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Neutron Structure of the Immunosuppressant Cyclosporin A

By R. B. Knott

Applications of Nuclear Physics Program, Australian Nuclear Science and Technology Organisation, Private Mail Bag, Menai, NSW 2234, *Australia*

and J. Schefer and B. P. Schoenborn

Centre for Structural Biology, Department of Biology, Brookhaven National Laboratory, Upton, NY 11973, *USA*

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Abstract. Cyclosporin A, $C_{62}H_{111}N_{11}O_{12}.H_2O$, M_r = 1220·6, orthorhombic, $P2_12_12_1$, a = 12·674 (1), b = 15·684 (2), c = 36·304 (30) Å, V = 7216·5 Å^3, Z = 4, D_x = 1·107 g cm^{-3}, λ(neutron) = 1·184 Å, $F(000)$ = 67·39, room temperature, final R = 0·074 for 4121 observed reflections. There is one cyclosporin A molecule and one water molecule per asymmetric unit.

Introduction. Cyclosporin A is a neutral, cyclic undecapeptide of fungal origin. Seven of the eleven amino acids are *N*-methylated (Fig. 1). Cyclosporin A is an immunosuppressant drug with wide clinical application primarily for solid organ and bone marrow transplantation. The ability of this drug to inhibit the activation of subpopulations of immunocompetent cells is a fundamental innovation in immunology. In order to investigate details of the molecular interactions involved in the pharmacological function of cyclosporin A, a detailed knowledge of the structure is required. The structure of cyclosporin A and eighteen derivatives have been determined by X-ray diffraction (Petcher, Weber & Ruegger, 1976; Loosli, Kessler, Oschkinat, Weber, Petcher & Widmer, 1985; Weber, 1986; Walkinshaw & Boelsterli, 1988), and various features investigated by two-dimensional NMR techniques (Loosli *et al.*, 1985). The molecular backbone of cyclosporin A forms a rigid structure with four hydrogen bonds holding the backbone in its folded configuration. Three of the four hydrogen bonds are involved in the formation of a short segment of β-sheet. The high-resolution X-ray studies indicate flexibility in a number of the side chains. Studies investigating the relation between the chemical structure and the pharmacological function have concentrated attention on the region around amino-acid residues MeBmt-1 and Abu-2 (Wenger, 1981; Loosli *et al.*, 1985; Wenger, 1985; Rich, Dhaon, Dunlap & Miller, 1986). However, the structural differences that lead to the dramatic changes in observed pharmacological function have yet to be defined. Because of the fundamental importance of this drug, a neutron diffraction diffraction study was undertaken for two main reasons. The first reason was to locate all hydrogen atoms particularly those involved in the four intramolecular hydrogen bonds. The second reason was to locate solvent molecules alluded to in the X-ray studies.

Experimental. Cyclosporin A is an extremely hydrophobic molecule (water solubility $< 0{\cdot}04$ mg ml^{-1}). It has been crystallized in different space groups ($P2_1$, $P4_1$, $P2_12_12_1$) depending primarily on the organic solvent(s) used (Petcher *et al.*, 1976; Loosli *et al.*, 1985; Weber, 1986). From the X-ray studies, only the $P2_12_12_1$ crystal form diffracted to 2·0 Å, and was therefore chosen for this study. A large crystal (approximate dimensions 2 × 2 × 5 mm) was crystallized from a mixture of oil, ethanol and a non-ionic surfactant (unpublished data), and supplied for this study by Dr Hans-Peter Weber (Sandoz, Switzerland). It is designated cyclosporin A mod III to distinguish it from the other crystal forms.

