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Cyclosporins of Symmetry $P2_1$ – a Series of Clathrates

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Abstract. A series of isomorphous clathrates (cyclosporin A: solvent = 1 : 1) is reported. The structure of the tetrahydrofuran (**1**) and dibutylether (**2**) clathrates have been determined by X-ray single crystal diffraction. The cyclosporin molecules in both structures are associated via van der Waals interactions forming cavities occupied by solvent molecules. The structure refinement based on room-temperature data sets show significant disorder of the side chains of the cyclosporin A molecule with no adequate model of the solvent molecule in both structures. In contrast, the position of dibutylether was successfully localised using a low-temperature data set for **2**.

Key words: cyclosporin A, X-ray structure analysis

1. Introduction

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, Figure 1) is a fungal metabolite widely used as an immunosupressive drug in transplantation surgery (Consupren®, Galena). The molecule of cyclosporin is highly lipophilic; hence it is practically insoluble in water, but freely soluble in many organic solvents (except *n*-hexane). CsA has been crystallised in different space groups depending on the solvent used for crystallisation. Three different polymorphs have been described in the literature: cyclosporin A dihydrate, CsA·2H_2O (tetragonal, $P4_1$) [1], cyclosporin A monohydrate, CsA·H_2O (orthorhombic, $P2_12_12_1$) [2, 3] and cyclosporin A dimethylisosorbide, CsA·DMI [4] (monoclinic, $P2_1$). In this work we report a new series of isomorphous clathrates of cyclosporin A with various ether guest molecules (1 : 1).

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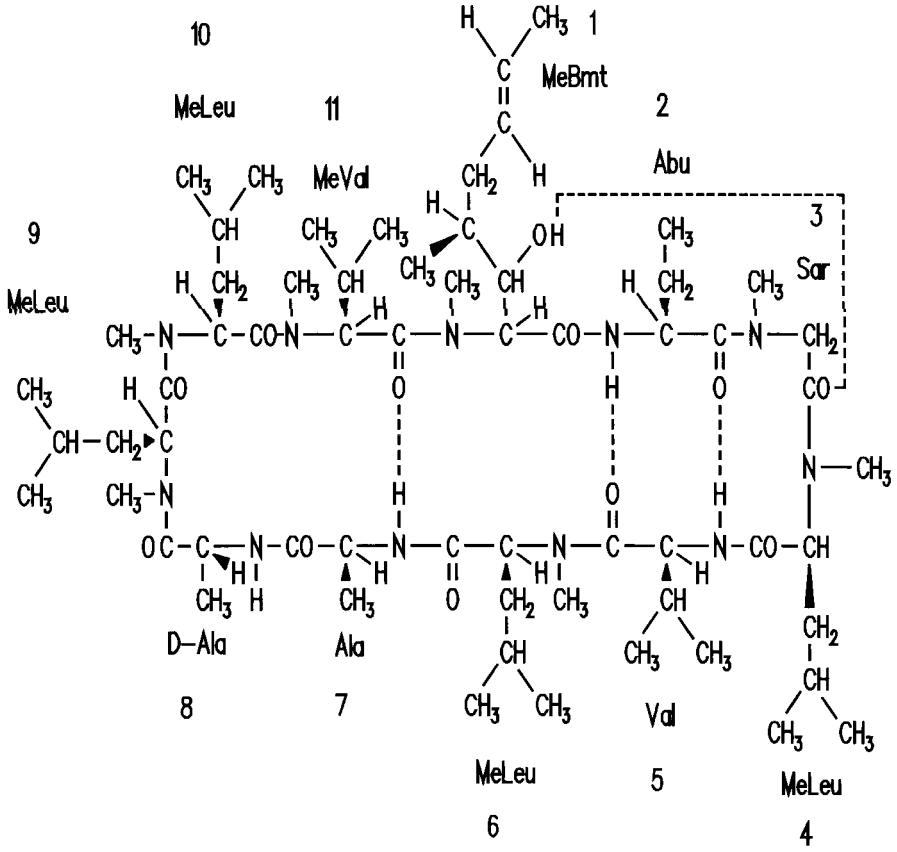

Figure 1. Schematic representation of the CsA hydrogen bond system in $P2_1$ crystal forms. Dashed lines indicate hydrogen bonds.

2. Experimental

2.1. CRYSTALLISATION

General procedure: Cyclosporin A (100 mg, dried dihydrate, Galena a.s, Czech Republic) was dissolved in an appropriate solvent (1 mL, tert.-butylmethyl ether = t-BME, di-*n*-butyl ether = DBE, terahydrofuran = THF, tetrahydropyran = THP) with short moderate heating (3 min, 50–80 °C), *n*-heptane (4 mL) was added in one portion with stirring, and the solution was allowed to evaporate at laboratory temperature. With CsA·THF (**1**), crystals were formed within two days. Since the majority of the clathrates are prone to desolvatation, crystals were mounted into glass capillaries with the residual amount of the mother liquor for the X-ray crystal structure determination (room-temperature data set of **1**). Since CsA·DBE (**2**) is stable in air, crystals formed overnight were filtered, dried in air and mounted without capillaries in order to obtain room-temperature and low-temperature data

sets of **2**. Two other clathrates CsA·THP and CsA·t-BME were characterised by cell parameters and NMR only.

