CRYSTAL STRUCTURES OF CYCLOSPORIN DERIVATIVES: *O*-ACETYL-(4*R*)-4-(*E*-2-BUTYL)-4,*N*-DIMETHYL-L-THREONYL-CYCLOSPORIN A AND *O*-ACETYL-(4*R*)-4-[*E*-2-(4-BROMOBUTYL)]-4,*N*-DIMETHYL-L-THREONYL-CYCLOSPORIN A

Bohumil KRATOCHVÍL[a1], Alexandr JEGOROV[b,*], Svetlana PAKHOMOVA[a2], Michal HUŠÁK[a3], Petr BULEJ[c1], Ladislav CVAK[c2], Petr SEDMERA[d1] and Vladimír HAVLÍČEK[d2]

[a] *Department of Solid State Chemistry, Prague Institute of Chemical Technology, Technická 5, 166 28 Prague 6, Czech Republic; e-mail: [1] kratochb@vscht.cz, [2] pachomos@vscht.cz, [3] husakm@vscht.cz*

[b] *Galena Co., Research Unit, Branišovská 31, 370 05 České Budějovice, Czech Republic; e-mail: husakm@marvin.jcu.cz*

[c] *Galena Co., R. D., 747 70 Opava-Komárov, Czech Republic; e-mail: [1] pbulej@ivax.com, [2] ladislav_cvak@ivax.com*

[d] *Institute of Microbiology, Academy of Sciences of the Czech Republic, Vídeňská 1083, 142 20 Prague 4, Czech Republic; e-mail: [1] sedmera@biomed.cas.cz, [2] vlhavlic@biomed.cas.cz*

Received June 19, 1998
Accepted September 2, 1998

The structures of *O*-acetyl-(4*R*)-4-(*E*-2-butyl)-4,*N*-dimethyl-L-threonyl-cyclosporin A (**1**) and *O*-acetyl-(4*R*)-4-[*E*-2-(4-bromobutyl)]-4,*N*-dimethyl-L-threonyl-cyclosporin A (**2**) were determined by X-ray diffraction methods and compared with the structure of related cyclosporins. In contrast to expectation, neither the acetylation nor the subsequent bromination of **1** affects the conformation and packing of cyclosporins in the solid state. Both compounds are isomorphous and crystallize in the orthorhombic space group $P2_12_12_1$ with a = 12.936(2) Å, b = 15.590(2) Å, c = 36.280(3) Å, and a = 12.916(3) Å, b = 15.675(4) Å, c = 36.715(7) Å, for **1** and **2**, respectively.

Key words: Cyclic peptides; Cyclosporins; Crystal structure determination; X-Ray diffraction; NMR spectroscopy; Immunosupressants.

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, Fig. 1). CsA is biologically active substance which is used as an immunosupressant for organ transplantations and treatment of various autoimmune diseases (*Consupren*®, Galena).

So far, three crystal structures of solvated cyclosporin A have been reported including: CsA monohydrate[1,2] (**3**, $P2_12_12_1$), CsA dihydrate[3] (**4**, $P4_1$) and CsA dimethyl isosorbide clathrate[4] (**5**, $P2_1$). The conformation of CsA in nonpolar solvents[5,6] is very similar to that found in various single crystal forms[3,7]. In more polar solvents, such as DMSO, the number of conformations is increasing[6], which is probably caused by the breaking of the intramolecular H-bonds and formation of H-bonds to the solvent molecules. However, the conformation of CsA found in the crystalline state also predominates in polar solvents. X-Ray structural studies demonstrated[8,9] that CsA after complexation with its transport protein – cyclophilin adopts the complete different all-*trans* conformation with the only intramolecular hydrogen bonds between the MeBmt[1] (OH) side chain and MeLeu[4] (CO). Another new backbone form of CsA in complex with lithium chloride in THF solution has been reported recently[10] in which the configuration of the peptide bond between MeLeu[9] and MeLeu[10] has changed from *cis* to *trans* similarly as in the complex with cyclophilin.