The neutron diffraction data were collected on the H3A Protein Crystallography Diffractometer located at the High Flux Beam Reactor, Brookhaven

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National Laboratory, USA (Schoenborn, 1984). A copper monochromator produced a neutron beam of wavelength 1·184 Å, and an external collimator defined the beam divergence at 0·08°. The crystal was treated as *water-free* and encapsulated in a quartz tube in a solvent-free environment. Using normal beam geometry, the bulk of the data was collected with the χ axis zero and rotating the crystal around the φ axis parallel to b^*. Reflections in the *blind region* were collected by moving the χ axis to 90° and rotating around the ω axis. The profile of the reflection on the two-dimensional position sensitive detector is dependent on experimental conditions (Schoenborn, 1983). The effects of beam divergence and wavelength spread, crystal size and mosaic, and detector resolution are convolved to produce the observed reflection profile. A profile evaluation computer program integrated the total intensity in a three-dimensional space and corrected for background. A total of 12 054 reflections were collected which yielded 4121 independent reflections. Data collection took thirty-two days of beam time. Neutrons cause negligible damage to protein crystals and the diffraction intensity was not monitored explicitly for decay. The diffractometer geometry limited the angular range of data collection to $\sin\theta/\lambda \leq 0{\cdot}546$ Å^{-1}, $0 \leq h \leq 12$, $0 \leq k \leq 16$, $0 \leq l \leq 38$. An absorption correction was applied to the observed data using the intensity of two strong reflections measured as a function of angle φ, and the semi-empirical algorithm of North, Phillips & Mathews (1968).

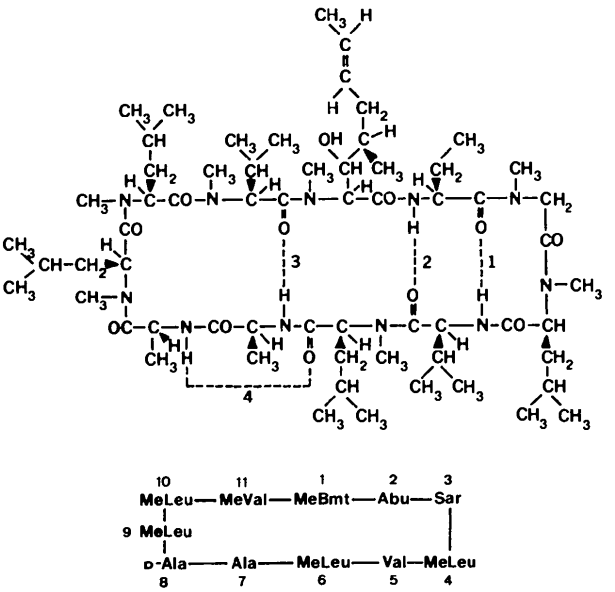

Fig. 1. Schematic of the cyclic undecapeptide cyclosporin A. All residues are in the L-configuration except for D-Ala residue 8. Standard nomenclature for amino acid residues is used together with the following: MeBmt = (4*R*)-4-[(*E*)-2-butenyl]-4,*N*-dimethyl-L-threonine; Abu = α-aminobutyric acid; Sar = sarcosine (*N*-methylglycine). The four intramolecular hydrogen bonds (1–4) are identified.

Refinement using F_o magnitudes was carried out using the 400-atom version of the *SHELX*76 computer code (Sheldrick, 1976). Atomic scattering factors were from Sears (1984). Cell parameters and atomic position parameters from the X-ray structure analysis (Weber, 1986) were used as starting values in a blocked full-matrix least-squares refinement. All parameters were used as variables. Despite the low observed-to-free-parameter ratio (2·3:1) the refinement was stable at all times. Geometric constraints were imposed on the hydrogen atoms in the initial refinement steps. The constraints were gradually relaxed until refinement for all atoms with anisotropic thermal parameters produced a final $R = 0{\cdot}074$, $wR = 0{\cdot}034$ where $w = 1/\sigma^2(F)$. All atomic coordinates and thermal parameters were unrestrained except for the coordinates of methylene group C2*B*, the methyl groups C5*C*2 and C8*B*, and the water molecule. For these, the interatomic distances were given fixed values: C—H 1·08, H—H (methyl) 1·747, O—H 0·965, H—H (water) 1·526 Å.

Discussion. Table 1 is a list of the final position parameters, and equivalent isotropic thermal parameters, B_{eq}, for all atoms in the asymmetric unit.* The non-conventional nomenclature for atom labelling follows that established in the X-ray structure analysis of cyclosporin-A (Petcher *et al*, 1976).