2.2. DATA COLLECTION AND PROCESSING

X-ray data collections were performed with an Enraf-Nonius CAD4 diffractometer using CuK_α(room-temperature data sets for **1, 2**) and a KUMA KM4 diffractometer with MoK_α radiation (150 K, data set for **2**). In reducing the data, Lorenz and polarisation factors were applied, no correction for absorption was made. Crystallographic data, data collection and structure refinement parameters for **1** and both data sets of **2** are given in Table I.

2.3. STRUCTURE SOLUTION AND REFINEMENT

2.3.1. *Room-temperature data set of CsA·THF (**1**)*

The structure was solved by direct methods and anisotropically refined by full-matrix least-squares, except for the C(9) and C(10) atoms of the MeBmt residue, which were refined isotropically due to their large thermal parameters. The C(62) atom is disordered over two positions with site-occupancy factors of 0.4 and 0.6. The parameters of the C(62) atom for both positions were refined with restrained geometry. H-atoms were included in calculated positions and were not refined. The electron density map calculated at this step indicated the presence of a disordered solvent molecule in the structure, which was further restrained using geometry from the low-temperature structure determination of tetrahydrofuran [5] and isotropically refined with distance restraints. Final atomic coordinates and equivalent isotropic thermal parameters of the non-hydrogen atoms of **1** are given in Table II. The relevant bond distances and angles, hydrogen atoms parameters, anisotropic thermal parameters and structure factors are summarised in the Supplementary Material.

2.3.2. *Room-temperature data set of CsA·DBE (**2**)*

A solution of the structure was realised with SIR92 [6] in which coordinates were obtained for the non-hydrogen atoms of the cyclosporin molecule. All non-H atoms were refined anisotropically against F^2 by the full-matrix least-squares method. Hydrogen atoms were included in calculated positions and were not refined. The side chains of residues 1, 8 and 10 were found to be disordered showing unreasonable values for the bond lengths and angles. Subsequent difference Fourier maps showed a significant electron density area associated with the solvate molecule, however, attempts failed to constrain any adequate model for the dibutylether molecule. Final atomic coordinates and equivalent isotropic thermal parameters of the non-hydrogen atoms of **2** obtained from room temperature data are given in Table III. The relevant bond distances and angles, hydrogen atoms parameters,

Table I. Data collection and structure refinement parameters for the CsA-guest compounds

Parameters	Tetrahydrofuran	Dibutylether	Dibutylether[a]
Formula	$C_{62}H_{111}N_{11}O_{12}$ C_4H_8O	$C_{62}H_{111}N_{11}O_{12}$ $C_8H_{18}O$	$C_{62}H_{111}N_{11}O_{12}$ $C_8H_{18}O$
Crystal system	monoclinic	monoclinic	monoclinic
Space group	$P2_1$	$P2_1$	$P2_1$
Unit cell dimensions	a = 15.551(5) Å	a = 15.472(10) Å	a = 15.372(10) Å
	b = 21.216(2) Å	b = 21.115(5) Å	b = 20.910(4) Å
	c = 12.862(7) Å	c = 12.843(7) Å	c = 12.496(6) Å
	β = 98.23(1)°	β = 98.96(4)°	β = 99.44(3)°
Volume (Å^3)	4200(3)	4144(4)	3962(3)
M_r	1274.72	1332.84	1332.84
D_x (mg m^{-3})	1.008	1.068	1.117
Crystal size (mm)	0.72 × 0.86 × 0.94	0.4 × 0.4 × 0.3	0.5 × 0.5 × 0.4
Radiation used	CuK_α, λ = 1.54178 Å	CuK_α, λ = 1.54178 Å	MoK_α, λ = 0.71073 Å
μ (mm^{-1})	0.57	0.59	0.08
Scan technique	$\omega/2\theta$	$\omega/2\theta$	$\omega/2\theta$
θ-range (°)	3.55–64.99	2.89–57.99	1.34–25.03
Range of h, k, l	$-18 \to 18$, $-24 \to 24$, $0 \to 15$	$0 \to 17$, $-23 \to 23$, $-14 \to 13$	$-14 \to 14$, $-24 \to 0$, $0 \to 14$
No. of reflections	9267	10934	6665
Used for refinements	9267	10518	6484
Function minimised	$\sum \omega(F_0^2 - F_c^2)$	$\sum \omega(F_0^2 - F_c^2)$	$\sum \omega(F_0^2 - F_c^2)$
Weighting scheme	$\omega = 1/[\sigma^2 F_o^2 + 0.01P^2]$,	$\omega = 1/[\sigma^2 F_o^2 + 0.01P^2]$,	$\omega = 1/[\sigma^2 F_o^2 + (0.1054)P^2 + 2.474P]$,
	where $P = (F_o^2 + 2F_c^2)/3$	where $P = (F_o^2 + 2F_c^2)/3$	where $P = (F_o^2 + 2F_c^2)/3$
Parameters refined	779	767	874
$R_1(F > 4\sigma(F))$	0.085	0.091	0.056
wR_2 (all data)	0.240	0.235	0.188
S	2.109	1.869	1.071
Ratio of max. least-squares shift to e.s.d. in the last cycle	<0.001	<0.009	<0.005
Max. and min. heights in final $\Delta\rho$-map (e Å^{-3})	0.34, −0.23	0.85, −0.33	0.71, −0.35
Programs used	SIR92 [6], SHELXL-97 [11], PARST [12]	SIR92 [6], SHELXL-97 [11], PARST [12]	SHELXL-97 [11], PARST [12]

[a]150 K.