Both [*O*-acetyl-MeBmt[1]]CsA (**1**) and [*O*-acetyl-ω-bromo-MeBmt[1]]CsA (**2**) are useful intermediates in the synthesis of human metabolite[11] AM1

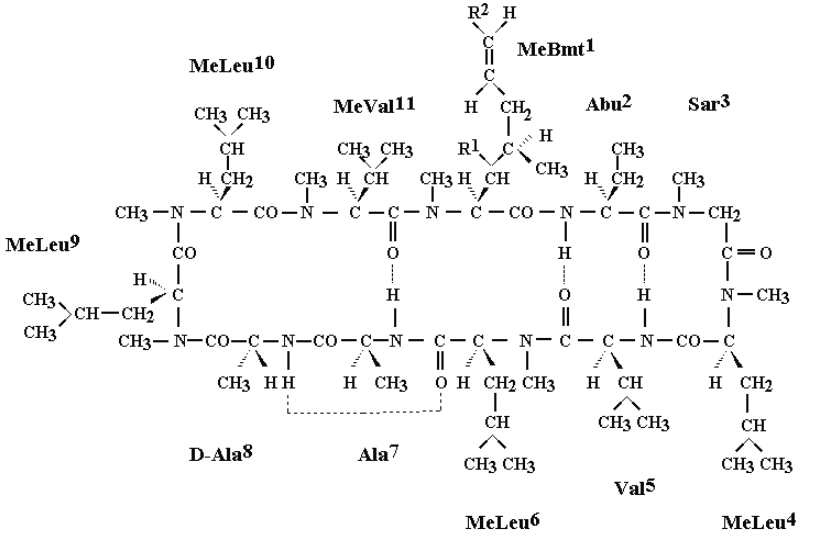

FIG. 1
Schematic representation of conformations and hydrogen bonds common for **1**–**4**, **6**. **1**: R^1 = CH_3COO–, R^2 = CH_3; **2**: R^1 = CH_3COO–, R^2 = –CH_2Br; **3**–**4**: R^1 = –OH, R^2 = –CH_3; **6**: R^1 = CH_3COS–, R^2 = CH_3

(M-17, **7**) and/or in the synthesis of tritium-labeled[12] cyclosporin A. Acetylation of cyclosporin A leads to a complete loss of its immunosuppressive activity[13], indicating that the introduction of acetyl group might influence the conformation of the cyclopeptide backbone. However, its ability to sensitize multidrug-resistant cells to chemotherapeutic agents is retained[13]. The aim of this paper is to evaluate the effect of acetylation and subsequent bromination of cyclosporin A on its conformation and hydrogen bonding in the solid state.