The estimated standard deviations of atomic coordinates calculated in the refinement procedure are: N 0·004, O 0·006, C (backbone) 0·005, C (side chain) 0·008, H (backbone) 0·01, H (side chain) 0·02 Å. These underestimate the true values and a more reliable indication is given by the estimated standard deviation of bond lengths calculated from the atomic coordinates: C—N 1·46, C—N (peptide) 1·35 (1), C—O 1·22, C—C 1·52 (3), C—H 1·06 (7), N—H 1·05 (2) Å. A histogram of C—H bond length (uncorrected for thermal motion) is given in Fig. 2(*a*). A histogram of the C—C—H bond angle calculated from the atomic coordinates is given in Fig. 2(*b*). The mean bond angle is 110 (3)°.

There is substantial agreement between the non-hydrogen atomic coordinates for the cyclosporin A mod III crystal structure determined by X-ray and by neutron diffraction. Approximately 74% of position differences are < 0·05 Å. Only two atoms have position differences that exceed 0·1 Å. They are

* Lists of structure factors and anisotropic thermal parameters have been deposited with the British Library Document Supply Centre as Supplementary Publication No. SUP 52784 (29 pp.). Copies may be obtained through The Technical Editor, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

Table 1. *A complete list of the fractional coordinates for the 199 atoms in the asymmetric unit, and equivalent isotropic temperature factors* B_{eq}

The e.s.d. of the atomic coordinates is calculated in the refinement procedures.