Table II. Atomic coordinates ($\times 10^4$) and equivalent isotropic displacement parameters ($Å^2 \times 10^3$) for non-H atoms of **1**

	x	*y*	*z*	U^*_{eq}
O(1)	8218(2)	1890(2)	6001(4)	93(1)
O(2)	7019(2)	1127(2)	4233(4)	87(1)
O(3)	5194(2)	2300(2)	6401(3)	74(1)
O(4)	5335(3)	1059(2)	4610(4)	84(1)
O(5)	3885(5)	2562(2)	2547(5)	116(2)
O(6)	6025(2)	3227(2)	4214(4)	82(1)
O(7)	6347(3)	4999(2)	3522(6)	115(2)
O(8)	8845(6)	5609(3)	5099(6)	145(3)
O(9)	9267(2)	4888(2)	1651(4)	89(1)
O(10)	1 2358(3)	4505(3)	2187(6)	123(2)
O(11)	1 0134(5)	2458(2)	1731(4)	117(2)
O(12)	8557(2)	3187(2)	3926(4)	86(1)
N(1)	8473(2)	2133(2)	3956(4)	72(1)
N(2)	6936(2)	2407(2)	5780(3)	64(1)
N(3)	5787(2)	1344(2)	6706(3)	69(1)
N(4)	3999(2)	1439(2)	4687(3)	65(1)
N(5)	4377(3)	2668(2)	4260(3)	70(1)
N(6)	5395(3)	3853(2)	2925(4)	77(1)
N(7)	7473(3)	4343(2)	3994(4)	73(1)
N(8)	8764(4)	5147(2)	3542(4)	86(1)
N(9)	1 0627(3)	5204(3)	2256(4)	89(1)
N(10)	1 1357(3)	3760(2)	1789(4)	79(1)
N(11)	1 0091(3)	3140(2)	3063(3)	69(1)
C(1)	8857(4)	1502(2)	3925(8)	108(3)
C(2)	7600(3)	2192(2)	4243(4)	66(1)
C(3)	7636(3)	2150(2)	5436(4)	69(1)
C(4)	6908(3)	1728(2)	3716(4)	72(1)
C(5)	6856(4)	1642(3)	2522(5)	90(2)
C(6)	6813(8)	2262(4)	1968(6)	122(3)
C(7)	6081(6)	1224(4)	2128(6)	114(3)
C(8)	6067(8)	1003(6)	1038(9)	143(4)
C(9)	5375(12)	1107(7)	283(14)	166(4)
C(10)	5505(18)	859(11)	−790(2)	233(8)
C(11)	6735(3)	2294(2)	6839(4)	68(1)
C(12)	5846(3)	1975(2)	6653(3)	63(1)
C(13)	6720(5)	2888(3)	7492(6)	96(2)
C(14)	6561(11)	2772(6)	8561(8)	161(5)

Table II. Continued

	x	y	z	U^*_{eq}
C(15)	6529(4)	921(3)	6884(6)	87(1)
C(16)	4925(3)	1083(2)	6305(4)	74(1)
C(17)	4754(3)	1188(2)	5118(4)	65(1)
C(18)	3279(3)	1576(3)	5281(5)	82(1)
C(19)	3908(3)	1617(2)	3575(4)	69(1)
C(20)	4043(3)	2338(2)	3418(4)	73(1)
C(21)	3049(5)	1424(4)	2898(5)	94(2)
C(22)	2899(8)	714(5)	2878(6)	134(4)
C(23)	3629(12)	340(5)	2505(11)	172(5)
C(24)	2031(11)	565(8)	2216(9)	202(8)
C(25)	4497(3)	3349(2)	4151(4)	71(1)
C(26)	5366(3)	3470(2)	3753(4)	67(1)
C(27)	4477(4)	3684(3)	5201(6)	93(2)
C(28)	4625(7)	4379(3)	510010)	129(3)
C(29)	3606(7)	3564(4)	5583(11)	152(5)
C(30)	4647(4)	4178(4)	2385(7)	106(2)
C(31)	6281(3)	3999(2)	2674(4)	70(1)
C(32)	6707(3)	4493(2)	3440(5)	74(1)
C(33)	6275(5)	4217(3)	1559(6)	95(2)
C(34)	7167(4)	4359(3)	1247(5)	88(1)
C(35)	7058(7)	4636(5)	133(7)	134(3)
C(36)	7745(5)	3774(3)	1297(6)	100(2)
C(37)	7907(4)	4736(3)	4820(5)	86(2)
C(38)	8543(4)	5205(3)	4480(6)	91(2)
C(39)	8312(7)	4342(5)	5722(6)	119(3)
C(40)	9410(6)	5544(3)	3159(8)	126(4)
C(41)	9761(4)	5178(2)	2273(6)	85(2)
C(42)	8988(12)	6148(4)	2658(15)	223(10)
C(43)	1 1225(7)	5540(5)	3040(8)	148(4)
C(44)	1 0976(3)	4886(2)	1397(4)	76(1)
C(45)	1 1612(4)	4364(3)	1831(5)	83(1)
C(46)	1 1450(4)	5352(3)	758(5)	85(1)
C(47)	1 0924(6)	5928(3)	336(7)	102(2)
C(48)	1 0127(8)	5772(6)	−401(10)	140(3)
C(49)	1 1513(8)	6375(4)	−175(11)	158(5)
C(50)	1 2020(4)	3285(4)	2098(8)	107(2)
C(51)	1 0484(3)	3512(2)	1367(4)	74(1)
C(52)	1 0213(3)	2997(2)	2062(4)	75(1)
C(53)	1 0476(5)	3276(3)	232(5)	93(2)