EXPERIMENTAL

Source of Materials

[*O*-Acetyl-MeBmt[1]]CsA (**1**) and [*O*-acetyl-ω-bromo-MeBmt[1]]CsA (**2**) were prepared by acetylation of cyclosporin A (99.5%, Galena, Czech Republic) with acetic anhydride and bromination of **1** with *N*-bromosuccinimide[11,12]. Identification: FAB MS (Finnigan MAT 90, *m*-nitrobenzyl alcohol): **1** *m/z* 1 244.9 $[M + H]^+$, 1 184.8 $[MH - CH_3CO_2H]^+$; **2** $[M + H]^+$ as a doublet *m/z* 1 322.8/1 324.8 ($^{79}Br/^{81}Br$ 1 : 1). For the comparison of conformations of **1** and **2** in the crystalline state and solution, so far unpublished full NMR assignment is provided for **2** (Varian VXR-400, 399.95 MHz for ^1H, 100.58 MHz for ^{13}C, $CDCl_3$, 25 °C). Carbon signal multiplicity was determined by APT and DEPT. The 2D NMR experiments on which the reported assignments are based (COSY, delay-COSY, ROESY, HOM2DJ and HETCOR) were performed using the manufacturer's software: δ 0.795 (3 H, d, *J* = 6.5 Hz, H-6δ_u); 0.807 (3 H, d, *J* = 6.8 Hz, H-5γ_u); 0.849 (3 H, d, *J* = 6.7 Hz, H-11γ_u); 0.853 (3 H, t, *J* = 7.4 Hz, H-2γ); 0.855 (3 H, d, *J* = 6.3 Hz, H-9δ_u); 0.885 (3 H, d, *J* = 6.8 Hz, H-11γ_d); (3 H, d, *J* = 6.4 Hz, H-9δ_d); 0.896 (3 H, d, *J* = 6.9 Hz, 1γ-Me); 0.951 (3 H, d, *J* = 6.6 Hz, H-10δ_u); 0.957 (3 H, d, *J* = 6.6 Hz, H-10δ_d); 0.985 (3 H, d, *J* = 6.6 Hz, H-4δ_u); 0.992 (3 H, d, *J* = 6.5 Hz, H-6δ_d); 1.019 (3 H, d, *J* = 6.6 Hz, H-5δ_d); 1.048 (3 H, d, *J* = 6.5 Hz, H-6δ_d); 1.182 (1 H, m, H-10β_u); 1.209 (1 H, m, H-9β_u); 1.263 (3 H, d, *J* = 6.8 Hz, H-8β); 1.311 (3 H, d, *J* = 7.2 Hz, H-7β); 1.333 (1 H, m, H-9γ); 1.421 (1 H, m, H-10γ); 1.449 (1 H, m, H-4γ); 1.650 (1 H, m, H-4β_u); 1.667 (1 H, m, H-1δ_u); 1.690 (2 H, m, H-2β); 1.899 (1 H, m, H-6γ); 1.903 (1 H, m, H-1γ); 2.022 (3 H, s, Ac); 2.022 (1 H, m, H-4β_d); 2.070 (1 H, m, H-10β_d); 2.139 (1 H, m, H-9β_d); 2.180 (1 H, m, H-1δ_u); 2.219 (1 H, m, H-6β_d); 2.418 (1 H, dqq, *J* = 9.0, 6.8, 6.6 Hz, H-5β); 2.651 (3 H, s, 10-Me); 2.674 (3 H, s, 11-Me); 3.106 (3 H, s, 4-Me); 3.180 (1 H, d, *J* = 13.8 Hz, H-3α_u); 3.208 (3 H, s, 9-Me); 3.255 (3 H, s, 6-Me); 3.263 (3 H, s, 3-Me); 3.453 (3 H, s, 1-Me); 3.894 (1 H, dd, *J* = 9.9, 7.8 Hz, H-1ω_u); 3.938 (1 H, dd, *J* = 9.9, 8.0 Hz, H-1ω_d); 4.412 (1 H, dq, *J* = 6.9, 7.2 Hz, H-7α); 4.647 (1 H, d, *J* = 13.8 Hz, H-3α_d); 4.738 (1 H, dd, *J* = 9.0, 9.0 Hz, H-5α); 4.837 (1 H, dq, *J* = 7.6, 6.8 Hz, H-8α); 4.952 (1 H, ddd, *J* = 9.7, 6.9, 6.9 Hz, H-2α); 4.972 (1 H, d, *J* = 11.1 Hz, H-11α); 5.143 (1 H, dd, *J* = 8.5, 5.6 Hz, H-10α); 5.276 (1 H, dd, *J* = 7.0, 3.6 Hz, H-6α); 5.306 (1 H, dd, *J* = 6.7, 3.8 Hz, H-4α); 5.464 (1 H, ddd, *J* = 15.2, 4.9, 4.8 Hz, H-1ε); 5.510 (1 H, dd, *J* = 11.6, 1.0, H-1β); 5.539 (1 H, d, *J* = 11.6 Hz, H-1α); 5.572 (1 H, ddd, *J* = 15.2, 8.0, 7.8 Hz, H-1η); 5.675 (1 H, dd, *J* = 11.1, 4.2 Hz, H-9α), 7.429 (1 H, d, *J* = 7.6 Hz, 8-NH); 7.568 (1 H, d, *J* = 9.0 Hz, 5-NH); 8.022 (1 H, d, *J* = 6.9 Hz, 7-NH); 8.551 (1 H, d, *J* = 9.7 Hz, 2-NH).

