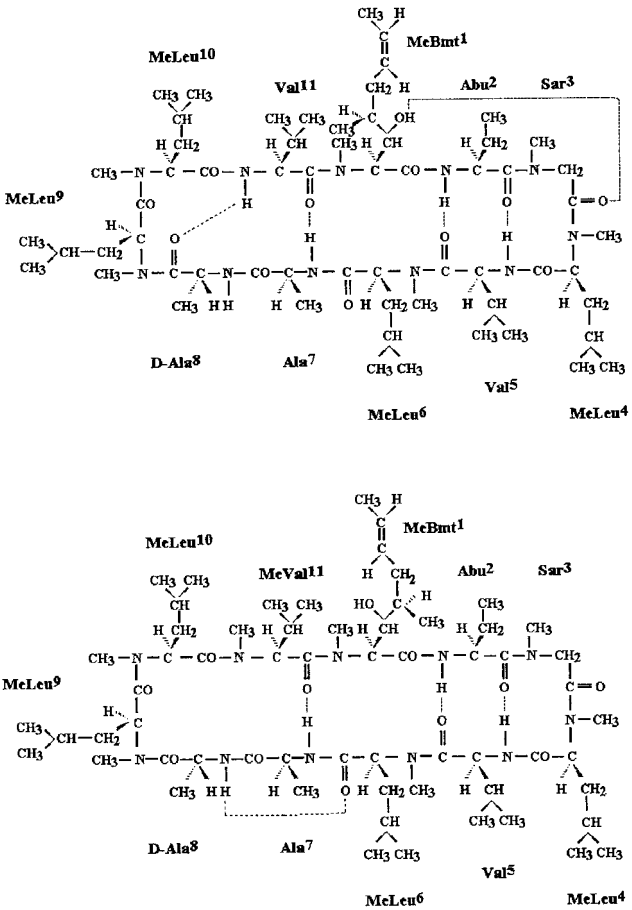

FIG. 1
Schematic representation of structure and hydrogen bonds in cyclosporin E acetone solvate monohydrate (top) and cyclosporin A dihydrate (bottom)

offer new possibilities for hydrogen bonding, its pronounced effect on the cyclosporin conformation was expected. Crystallization of cyclosporin E was attempted from several solvents. Cyclosporin E crystallized in well developed single crystals either from acetone–diethyl ether or acetone–heptane. First examination of crystals from acetone–diethyl ether indicated that the loss of solvent causes considerable decomposition of crystals even in a sealed capillary. Since diethyl ether also contributed to difficulties in the manipulation with crystals, the acetone–heptane mixture was choosen as the more convenient alternative. Examination of new single crystals revealed that both crystals have the same cell parameters and might eventually differ only in a solvated molecule. Hence, crystals obtained from acetone–heptane were studied in detail.

The final positional and thermal parameters of the non-H are deposited. All bond distances and angles are unexceptional and are, therefore, also deposited. Figure 2 shows the ORTEP drawing of cyclosporin E molecule in the structure, including the atom numbering. Whereas cyclosporin A crystallizes from acetone as tetragonal dihydrate, cyclosporin E crystallizes under the same conditions in a slightly different conformation. This conformation is characterized by the torsion angles ψ_1(N1C2C3N2) = 159.0(4)°, ψ_7(N7C37C38N8) = 12.9(9)°, ϕ_8(C38N8C40C41) = 163.0(6)°, and by MeBmt[1] side chain orientation χ_1(N1C2C4C5) = –46.2(6)°. The side chain orientation of Val[11] differs from related structures by χ_{11}(N11C58C60C61) = –176.8(7)° (χ_{11} = –33(1)° for cyclosporin A dimethylisosorbide solvate[4]). The crystal packing is dictated by the hydrogen bonds: Ala[7]NH-Val[11]CO, Abu[2]NH-Val[5]CO, Val[5]NH-Abu[2]CO, MeBmt[1]OH-Sar[3]CO, and Val[11]NH-D-Ala[8]CO, in which the new N–H bond participates (Fig. 1). The Ala[7] carbonyl group forms a H-bond with the water molecule. This conformation is not,

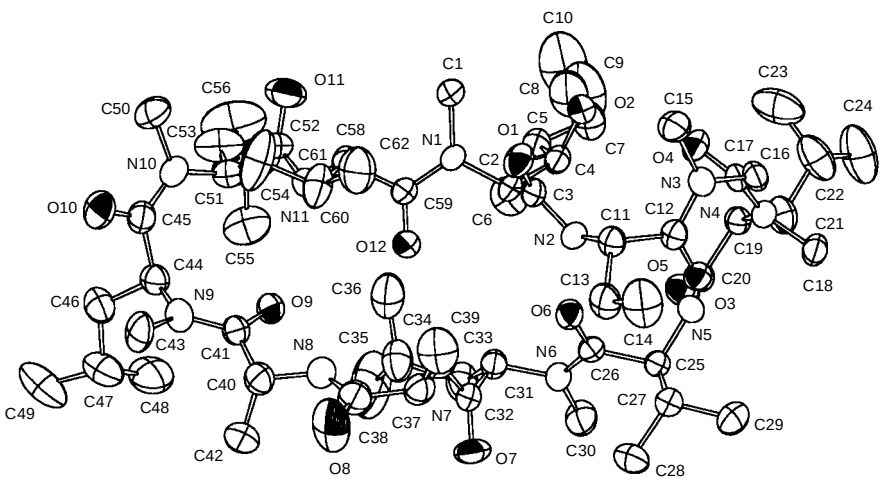

FIG. 2
ORTEP drawing of cyclosporin E (thermal ellipsoids at 50% probability)