Table II. Continued

	x	y	z	U^*_{eq}
C(54)	9588(6)	3216(4)	−431(6)	107(2)
C(55)	9217(7)	3855(4)	−847(6)	117(2)
C(56)	9623(10)	2733(6)	−1333(8)	158(4)
C(57)	1 0147(4)	3787(3)	3446(5)	86(2)
C(58)	9871(3)	2637(3)	3753(5)	80(1)
C(59)	8893(3)	2671(2)	3866(4)	68(1)
C(60)	1 0411(5)	2598(5)	4747(6)	151(5)
C(61)	1 1384(5)	2596(8)	4740(10)	173(6)
C(62)	1 0195(13)	2865(9)	5783(11)	98(7)
C(62')	1 0242(14)	2279(12)	5765(12)	153(9)
O(100)	8910(3)	9799(11)	3970(3)	430(19)
C(101)	9230(2)	9779(12)	3020(3)	282(12)
C(102)	8600(3)	9532(16)	2280(3)	339(17)
C(103)	7790(2)	9572(18)	2660(3)	390(2)
C(104)	8060(3)	9598(12)	3800(3)	334(16)

*$U = 1/3 \sum_i \sum_j U_{ij} a_i^* a_j^* \mathbf{a}_i \mathbf{a}_j$.

anisotropic thermal parameters and structure factors are summarised in the Supplementary Material.

2.3.3. *Low-temperature data set of CsA·DBE (**2**)*

The coordinates of the non-H atoms of cyclosporin A from the room-temperature data set of **2** were used for the refinement. The structure was anisotropically refined by full-matrix least-squares. The dibutylether molecule was successfully localised from the $\Delta\rho$-map and anisotropically refined. H-atoms were included in calculated positions and were not refined. Final atomic coordinates and equivalent isotropic thermal parameters of the non-hydrogen atoms are given in Table IV. The relevant bond distances and angles, hydrogen atom parameters, anisotropic thermal parameters and structure factors are in the Supplementary Material.

3. Results and Discussion

When we reported recently the structure of CsA crystallised with one molecule of dimethylisosorbide [4], we assumed that such a structure was unique because of the rather unusual solvent used for crystallisation. However, additional experiments with various solvents revealed that this type of host-guest complex is not unique, but common for at least some members of the cyclosporin family like CsE [7, 8]. Similar to dimethylisosorbide, CsA crystallises with a number of eth-

Table III. Atomic coordinates ($\times 10^4$) and equivalent isotropic displacement parameters ($Å^2 \times 10^3$) for non-H atoms for the room-temperature data set of **2**

	x	y	z	U^*_{eq}
O(1)	8235(2)	1884(2)	6060(3)	95(1)
O(2)	7035(2)	1111(2)	4283(4)	94(1)
O(3)	5202(2)	2310(2)	6456(3)	78(1)
O(4)	5336(2)	1071(2)	4669(3)	82(1)
O(5)	3887(4)	2574(2)	2580(4)	120(2)
O(6)	6029(2)	3230(2)	4247(3)	85(1)
O(7)	6362(3)	5023(2)	3502(5)	112(2)
O(8)	8850(4)	5664(3)	4999(5)	14(2)
O(9)	9220(2)	4878(2)	1536(4)	92(1)
O(10)	12338(3)	4487(3)	2226(4)	19(2)
O(11)	10090(4)	2444(2)	1818(4)	118(2)
O(12)	8552(2)	3192(2)	4007(3)	84(1)
N(1)	8475(2)	2134(2)	3999(3)	71(1)
N(2)	6948(2)	2403(2)	5829(3)	66(1)
N(3)	5800(2)	1355(2)	6767(3)	74(1)
N(4)	3989(2)	1448(2)	4723(3)	68(1)
N(5)	4360(3)	2692(2)	4319(4)	75(1)
N(6)	5388(2)	3867(3)	2963(4)	78(1)
N(7)	7479(3)	4359(2)	3991(3)	72(1)
N(8)	8725(3)	5174(2)	3447(4)	84(1)
N(9)	10578(3)	5197(2)	2203(3)	80(1)
N(10)	11329(3)	3744(2)	1820(4)	82(1)
N(11)	10078(2)	3146(2)	3109(3)	69(1)
C(1)	8888(4)	1501(2)	3959(6)	102(2)
C(2)	7595(3)	2178(2)	4269(4)	65(1)
C(3)	7636(3)	2143(2)	5475(4)	70(1)
C(4)	6918(3)	1702(2)	3759(4)	75(1)
C(5)	6852(4)	1627(3)	2569(5)	88(2)
C(6)	6814(6)	2244(4)	2008(5)	121(2)
C(7)	6047(6)	1212(4)	2137(6)	124(3)
C(8)	6001(8)	1040(6)	1071(8)	159(4)
C(9)	5422(19)	1040(14)	434(12)	390(20)
C(10)	5399(18)	801(13)	−806(11)	390(20)
C(11)	6758(3)	2301(2)	6880(4)	71(1)
C(12)	5859(3)	1987(2)	6714(4)	64(1)
C(13)	6726(5)	2922(3)	7506(6)	108(2)
C(14)	6567(9)	2798(7)	8623(8)	190(5)