^{13}C NMR (100.58 MHz, $CDCl_3$, 25 °C): δ 9.88 q (C-2γ), 15.02 q (C-7β), 17.67 q (C1γ-Me), 17.90 q (C-8β), 18.08 q (C-5γ_u), 18.62 q (C-11γ_u), 19.69 q (C-5γ_d), 20.46 q (C-11γ_d), 20.7 + q

(Ac), 21.15 q (C-6δ_u), 21.28 q (C-4δ_u), 21.81 q (C-9δ_u), 23.50 q (C-4δ_d), 23.52 q (C-10δ_u), 23.76 q (3 C, C-6δ_d, C-9δ_d, C-10δ_d), 24.22 d (C-10γ), 24.50 d (C-4γ), 24.66 d (C-9γ), 24.75 d (C-6γ), 24.90 t (C-2β), 29.47 d (C-11β), 29.68 q (9-Me), 29.86 q (10-Me), 30.17 q (11-Me), 31.35 q (4-Me), 31.45 q (6-Me), 31.66 d (C-5β), 32.33 q (3-Me), 32.87 t (C-1ω), 32.99 d (C-1γ), 33.64 t (C-1δ), 35.93 t (C-4β), 37.14 t (C-6β), 39.23 t (C-9β), 39.29 q (1-Me), 40.98 t (C-10β), 44.68 d (C-8α), 47.87 d (C-9α), 48.24 d (C-7α), 48.73 d (C-2α), 50.02 t (C-3α), 54.25 (C-6α), 54.86 d (C-5α), 55.28 d (C-4α), 56.04 d (C-1α), 57.29 d (C-10α), 58.28 d (C-11α), 72.97 d (C-1β), 128.49 d (C-1η), 134.12 d (C-1ε), 167.95 s (Ac C=O), 170.09 s, 170.33 s, 170.44 s, 170.77 s, 170.94 s, 171.19 s, 171.31 s, 172.78 s, 172.96 s, 173.45 s, 173.74 s (11 × C=O). Note: subscripts u and d denote the upfield and downfield resonating atom in diastereotopic pairs.

Crystal Structure Determination

Compounds **1** or **2** (1 g) were dissolved in acetone (5 ml) and heptane (50 ml) was added under vigorous stirring. The solution was allowed to stand in an open flask to slowly evaporate. Crystals were filtered off , washed with an acetone–heptane mixture (1 : 20, v/v), and dried in air.

Compound **1**: $C_{64}H_{113}N_{11}O_{13}$; M_r = 1 244.7, orthorhombic system, space group $P2_12_12_1$ (No. 19), a = 12.936(2) Å, b = 15.590(2) Å, c = 36.280(3) Å, Z = 4, V = 7 317(2) Å^3, D_{calc} = 1.130 g cm^{-3}, μ(Cu$K\alpha$) = 0.64 mm^{-1}, F(000) = 2 712. The structure was solved by direct methods and anisotropically refined by full-matrix least-squares. Hydrogen atoms were found from expected geometry and were not refined. Absorption was neglected. The absolute configuration was assigned based on that of cyclosporin A.

Compound **2**: $C_{64}H_{112}BrN_{11}O_{13}$; M_r = 1 323.6, orthorhombic system, space group $P2_12_12_1$ (No. 19), a = 12.926(3) Å, b = 15.675(4) Å, c = 36.715(7) Å, Z = 4, V = 7 433(3) Å^3, D_{calc} = 1.183 g cm^{-3}, μ(Cu$K\alpha$) = 1.24 mm^{-1}, F(000) = 2 848. The structure was solved by direct methods. The semi-empirical absorption correction based on ψ-scan of six reflections[14] was applied. H-atoms were included in calculated positions. The C14 carbon (the terminal atom of Abu[2]) was found disordered over two positions with the same site-occupancy factors of 0.5. Its parameters for both positions were refined isotropically with restrained geometry. The positions of the remaining atoms were refined anisotropically by full-matrix least-squares. The large residual electron density and decrease of standard reflections indicated a possible presence of a water molecule in the structure, but it was impossible to interpret difference Fourier maxima in this way. Absolute configuration was determined using refinement of Flack's enantiopole parameter to final value of x = 0.11(4).