	x	*y*	*z*	B_{eq}
N1	−0·3513 (3)	0·5401 (2)	0·8412 (1)	4·6505
C1*N*	−0·4395 (6)	0·5026 (6)	0·8207 (2)	6·0586
C1*A*	−0·3296 (4)	0·5072 (3)	0·8788 (1)	4·1584
C1	−0·2865 (5)	0·4177 (3)	0·8745 (1)	4·4426
O1	−0·3452 (6)	0·3585 (4)	0·8656 (2)	7·1166
C1*B*	−0·4290 (4)	0·5100 (4)	0·9037 (1)	4·5321
O1*B*	−0·4798 (7)	0·5903 (5)	0·9013 (2)	6·9061
C1*C*	−0·4021 (5)	0·4925 (4)	0·9443 (1)	5·1374
C1*CMe*	−0·5015 (7)	0·4801 (7)	0·9650 (2)	8·0509
C1*D*	−0·3340 (6)	0·5641 (5)	0·9611 (2)	6·5113
C1*E*	−0·2902 (6)	0·5442 (5)	0·9979 (1)	7·9351
C1*F*	−0·3040 (7)	0·5834 (5)	1·0276 (2)	9·3274
C1*G*	−0·2572 (8)	0·5677 (9)	1·0651 (3)	12·1356
N2	−0·1811 (3)	0·4105 (2)	0·8786 (1)	4·4189
C2*A*	−0·1232 (4)	0·3348 (3)	0·8695 (1)	4·6927
C2	−0·0884 (4)	0·2916 (3)	0·9056 (1)	4·5637
O2	−0·0165 (5)	0·3238 (4)	0·9234 (1)	5·3006
C2*B*	−0·312 (6)	0·3612 (5)	0·8457 (2)	7·2877
C2*C*	0·0297 (10)	0·2843 (8)	0·8308 (3)	9·9591
N3	−0·1376 (3)	0·2206 (2)	0·9180 (1)	5·3559
C3*N*	−0·2368 (7)	0·1817 (5)	0·9029 (2)	6·6403
C3*A*	−0·1018 (7)	0·1907 (5)	0·9543 (2)	5·8875
C3	−0·1366 (4)	0·2621 (3)	0·9826 (1)	4·9058
O3	−0·2247 (5)	0·2922 (4)	0·9805 (1)	5·5059
N4	−0·0651 (3)	0·2862 (2)	1·0083 (1)	4·7953
C4*N*	0·0402 (6)	0·2516 (5)	1·0110 (2)	5·7849
C4*A*	−0·0923 (4)	0·3589 (3)	1·0318 (1)	4·1136
C4	−0·0321 (4)	0·4382 (3)	1·0209 (1)	4·7453
O4	−0·0078 (6)	0·4928 (4)	1·0428 (2)	7·3061
C4*B*	−0·0820 (5)	0·3372 (5)	1·0730 (1)	5·6428
C4*C*	−0·1569 (6)	0·2674 (5)	1·0859 (1)	7·9562
C4*D*1	−0·1282 (11)	0·2345 (9)	1·1224 (3)	11·7303
C4*D*2	−0·2670 (7)	0·2891 (7)	1·0843 (3)	9·6222
N5	−0·0101 (3)	0·4486 (2)	0·9852 (1)	4·2873
C5*A*	0·0418 (4)	0·5230 (3)	0·9703 (1)	4·5611
C5	−0·0339 (4)	0·5759 (3)	0·9483 (1)	4·3452
O5	−0·0740 (5)	0·5465 (3)	0·9202 (1)	4·7716
C5*B*	0·1379 (5)	0·4987 (4)	0·9464 (1)	6·2665
C5*C*1	0·2156 (6)	0·4389 (7)	0·9645 (3)	8·6800
C5*C*2	0·1899 (5)	0·5791 (5)	0·9318 (2)	10·2907
N6	−0·0528 (3)	0·6578 (2)	0·9586 (1)	4·5979
C6*N*	−0·0106 (8)	0·6980 (5)	0·9913 (2)	7·9167
C6*A*	−0·1098 (4)	0·7137 (3)	0·9318 (1)	4·1005
C6	−0·0261 (4)	0·7526 (3)	0·9064 (1)	4·3742
O6	0·0331 (5)	0·8086 (4)	0·9174 (1)	6·3139
C6*B*	−0·1771 (5)	0·7843 (4)	0·9489 (1)	5·1796
C6*C*	−0·2369 (4)	0·8367 (3)	0·9202 (1)	5·4348
C6*D*1	−0·2735 (9)	0·9185 (6)	0·9357 (2)	7·9588
C6*D*2	−0·3273 (9)	0·7858 (8)	0·9037 (3)	9·1195
N7	−0·0216 (3)	0·7197 (2)	0·8723 (1)	3·9241
C7*A*	0·0603 (4)	0·7435 (3)	0·8465 (1)	4·1689
C7	0·0330 (4)	0·8251 (3)	0·8246 (1)	4·3005
O7	0·0381 (5)	0·8293 (4)	0·7914 (1)	6·2060
C7*B*	0·0847 (6)	0·6704 (5)	0·8217 (2)	6·3823
N8	0·0048 (3)	0·8912 (2)	0·8452 (1)	4·9901
C8*A*	−0·0239 (4)	0·9738 (3)	0·8295 (1)	4·9322
C8	−0·1396 (4)	0·9735 (3)	0·8168 (1)	4·6663
O8	−0·2093 (5)	0·9569 (4)	0·8400 (1)	5·2901
C8*B*	−0·0116 (5)	1·0419 (4)	0·8574 (1)	8·0878
N9	−0·1633 (3)	0·9968 (2)	0·7822 (1)	4·5874
C9*N*	−0·0850 (6)	1·0121 (6)	0·7544 (2)	6·6955
C9*A*	−0·2738 (4)	1·0110 (3)	0·7718 (1)	3·8952
C9	−0·3066 (4)	0·9507 (3)	0·7407 (1)	4·8611
O9	−0·2822 (7)	0·9684 (4)	0·7090 (1)	8·4694
C9*B*	−0·2935 (5)	1·1025 (3)	0·7598 (1)	4·7611
C9*C*	−0·2858 (4)	1·1681 (3)	0·7906 (1)	4·5953
C9*D*1	−0·2747 (9)	1·2573 (5)	0·7739 (2)	7·5930
C9*D*2	−0·3822 (6)	1·1626 (5)	0·8152 (2)	6·7218
N10	−0·3617 (3)	0·8800 (2)	0·7483 (1)	4·0768
C10*N*	−0·3991 (6)	0·8316 (4)	0·7161 (1)	5·0111
C10*A*	−0·4012 (4)	0·8531 (3)	0·7846 (1)	3·6899
C10	−0·3973 (4)	0·7550 (3)	0·7870 (1)	4·0768
O10	−0·4815 (5)	0·7151 (4)	0·7877 (2)	5·6112
C10*B*	−0·5112 (4)	0·8879 (4)	0·7919 (1)	4·5926
C10*C*	−0·5538 (4)	0·8799 (4)	0·8317 (1)	4·9058
C10*D*1	−0·6689 (7)	0·8763 (7)	0·8328 (2)	8·7773
C10*D*2	−0·5136 (6)	0·9495 (5)	0·8558 (2)	6·5692
N11	−0·3023 (3)	0·7185 (2)	0·7866 (1)	4·0742
C11*N*	−0·1996 (5)	0·7644 (4)	0·7903 (2)	4·6269