Table III. Continued

	x	y	z	U^*_{eq}
C(15)	6543(4)	915(3)	6949(6)	94(2)
C(16)	4939(3)	1086(2)	6360(4)	78(1)
C(17)	4764(3)	1197(2)	5188(4)	65(1)
C(18)	3281(3)	1596(3)	5323(5)	92(1)
C(19)	3896(3)	1639(2)	3622(4)	74(1)
C(20)	4044(3)	2339(3)	3475(5)	80(1)
C(21)	3041(4)	1435(3)	2949(6)	106(2)
C(22)	2927(8)	700(6)	2873(7)	158(5)
C(23)	3644(10)	364(5)	2490(11)	177(5)
C(24)	2016(10)	626(9)	2150(10)	269(10)
C(25)	4501(3)	3367(2)	4207(5)	82(2)
C(26)	5359(3)	3476(2)	3788(4)	70(1)
C(27)	4508(4)	3703(3)	5273(6)	97(2)
C(28)	4728(7)	4398(3)	5203(9)	150(3)
C(29)	3644(6)	3598(4)	5664(10)	157(4)
C(30)	4605(4)	4175(4)	2381(7)	119(3)
C(31)	6267(3)	4000(2)	2675(4)	72(1)
C(32)	6701(3)	4507(2)	3420(5)	76(1)
C(33)	6233(4)	4196(3)	1546(5)	97(2)
C(34)	7125(4)	4329(3)	1228(5)	95(2)
C(35)	7009(6)	4601(5)	111(6)	140(3)
C(36)	7706(4)	3769(4)	1297(5)	103(2)
C(37)	7928(4)	4775(3)	4805(4)	81(1)
C(38)	8554(4)	5230(3)	4417(5)	86(2)
C(39)	8385(5)	4393(4)	5733(5)	119(2)
C(40)	9313(5)	5576(3)	2981(6)	107(2)
C(41)	9706(4)	5188(2)	2178(5)	78(2)
C(42)	8763(9)	6116(4)	2321(12)	207(7)
C(43)	11184(5)	5538(5)	3018(7)	138(3)
C(44)	10957(3)	4869(3)	1369(4)	75(1)
C(45)	11585(3)	4349(3)	1838(5)	83(1)
C(46)	11431(4)	5330(3)	736(5)	89(2)
C(47)	10921(4)	5896(3)	262(6)	101(2)
C(48)	10164(7)	5707(6)	−520(10)	165(4)
C(49)	11540(7)	6329(5)	−253(10)	169(4)
C(50)	12006(4)	3259(4)	2174(6)	113(2)
C(51)	10455(3)	3497(2)	1381(4)	73(1)
C(52)	10185(3)	2983(2)	2132(4)	75(1)
C(53)	10446(5)	3235(3)	270(5)	101(2)

Table III. Continued

	x	*y*	*z*	U^*_{eq}
C(54)	9595(5)	3171(4)	−412(6)	115(2)
C(55)	9237(6)	3823(4)	−858(6)	127(3)
C(56)	9637(9)	2698(6)	−1308(8)	176(4)
C(57)	10104(4)	3820(3)	3458(5)	87(2)
C(58)	9880(3)	2659(3)	3847(5)	82(2)
C(59)	8913(3)	2678(2)	3954(4)	69(1)
C(60)	10448(4)	2741(5)	4953(6)	136(3)
C(61)	11426(4)	2743(8)	4845(8)	198(6)
C(62)	10275(7)	2491(14)	5773(8)	318(2)

$^*U = 1/3 \sum_i \sum_j U_{ij} a_i^* a_j^* \mathbf{a}_i \mathbf{a}_j$.

ers including, e.g., tert.-butylmethyl ether, di-*n*-butyl ether, terahydrofuran, and/or tetrahydropyran. Thus, similar to the cyclodextrins, cyclosporins crystallising in symmetry $P2_1$ represent a series of clathrates. However, in contrast to cyclodextrins, the guest molecule is not located inside the ring, but in the cavity created by several cyclosporin molecules.

The conformation of CsA in the crystal structures of the monohydrate, dihydrate and all clathrates described in this work is related. A large portion of the backbone (residues 1–5) adopts an antiparallel twisted β-pleated sheet conformation, the remaining residues 7–11 form an open loop with the only *cis*-amide linkage in the molecule between residues 9 and 10. As implied from Table V, the conformation of the CsA backbone is identical within experimental error in all clathrates. The ORTEP view of **2** with the atom numbering scheme is given as a typical representative of the clathrate series (Figure 2). The hydrogen bond network and orientation of the MeBmt moiety in the clathrates slightly differs from that in CsA dihydrate or monohydrate (Figure 1) [4].