The complete X-ray data can be obtained from the fourth author upon request.

RESULTS AND DISCUSSION

Data collection and structure refinement parameters for compounds **1** and **2** are listed in Table I. Figure 2 shows ORTEP drawing of **1** and **2**. The crystal structures and the numbering of the peptide backbone are shown in Fig. 2. Both **1** and **2** crystallize in the orthorhombic space group $P2_12_12_1$ and exhibit a compact antiparallel β-sheet structure with four intramolecular hydrogen bonds involving NH groups, and the peptide bond between MeLeu[9]

and MeLeu[10] in a *cis*-conformation (Fig. 1). The crystal structure determination of the title compounds has revealed that they are isostructural with cyclosporin A monohydrate[1,2] (**3**) and [*S*-acetyl-MeBmt[1]]CsA (**6**) (ref.[15]). Consequently, the molecular structure, backbone conformation as well as the conformation of side chains in **1–4** and **6** are very similar (for the backbone conformational angles see Table II). As implies from the comparison of structures, the conformation of the cyclosporin backbone is highly conserved and is not affected even by the introduction of the bulky substituents. In **3** the solvent water is hydrogen bonded to the hydroxy group of

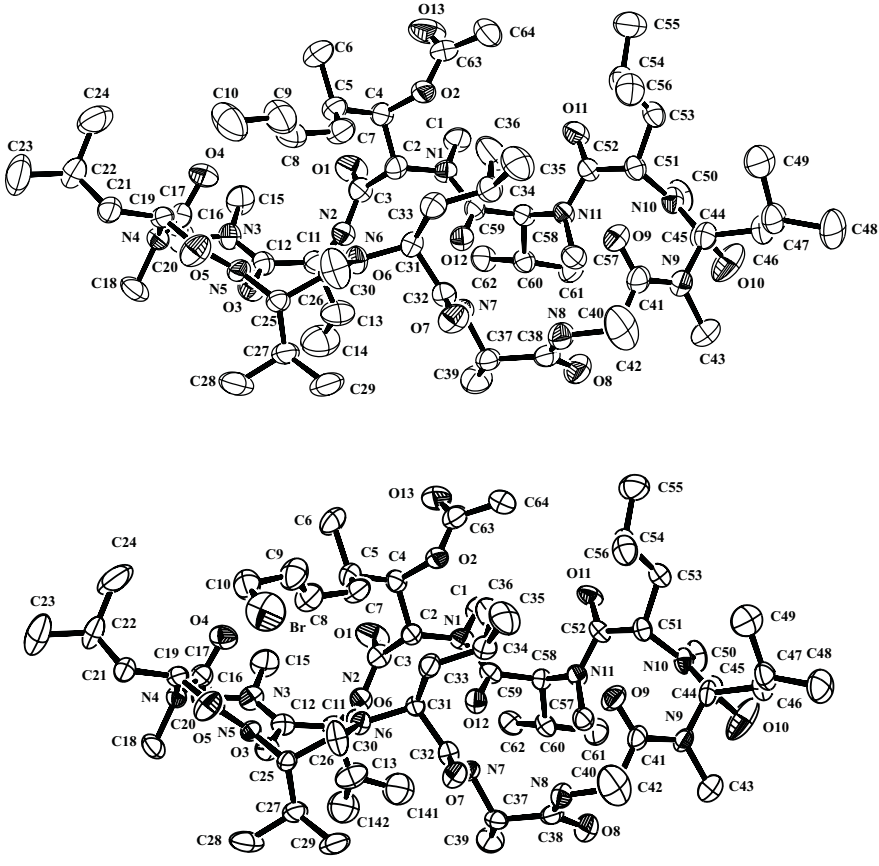

FIG. 2

Ortep drawings of [*O*-acetyl-MeBmt[1]]CsA (**1**, upper) and [*O*-acetyl-ω-bromo-MeBmt[1]]CsA (**2**, bottom)