Table 1 (*cont.*)

	x	*y*	*z*	B_{eq}
C11*A*	−0·2995 (4)	0·6253 (3)	0·7862 (1)	4·0321
C11	−0·2810 (4)	0·5925 (3)	0·8263 (1)	3·9136
O11	−0·1970 (5)	0·6159 (3)	0·8417 (1)	5·2532
C11*B*	−0·2139 (4)	0·5859 (3)	0·7605 (1)	4·3242
C11*C*1	−0·2212 (7)	0·4896 (4)	0·7606 (2)	5·7480
C11*C*2	−0·2264 (9)	0·6175 (6)	0·7213 (2)	7·7588
OSOL	−0·6279 (11)	0·6279 (8)	0·8420 (3)	16·4124
*HS*1	−0·5935 (19)	0·6675 (13)	0·8256 (5)	22·9158
*HS*2	−0·6544 (19)	0·5836 (13)	0·8260 (4)	19·4628
H1*Na*	−0·4877 (12)	0·4696 (10)	0·8392 (3)	12·6304
H1*Nb*	−0·4138 (13)	0·4592 (10)	0·8020 (4)	10·9092
H1*Nc*	−0·4768 (13)	0·5451 (10)	0·8072 (5)	12·0040
H1*a*	−0·2690 (7)	0·5518 (5)	0·8902 (2)	4·5505
H1*b*	−0·4810 (9)	0·4628 (7)	0·8942 (3)	6·2639
H1*OB*	−0·5184 (14)	0·6014 (11)	0·8814 (3)	10·8355
H1*C*	−0·3513 (10)	0·4331 (7)	0·9443 (3)	8·0615
H1*CMea*	−0·5465 (13)	0·5332 (11)	0·9639 (5)	11·0276
H1*CMeb*	−0·4750 (13)	0·4671 (9)	0·9949 (4)	11·9830
H1*CMec*	−0·5441 (15)	0·4296 (14)	0·9561 (5)	14·0069
H1*Da*	−0·3799 (13)	0·6212 (8)	0·9629 (3)	8·0667
H1*Db*	−0·2727 (12)	0·5768 (11)	0·9426 (3)	12·4936
H1*E*	−0·2403 (14)	0·4942 (13)	1·0000 (4)	17·1573
H1*F*	−0·3632 (15)	0·6377 (11)	1·0258 (4)	16·6493
H1*Ga*	−0·1990 (24)	0·6048 (25)	1·0703 (8)	22·5053
H1*Gb*	−0·3086 (16)	0·5812 (16)	1·0853 (3)	16·9020
H1*Gc*	−0·2384 (34)	0·5145 (16)	1·0677 (7)	29·5482
H2	−0·1385 (9)	0·4601 (7)	0·8897 (3)	6·3271
H2*A*	−0·1845 (10)	0·2883 (6)	0·8545 (2)	7·8799
H2*Ba*	0·0200 (14)	0·4008 (9)	0·8626 (4)	11·6119
H2*Bb*	−0·0693 (11)	0·4002 (9)	0·8250 (3)	11·0408
H2*Ca*	0·0499 (14)	0·2503 (10)	0·8505 (4)	8·7958
H2*Cb*	0·0759 (21)	0·3059 (15)	0·8102 (6)	20·2419
H2*Cc*	−0·0234 (16)	0·2427 (12)	0·8111 (6)	14·3754
H3*Na*	−0·2524 (21)	0·2071 (16)	0·8817 (5)	20·4734
H3*Nb*	−0·2895 (13)	0·1792 (21)	0·9177 (5)	15·8545
H3*Nc*	−0·2237 (17)	0·1247 (11)	0·8969 (8)	15·8334
H3*Aa*	−0·1485 (11)	0·1353 (8)	0·9614 (3)	8·3220
H3*Ab*	−0·0286 (11)	0·1773 (10)	0·9539 (3)	6·9298
H4*Na*	0·0807 (11)	0·2582 (12)	0·9839 (4)	13·2068
H4*Nb*	0·0471 (16)	0·2010 (10)	1·0183 (7)	15·2228
H4*Nc*	0·0909 (12)	0·2942 (12)	1·0259 (4)	13·5226
H4*A*	−0·1731 (8)	0·3716 (7)	1·0253 (2)	5·4533
H4*Ba*	−0·0934 (10)	0·3970 (8)	1·0884 (3)	7·1903