The shape and the volume of the cavity which is occupied by a guest molecule in the clathrates was calculated for several $P2_1$ forms of cyclosporins by the programme PLATON [9]. The results of this calculation are summarised in Table VI. The position of the solvent in the crystal packing for the low temperature data of **2** is shown in Figure 3. In contrast, the correct location of the solvent from the room temperature data is almost impossible. In the case of cyclosporin clathrates, the vectors of the free space and the free space volume indicate that the shape of the cavity is identical in all structures. The computed maximal continuous solvent suitable area was found in the range from 391 to 562 Å^3. Under the assumption that the average packing coefficient for non-hydrogen atoms is 19.4 in such type of organic compounds [10], it can be calculated that about 12–18 non-hydrogen solvent atoms (in addition to 85 ones in CsA) could be incorporated into the asymmetric unit of the Cs $P2_1$ form to achieve this value. In the particular case of the CsA dibutylether

Table IV. Atomic coordinates ($\times 10^4$) and equivalent isotropic displacement parameters (Å^2 $\times$ 10^3) for non-H atoms for the low-temperature data set of **2**

	x	y	z	U^*_{eq}
O(1)	8185(3)	1885(2)	6032(3)	36(1)
O(2)	6984(3)	1079(2)	4204(4)	36(1)
O(3)	5123(3)	2341(2)	6390(3)	30(1)
O(4)	5269(3)	1069(2)	4548(3)	35(1)
O(5)	3934(3)	2546(2)	2374(3)	37(1)
O(6)	5965(3)	3229(2)	4137(3)	36(1)
O(7)	6365(3)	5039(2)	3385(4)	40(1)
O(8)	8874(4)	5652(3)	5036(5)	56(2)
O(9)	9277(3)	4899(2)	1422(4)	44(1)
O(10)	1 2428(3)	4416(3)	2295(4)	47(1)
O(11)	1 0020(4)	2418(2)	1728(4)	50(1)
O(12)	8537(3)	3184(2)	4002(4)	42(1)
N(1)	8432(3)	2113(2)	3909(4)	27(1)
N(2)	6902(3)	2444(2)	5768(4)	25(1)
N(3)	5762(3)	1376(2)	6710(4)	26(1)
N(4)	3909(3)	1451(2)	4633(4)	27(1)
N(5)	4276(3)	2708(2)	4176(4)	29(1)
N(6)	5355(3)	3877(2)	2798(4)	28(1)
N(7)	7468(3)	4338(2)	3941(4)	28(1)
N(8)	8757(4)	5143(3)	3425(5)	38(1)
N(9)	1 0654(3)	5165(3)	2256(4)	31(1)
N(10)	1 1359(3)	3690(3)	1775(4)	30(1)
N(11)	1 0071(3)	3136(3)	3076(4)	31(1)
C(1)	8819(5)	1477(3)	3839(6)	41(2)
C(2)	7539(4)	2179(3)	4195(5)	28(1)
C(3)	7587(4)	2153(3)	5424(5)	29(1)
C(4)	6853(4)	1688(3)	3670(5)	27(1)
C(5)	6762(4)	1603(3)	2438(5)	35(2)
C(6)	6674(6)	2251(4)	1864(6)	49(2)
C(7)	5962(5)	1179(4)	2070(5)	41(2)
C(8)	5792(5)	1046(4)	867(6)	46(2)
C(9)	5069(5)	1187(4)	219(5)	51(2)
C(10)	4895(7)	1041(5)	−987(6)	72(3)
C(11)	6693(4)	2356(3)	6852(5)	25(1)
C(12)	5798(4)	2008(3)	6670(4)	23(1)
C(13)	6644(4)	2986(3)	7459(5)	38(2)
C(14)	6451(6)	2865(4)	8594(6)	57(2)

Table IV. Continued

	x	y	z	U^*_{eq}
C(15)	6515(4)	947(3)	6925(6)	35(2)
C(16)	4892(4)	1087(3)	6301(5)	29(1)
C(17)	4707(4)	1195(3)	5097(5)	23(1)
C(18)	3222(4)	1593(3)	5262(5)	35(2)
C(19)	3821(4)	1630(3)	3483(5)	28(1)
C(20)	4019(4)	2328(3)	3296(5)	28(1)
C(21)	2911(4)	1470(3)	2823(5)	33(2)
C(22)	2631(5)	775(4)	2869(5)	45(2)
C(23)	3329(7)	314(4)	2663(7)	64(3)
C(24)	1762(7)	684(5)	2069(7)	78(3)
C(25)	4409(4)	3384(3)	4027(5)	29(1)
C(26)	5294(4)	3494(3)	3646(5)	28(1)
C(27)	4426(4)	3740(3)	5129(6)	35(2)
C(28)	4633(6)	4439(4)	5043(7	52(2)
C(29)	3544(5)	3649(4)	5530(8)	56(2)
C(30)	4595(4)	4217(4)	2179(6)	41(2)
C(31)	6238(4)	4001(3)	2550(5)	26(1)
C(32)	6683(4)	4503(3)	3321(5)	28(1)
C(33)	6246(4)	4211(3)	1377(5)	34(2)
C(34)	7160(4)	4356(3)	1130(5)	35(2)
C(35)	7081(5)	4660(4)	−5(6)	49(2)
C(36)	7764(5)	3774(3)	1237(5)	37(2)
C(37)	7898(4)	4755(3)	4787(5)	30(1)
C(38)	8555(4)	5224(3)	4415(5)	34(2)
C(39)	8365(5)	4363(4)	5739(5)	44(2)
C(40)	9320(5)	5571(4)	2942(7)	47(2)
C(41)	9752(4)	5184(3)	2142(6)	35(2)
C(42)	8754(7)	6098(5)	2255(9)	79(3)
C(43)	11236(5)	5461(4)	3177(6)	48(2)
C(44)	11054(4)	4857(3)	1403(5)	28(1)
C(45)	11654(4)	4295(3)	1859(5)	32(2)
C(46)	11587(4)	5337(3)	842(5)	33(2)
C(47)	11090(5)	5933(3)	401(6)	40(2)
C(48)	10327(5)	5791(4)	−499(8)	65(3)
C(49)	11717(5)	6406(4)	−26(7)	56(2)
C(50)	12014(4)	3185(4)	2117(6)	40(2)
C(51)	10460(4)	3485(3)	1319(5)	29(1)
C(52)	10154(4)	2959(3)	2057(5)	32(1)
C(53)	10439(5)	3234(4)	157(5)	37(2)