TABLE I
Data collection and structure refinement parameters for **1** and **2**

Parameter	**1**	**2**
Crystal dimensions	0.7 × 0.6 × 0.6 mm	0.8 × 0.8 × 0.1 mm
Diffractometer and radiation used	Enraf–Nonius CAD4, CuKα, λ = 1.54187 Å	
Scan technique	ω/2θ	ω/2θ
Temperature	293 K	293 K
No. and θ range of reflections for lattice parameter refinement	15; 48.82–49.80°	16; 15–50°
Range of *h*, *k* and *l*	0→15, 0→18, –43→0	0→12, 0→15, –36→36
Standard reflections monitored in interval; intensity fluctuation	120 min; –1.19%	60 min; –8.26%
Total number of reflections measured; θ range	5 608; 2.44–67.0°	8 201; 0–60°
No. of observed independent reflections	5 513	6 756
Criterion for observed reflections	$I \geq 1.96\sigma(I)$	$I \geq 1.96\sigma(I)$
Function minimized	$\sum w (F_o^2 - F_c^2)^2$	$\sum w (F_o - F_c)^2$
Weighting scheme	$w = 1/[\sigma^2(F_o^2) + (0.0751P)^2 + 6.5518P$, where $P = (F_o^2 + F_c^2)/3$	Chebychev polynomial[21]
Parameters refined	795	802
Value of *R*, *wR* and *S*	0.076, 0.179, 1.13	0.094, 0.116, 1.14
Ratio of maximum least-squares shift to e.s.d. in the last cycle	0.001	0.001
Maximum and minimum heights in final Δρ map	0.42; –0.19 e Å^{-3}	1.34; –1.45 e Å^{-3}
Source of atomic scattering factors	ref.[17]	ref.[17]
Programs used	SDP ref.[18], SIR92 ref.[19], SHELX97 ref.[20]	SDP ref.[18], CRYSTALS ref.[21], PARST ref.[22], SIR92 ref.[19]

TABLE II
A comparison of cyclosporin solid state conformations of compounds **1**–**6**

	φ_1	ψ_1	ω_1	φ_2	ψ_2	ω_2	φ_3	ψ_3	ω_3	φ_4	ψ_4	ω_4	φ_5	ψ_5	ω_5	φ_6	ψ_6
[*O*-acetyl-MeBmt[1]]CsA **1**	–99	105	–172	–108	106	–176	63	–132	175	–105	33	179	–112	122	165	–88	107
[*O*-acetyl–ω-bromoMeBmt[1]]CsA **2**	–104	104	–173	–106	107	–175	62	–133	176	–101	25	–179	–106	123	168	–92	104
[*S*-acetyl-MeBmt[1]]CsA **6**[a] (ref.[15])	–103	102	–169	–107	105	–176	63	–133	176	–107	35	180	–114	120	167	–85	110
CsA·H_2O **3**[a] (ref.[2])	–100	103	–169	–108	105	–175	66	–136	173	–105	33	177	–109	120	167	–87	105
CsA·2 H_2O **4**[a] (ref.[3])	–84	122	–175	–120	90	–177	71	–128	173	–99	22	–180	–113	125	167	–90	100
CsA · dimethyl isosorbide **5**[a] (ref.[4])	–93	156	167	–118	99	–167	68	–133	172	–98	9	–177	–85	134	176	–79	128