however, unknown and is related to the structures of cyclosporin A dimethylisosorbide solvate[4] and thiocyclosporin diethyl ether solvate[7] ($C_{62}H_{111}N_{11}O_{10}S_2 \cdot C_4H_{10}O$), where two amide O-atoms were replaced by two S-atoms ([$^4\psi^5$, CS–NH; $^7\psi^8$, CS–NH]CsA). Hence, our results indicate that this cyclosporin conformation is not unique, affected by *e.g.* some exotic solvent or unusual structural modification, but might be the common conformation of cyclosporins in non-polar solvents. The structural type of $P2_1$ cyclosporin might be also considered as a type of cyclopeptidic clathrate with highly conserved backbone conformation creating the cavity in which various solvent molecules are entraped. In the present case of cyclosporin E, the diameters of the cavity are roughly 10 × 3 × 2.5 Å and the internal volume 200 Å^3 (Fig. 3). The shape and the volume of the cavity was determined as the space for arbitrary carbon atoms not being in van der Waals contacts with another atom. The very low calculated density for this crystal (D_{calc} = 0.976 g cm^{-3}) indicates that the presence of an additional disordered

TABLE II
Important packing constants for individual cyclosporin crystalline forms, V_m = molecular volume calculated by program GEPOL (ref.[15]) (solvents were not included), Kitaigorodskii packing coefficient[16] $C_K = V_m Z/V$

Compound	V_m, Å^3	V, Å^3	Z	C_K
Cyclosporin E	1 113	4 302	2	0.517
Cyclosporin A dihydrate (ref.[2])	1 151	7 896	4	0.583
Cyclosporin A monohydrate (ref.[3])	1 156	7 216	4	0.641
Cyclosporin A dimethylisosorbide solvate (ref.[4])	1 129	4 121	2	0.548

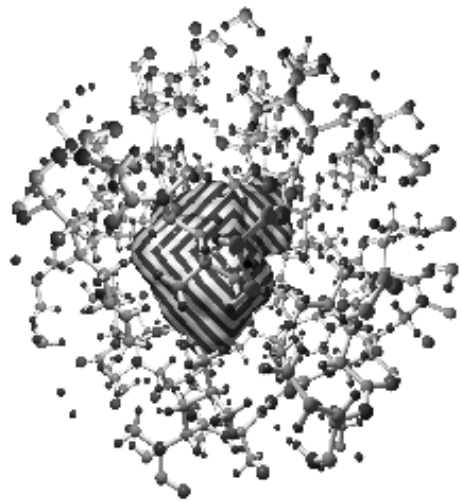

FIG. 3
Visualization of the cavity in the structure of cyclosporin E

water molecule in the structure cannot be excluded. The tendency of this cyclosporin modification to icorporate small host molecules is indicated also by the low value of Kitaigorodskii packing coefficient in comparison with related compounds (Table II).

This work was in part supported by the grant No. VS 96085 of Ministry of Education, and by the grants No. 203/97/0623 and No. 203/96/0111 of the Grant Agency of the Czech Republic.

REFERENCES

1. Wenger R. M.: *Prog. Chem. Org. Nat. Prod.* **1986**, *50*, 123.
2. Loosli H. R., Kessler H., Oschkinat H., Weber H. P., Petcher T. J., Widmer A.: *Helv. Chim. Acta* **1985**, *68*, 682.
3. Knott R. B., Schefer J., Schoenborn B. P.: *Acta Crystallogr., Sect. C: Cryst. Struct. Commun.* **1990**, *46*, 1528.
4. Husak M., Kratochvil B., Jegorov A., Matha V., Stuchlik M., Andrysek T.: *Z. Kristallogr.* **1996**, *211*, 313.
5. Pohl E., Sheldrick G. M., Bolsterli J. J., Kallen J., Traber R., Walkinshaw M. D.: *Helv. Chim. Acta* **1996**, *79*, 1635.
6. Petcher T. J., Weber H.-P., Ruegger A.: *Helv. Chim. Acta* **1976**, *59*, 1480.
7. Seebach D., Ko S. Y., Kessler H., Kock M., Reggelin M., Schmieder P., Walkinshaw M. D., Bolsterli J. J., Bevec D.: *Helv. Chim. Acta* **1991**, *74*, 1953.
8. Husak M., Ondracek J., Jegorov A.: *IUCr XVII Congress, Seattle, Collected Abstracts* **1996**, PS05.02.06.
9. Pohl E., Herbst-Irmer R., Sheldrick G. M., Dauter Z., Wilson K. S., Bolsterli J. J., Bollinger P., Kallen J., Walkinshaw M. D.: *Helv. Chim. Acta* **1995**, *78*, 355.
10. Carruthers J. R., Watkin D. J.: *Acta Crystallogr., Sect. A: Fundam. Crystallogr.* **1979**, *35*, 698.
11. *International Tables for X-Ray Crystallography*, Vol. IV. Kynoch Press, Birmingham 1974.
12. Watkin D. J.: *CRYSTALS. Crystallographic package*. University of Oxford, Oxford 1989.
13. Nardelli M.: *PARST. System of Computer Routines for Calculating Molecular Parameters from the Results of Crystal Structure*. University of Parma, Parma 1991.
14. Altomare A., Burla M. C., Camalli M., Cascarano G., Giaccovazzo C., Guagliardi A., Polidori G.: *J. Appl. Crystallogr.* **1994**, *27*, 435.
15. Silla E., Tunon I., Pascual-Ahuir J. L.: *J. Comput. Chem.* **1991**, *12*, 1077.
16. Alkorta I., Rozas I., Elguero J., Foces-Foces C., Cano F. H.: *J. Mol. Struct.* **1996**, *382*, 205.