H4*Bb*	0·0023 (10)	0·3217 (9)	1·0798 (3)	9·2300
H4*C*	−0·1387 (14)	0·2106 (7)	1·0659 (3)	11·7961
H4*D*1*a*	−0·1187 (16)	0·2883 (11)	1·1429 (3)	14·0201
H4*D*1*b*	−0·0374 (15)	0·2134 (15)	1·1225 (6)	14·8912
H4*D*1*c*	−0·1701 (19)	0·1906 (12)	1·1327 (5)	14·4201
H4*D*2*a*	−0·2786 (18)	0·3443 (18)	1·1052 (5)	20·9104
H4*D*2*b*	−0·3200 (17)	0·2412 (11)	1·0922 (5)	13·5174
H4*D*2*c*	−0·2915 (11)	0·3089 (14)	1·0566 (4)	15·9492
H5	−0·0376 (9)	0·4011 (6)	0·9658 (2)	6·0612
H5*A*	0·0709 (8)	0·5564 (6)	0·9962 (3)	5·9112
H5*B*	0·0989 (9)	0·4634 (8)	0·9220 (3)	8·8458
H5*C*1*a*	0·2427 (17)	0·4699 (12)	0·9915 (5)	15·6887
H5*C*1*b*	0·2728 (16)	0·4211 (13)	0·9484 (5)	13·6016
H5*C*1*c*	0·1656 (13)	0·3862 (11)	0·9764 (6)	14·7965
H5*C*2*a*	0·2177 (16)	0·6161 (10)	0·9549 (4)	20·6998
H5*C*2*b*	0·1376 (8)	0·6189 (11)	0·9158 (5)	14·9044
H5*C*2*c*	0·2571 (9)	0·5635 (9)	0·9148 (4)	16·1361
H6*Na*	−0·0740 (16)	0·7358 (13)	1·0046 (5)	14·5938
H6*Nb*	0·0495 (19)	0·7321 (16)	0·9874 (5)	17·7573
H6*Nc*	−0·0063 (16)	0·6509 (9)	1·0131 (3)	13·9148
H6*A*	−0·1582 (7)	0·6698 (5)	0·9155 (2)	4·3900
H6*Ba*	−0·2300 (9)	0·7547 (6)	0·9690 (3)	7·4851
H6*Bb*	−0·1263 (9)	0·8262 (6)	0·9638 (3)	7·0271
H6*C*	−0·1813 (8)	0·8539 (6)	0·8970 (2)	6·6850
H6*D*1*a*	−0·3291 (14)	0·9098 (12)	0·9533 (5)	11·0908
H6*D*1*b*	−0·3076 (13)	0·9622 (8)	0·9138 (3)	11·4566
H6*D*1*c*	−0·2154 (17)	0·9568 (10)	0·9453 (6)	14·7280
H6*D*2*a*	−0·3726 (19)	0·7679 (19)	0·9221 (6)	15·1886
H6*D*2*b*	−0·2994 (18)	0·7311 (10)	0·8877 (5)	16·4861
H6*D*2*c*	−0·3644 (12)	0·8233 (10)	0·8840 (5)	13·7095
H7	−0·0780 (8)	0·6742 (5)	0·8640 (2)	4·8269
H7*A*	0·1281 (8)	0·7615 (6)	0·8648 (2)	6·0060
H7*Ba*	0·0165 (10)	0·6509 (9)	0·8063 (4)	10·1275
H7*Bb*	0·1099 (14)	0·6156 (8)	0·8373 (3)	12·0014
H7*Bc*	0·1442 (11)	0·6851 (9)	0·8030 (3)	11·5935
H8	0·0017 (9)	0·8865 (6)	0·8744 (2)	6·8587
H8*A*	0·0262 (10)	0·9851 (7)	0·8057 (3)	9·3274
H8*Ba*	−0·0604 (9)	1·0255 (8)	0·8807 (2)	10·0091
H8*Bb*	−0·0378 (9)	1·1030 (5)	0·8471 (3)	13·9543
H8*Bc*	0·0685 (6)	1·0490 (8)	0·8670 (3)	11·8488
H9*Na*	−0·1206 (12)	1·0146 (10)	0·7299 (3)	10·1380
H9*Nb*	−0·0279 (10)	0·9566 (11)	0·7546 (4)	11·9251
H9*Nc*	−0·0348 (12)	1·0696 (11)	0·7595 (4)	11·9909