	ω_6	φ_7	ψ_7	ω_7	φ_8	ψ_8	ω_8	φ_9	ψ_9	ω_9	φ_{10}	ψ_{10}	ω_{10}	φ_{11}	ψ_{11}	ω_{11}
[*O*-acetyl-MeBmt[1]]CsA **1**	–174	–87	53	180	84	–126	–173	–117	112	–12	–134	64	–174	–98	122	177
[*O*-acetyl–ω-bromoMeBmt[1]]CsA **2**	–172	–84	54	180	82	–126	–173	–117	109	–8	–138	65	–174	–97	124	180
[*S*-acetyl-MeBmt[1]]CsA **6**[a] (ref.[15])	–173	–88	49	–180	83	–128	–174	–114	111	–10	–136	68	–176	–102	125	–180
CsA·H_2O **3**[a] (ref.[2])	–173	–86	52	–180	82	–127	–169	–120	100	3	–146	66	–176	–98	122	178
CsA·2 H_2O **4**[a] (ref.[3])	–165	–83	52	178	88	–125	–167	–119	100	–6	–139	65	–167	–103	125	173
CsA·dimethyl isosorbide **5**[a] (ref.[4])	–174	–98	–5	180	150	–133	–173	–120	99	–1	–139	64	–178	–95	142	166

[a] Data for **3**, **4** and **6** are from Cambridge Structural Database (CsA·H_2O, KEPNAU; CsA·2 H_2O, DEKSAN; [*S*-acetyl-MeBmt[1]]CsA, ZAJDUJ).

MeBmt. In **1**, **2** and **6** the same position is occupied by an acetyl group giving further argument for isostructurality of all these compounds.

However, the side chain conformation of MeBmt residue is changed. An increase in $J(\alpha,\beta)$ from 6.1 Hz in CsA to 11.6 Hz in **1** indicates an antiperiplanar arrangement, $J(\beta,\gamma)$ decreases from 5.5 to 1.0 Hz. Only few most intense crosspeaks observed in ROESY spectra at room temperature (corresponding to geminal, vicinal sequential and some others – H-9α *vs* H-10α, 2-NH *vs* H-1β) are insufficient for a detailed analysis.

TABLE III
Hydrogen bonds for **1** and **2**

Bonds	**1**			**2**		
	H···A Å	D···A Å	D–H···A °	H···A Å	D···A Å	D–H···A °
Abu^2(NH)···Val^5(CO)	2.10	2.934(7)	163.2	1.99	2.939(9)	158.0
Val^3(NH)···$MeLeu^4$(N)	2.21	2.753(7)	120.6	2.37	2.755(9)	102.1
Val^3(NH)···Abu^2(CO)	2.22	2.979(7)	146.2	2.01	2.974(9)	160.6
Ala^7(NH)···$MeVal^{11}$(CO)	2.14	2.991(7)	168.5	2.04	3.024(8)	166.0
D-Ala^8(NH)···$MeLeu^6$(CO)	2.31	2.934(8)	133.0	1.99	2.893(8)	148.3

TABLE IV
Comparison of volumes for potential solvent[23] in cyclosporins **1**–**5** (solvent molecules excluded from the calculations)

Compound	Total solvent area in unit cell, Å^3	Total solvent area in unit cell/V	Maximal continuous solvent area, Å^3
[*O*-acetyl-$MeBMT^1$]CsA **1**	81.7	0.01	13
[*O*-acetyl-ω-bromo-$MeBmt^1$]CsA **2**	177.5	0.04	27
CsA·H_2O **3** (ref.[2])	144.5	0.02	27
CsA·H_2O **4** (ref.[3])	875.6	0.21	332
CsA·DMI **5** (ref.[4])	1 326.2	0.16	438

The only difference we have found is concerned to the hydrogen bonding in solid **1**. The molecule of cyclosporin with 11 carbonyl oxygen atoms and only one OH and four NH groups is a typical example of the H-deficient compounds. In such systems the tendency to the formation of three-center (bifurcated) hydrogen bonds might be expected[16]. So far, the bifurcated H-bonds of D-Ala[8] (NH) to D-Ala[8] (CO) and MeLeu[6] (CO) have been observed in the structure of CsA in the nonpolar solvents[3]. Compound **1** is the first example of solid state conformation of cyclosporin with clear three-center hydrogen bonds from Val[5] (NH) to MeLeu[4] (CO) and Abu[2] (CO) (hydrogen bonding parameters are presented in Table III).