Table IV. Continued

	x	*y*	*z*	U^*_{eq}
C(54)	9532(5)	3193(4)	−538(6)	46(2)
C(55)	9214(5)	3858(4)	−959(6)	54(2)
C(56)	9554(7)	2725(5)	−1456(8)	76(3)
C(57)	10119(5)	3796(4)	3436(6)	42(2)
C(58)	9873(4)	2640(4)	3827(6)	38(2)
C(59)	8879(4)	2657(3)	3902(5)	32(2)
C(60)	10429(5)	2722(6)	4979(7)	65(3)
C(61)	11409(5)	2802(7)	4880(9)	99(5)
C(62)	10301(6)	2236(6)	5782(8)	77(3)
O(13)	6811(6)	8776(4)	1674(6)	96(2)
C(63)	7322(9)	7312(10)	457(10)	140(7)
C(64)	6944(8)	7374(7)	1470(10)	96(4)
C(65)	6099(8)	7756(7)	1300(11)	99(4)
C(66)	6190(8)	8424(7)	884(11)	99(4)
C(67)	7031(9)	9383(6)	1371(12)	102(4)
C(68)	7648(10)	9698(6)	2186(10)	98(4)
C(69)	8412(9)	9349(6)	2628(17)	142(7)
C(70)	9076(10)	9674(7)	3567(12)	120(5)

*$U = 1/3 \sum_i \sum_j U_{ij} a_i^* a_j^* \mathbf{a}_i \mathbf{a}_j$.

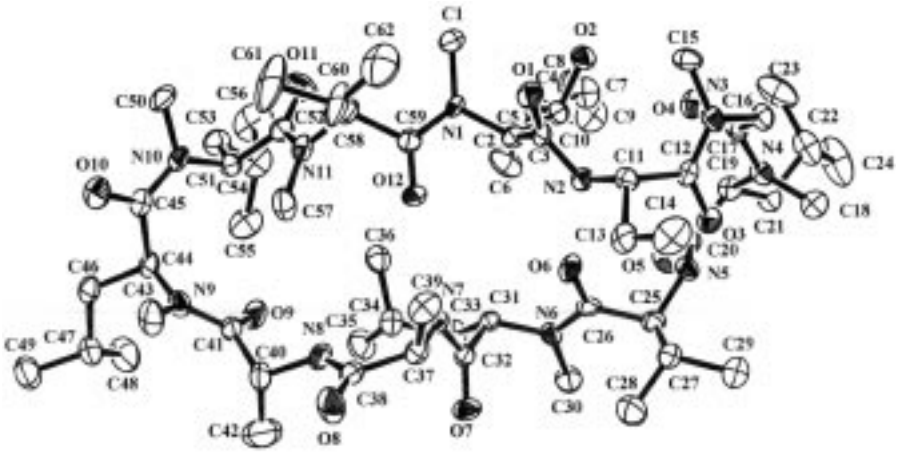

Figure 2. ORTEP drawing of the cyclosporin A dibutylether clathrate with the atom numbering scheme. Thermal ellipsoids are drawn at the 50% probability level. Hydrogen and solvent atoms are omitted for clarity.

Table V. A comparison of cyclosporin A solid state conformations in isomorphous clathrates [°]

Clathrate	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
CsA·THF (**1**)	−93	159	166	−119	98	−168	68	−131	171	−100	13	−179	−85	130	173	−78	124
CsA·DBE (**2**)[a]	−87	156	165	−114	93	−168	68	−130	171	−95	2	−176	−78	132	175	−79	122
CsA·DBE (**2**)[b]	−89	157	166	−119	97	−166	67	−131	171	−97	9	−179	−80	131	174	−81	123
CsA·DMI (**3**) [4]	−93	156	167	−118	99	−167	68	−133	172	−98	9	−177	−85	134	176	−79	128

Clathrate	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}
CsA·THF (**1**)	−174	−92	−12	−176	157	−138	−177	−118	101	−1	−139	64	−177	−104	145	170
CsA·DBE (**2**)[a]	−173	−91	−9	176	152	−123	−172	−121	98	−4	−137	64	−174	−101	138	167
CsA·DBE (**2**)[b]	−174	−92	−8	180	150	−131	−174	−120	99	0	−139	63	−176	−103	139	171
CsA·DMI (**3**) [4]	−174	−98	−5	180	150	−133	−173	−120	99	−1	−139	64	−178	−95	142	166

[a]Low temperature measurement.
[b]Ambient temperature measurement.