A comparison of the space available for solvent molecules in different cyclosporin modifications is made in Table IV. From the maximal continuous space aviable for solvent it shoud be deduced that the $P2_12_12_1$ packing in **1**, **2** and **3** is not suitable for incorporating solvent molecules in the structure in contradistinction to packing of **4** and **5**.

This work was supported by the Grant Agency of the Czech Republic (grants No. 203/97/0623, No. 203/99/0067 and No. 203/97/P045).

REFERENCES

1. Knott R. B., Schefer J., Schoenborn B. P.: *Mater. Sci. Forum* **1988**, *27/28*, 151 .
2. Knott R. B., Schefer J., Schoenborn B. P.: *Acta Crystallogr., Sect. C: Cryst. Struct. Commun.* **1990**, *46*, 1528.
3. Loosli H. R., Kessler H., Oschkinat H., Weber H. P., Petcher T. J., Widmer A.: *Helv. Chim. Acta* **1985**, *68*, 682.
4. Hušák M., Kratochvíl B., Jegorov A., Maťha V., Stuchlík M., Andrýsek T.: *Z. Kristallogr.* **1996**, *211*, 313.
5. Kessler H., Köck M., Wein T., Gerke M.: *Helv. Chim. Acta* **1990**, *73*, 1818.
6. Seebach D., Ko S. Y., Kessler H., Köck M., Reggelin M., Schmieder P., Walkinshaw M. D., Bölsterli J. J., Bevec D.: *Helv. Chim. Acta* **1991**, *74*, 1953.
7. Kessler H., Loosli H.-R., Oschkinat H.: *Helv. Chim. Acta* **1985**, *68*, 661.
8. Pflügl G., Kallen J., Schrimer T., Jansonius J. N., Zurini M. G. M., Walkinshaw M. D.: *Nature* **1993**, *361*, 91.
9. Mikol V., Kallen J., Pflügl G., Walkinshaw M. D.: *J. Mol. Biol.* **1993**, *234*, 1119.
10. Köck M., Kessler H., Seebach D., Thaler A.: *J. Am. Chem. Soc.* **1992**, *114*, 2676.
11. Eberle M. K., Nuninger F.: *J. Org. Chem.* **1992**, *57*, 2689.
12. Černý B., Jegorov A., Polívková J., Sedmera P., Havlíček V.: *J. Labelled Compd. Radiopharm.* **1998**, *41*, 267.
13. Gavériaux C., Boesch D., Boelsterli J. J., Bollinger P., Eberle M. K., Hiestand P., Payne T., Traber R., Wenger R., Loor F.: *Br. J. Cancer* **1989**, *60*, 867.
14. North A. C. T., Phillips D. C., Mathews F. S.: *Acta Crystallogr., Sect. A: Cryst. Phys., Diffr., Theor. Gen. Crystallogr.* **1968**, *24*, 351.

15. Eberle M. E., Nuninger F., Weber H.-P.: *J. Org. Chem.* **1995**, *60*, 2610.
16. Taylor R., Kennard O., Versichel W.: *J. Am. Chem. Soc.* **1984**, *106*, 244.
17. *International Tables for X-Ray Crystallography*, Vol. IV. Kynoch Press, Birmingham 1974.
18. Frenz B. A. and Associated, Inc.: *SDP. Structure Determination Package*. Enraf–Nonius, Delf 1985.
19. Altomare A., Burla M. C., Camalli M., Cascarano G., Giaccovazzo C., Guagliardi A., Polidori G.: *J. Appl. Crystallogr.* **1994**, *27*, 435.
20. Sheldrick G. M.: *SHELX97. Program for Crystal Structure Determination*. University of Göttingen, Göttingen 1997.
21. Watkin D. J.: *CRYSTALS. Crystallographic Package*. University of Oxford, Oxford 1989.
22. Nardelli M.: *PARST. System of Computer Routines for Calculating Molecular Parameters from the Results of Crystal Structure*. University of Parma, Parma 1991.
23. Vandersluis P., Spek A. L.: *Acta Crystallogr., Sect. A: Fundam. Crystallogr.* **1990**, *46*, 194.