Table VI. Comparison of different $P2_1$ cyclosporin crystal forms

Type of cyclosporin	Solvent [reference]	Temperature of measurement	*a* [Å]	*b* [Å]	*c* [Å]	*β* [°]	Total solvent volume in the unit cell [Å^3 (%)]	Max. continuous volume area in the unit cell, $Z = 2$ [Å^3]
CsA (**3**)	dimethyl-isosorbide [4]	ambient temperature	15.521(2)	20.833(3)	12.949(3)	100.21(1)	875 (21.23)	438
CsA (**1**)	tetrahydrofurane [*]	ambient temperature	15.551(2)	21.216(7)	12.862(2)	98.23(1)	974 (23.19)	487
CsA (**2**)	di-*n*-butylether [*]	ambient temperature	15.47(1)	21.115(5)	12.843(7)	98.96(4)	926 (22.35)	463
CsA (**2**)	di-*n*-butylether [*]	150 K	15.37(1)	20.910(4)	12.496(6)	99.44(4)	781 (19.71)	391
CsE	acetone, water [7]	ambient temperature	15.698(2)	21.333(3)	13.224(2)	103.74(1)	1142 (26.55)	562
CsE	2-butanol [8]	150 K	15.575(6)	20.584(9)	13.280(5)	105.97(3)	949 (23.19)	453

[*] This work.

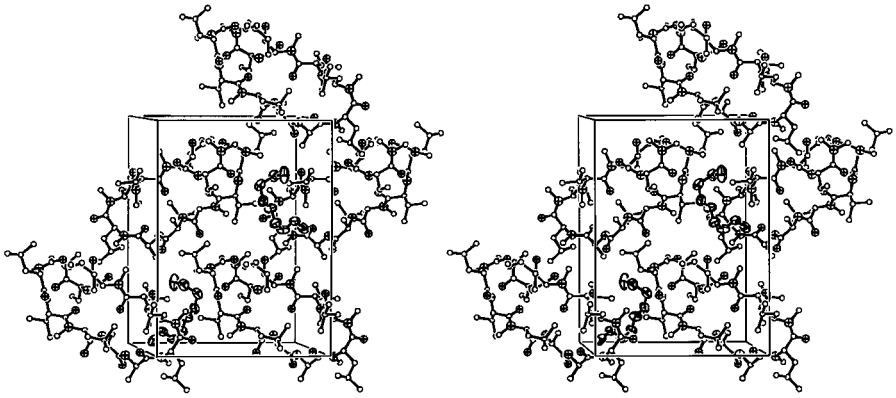

Figure 3. Stereo picture of the CsA dibutylether clathrate packing (low temperature data set) down the c axis (b is horizontal). Thermal ellipsoids are used for oxygen, nitrogen and solvent atoms in order to provide better resolution.

clathrate (low temperature data set of **2**), the evaluation of the volume available in the cavity makes it possible to estimate that the largest molecule which might be incorporated could be, e.g., hexyl-2-pentylether or butyl-2-heptylether. From non-polar solvents having larger molecules, e.g., from oleylalcohol, cyclosporin A crystallises as the orthorhombic monohydrate. Since the oxygen atom of the solvent is not involved in any H-bond (see also Figure 3), the type of molecule which can be incorporated is not restricted solely to ethers [9].

4. Conclusions

The inclusion behaviour of CsA was examined with various organic solvents. It has been found that various ethers provide clathrates with the molecular ratio CsA: guest = 1 : 1. The solvents have negligible influence on the conformation of the cyclosporin A molecule in the single crystal.

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References

1. H. R. Loosli, H. Kessler, H. Oschkinat, H. P. Weber, T. J. Petcher, and A. Widmer: *Helv. Chim. Acta* **68**, 682 (1985).
2. D. Giron, M. List, F. Richter, Y. Vike, and H. P. Weber: Ger. Offen. DE 3843054 (1989).
3. R. B. Knott, L. Schefer, and B. P. Schoenborn: *Acta Crystallogr*. **C46**, 1528 (1990).

4. M. Hušák, B. Kratochvíl, A. Jegorov, V. Mat'ha, M. Stuchlík, and T. Andrýsek: *Z. Kristallogr.* **211**, 313 (1996).
5. P. Luger and J. Buschmann: *Angew. Chem.* **22**, 410 (1983).
6. A. Altomare, M. C. Burla, M. Camalli, G. Cascarano, C. Giaccovazzo, A. Guagliardi, and G. Polidori: *J. Appl. Crystallogr*. **27,** 435 (1994).
7. M. Hušák, B. Kratochvíl, M. Buchta, L. Cvak, and A. Jegorov: *Coll. Czech Chem. Commun.* **63**, 115 (1998).
8. Crystal structure of the cyclosporin E bis(2-butanol) clathrate measured at 150 K. To be published.
9. A. L. Speak: *Acta Crystallogr.* **A46**, C34 (1990).
10. I. Alkorta, I. Rozas, J. Elguero, C. Foces-Foces, and F. H. Cano: *J. Mol. Struct*. **382**, 205 (1996).
11. G. M. Sheldrick: *SHELX-97. Program for Crystal Structure Determination.* University of Göttingen, Göttingen (1997).
12. M. Nardelli: *PARST. System of Computer Routines for Calculating Molecular Parameters from the Results of Crystal Structure.* University of Parma, Parma (1991).