Table 1 (*cont.*)

	x	*y*	*z*	B_{eq}
H9*A*	−0·3163 (7)	0·9973 (5)	0·7967 (2)	4·9716
H9*Ba*	−0·3696 (10)	1·1056 (7)	0·7468 (3)	7·2166
H9*Bb*	−0·2372 (9)	1·1191 (6)	0·7365 (2)	6·8561
H9*C*	−0·2169 (8)	1·1539 (6)	0·8080 (2)	6·0876
H9*D1a*	−0·3386 (14)	1·2708 (8)	0·7562 (4)	12·3883
H9*D1b*	−0·2048 (12)	1·2687 (9)	0·7586 (4)	8·2247
H9*D1c*	−0·2724 (11)	1·3037 (7)	0·7964 (3)	9·4669
H9*D2a*	−0·4458 (12)	1·1657 (14)	0·7998 (5)	11·7645
H9*D2b*	−0·3748 (13)	1·2158 (7)	0·8349 (3)	11·1381
H9*D2c*	−0·3753 (14)	1·1050 (8)	0·8309 (3)	11·4908
H10*Na*	−0·4770 (12)	0·8083 (16)	0·7195 (4)	15·6650
H10*Nb*	−0·3507 (16)	0·7821 (11)	0·7101 (5)	14·1938
H10*Nc*	−0·3958 (19)	0·8629 (8)	0·6945 (3)	14·1964
H10*A*	−0·3467 (7)	0·8799 (5)	0·8065 (2)	4·5032
H10*Ba*	−0·5134 (8)	0·9569 (6)	0·7837 (3)	5·8217
H10*Bb*	−0·5608 (8)	0·8536 (6)	0·7728 (2)	6·0586
H10*C*	−0·5089 (10)	0·8183 (7)	0·8430 (3)	7·9378
H10*D1a*	−0·7001 (14)	0·9423 (14)	0·8228 (5)	14·9991
H10*D1b*	−0·6985 (13)	0·8675 (11)	0·8607 (3)	13·3147
H10*D1c*	−0·7040 (15)	0·8279 (15)	0·8169 (5)	15·5808
H10*D2a*	−0·5377 (15)	1·0125 (7)	0·8467 (4)	12·2462
H10*D2b*	−0·4166 (9)	0·9539 (8)	0·8554 (3)	8·2957
H10*D2c*	−0·5332 (11)	0·9420 (8)	0·8833 (3)	9·0326
H11*Na*	−0·1410 (9)	0·7293 (8)	0·7925 (5)	8·8221
H11*Nb*	−0·2083 (13)	0·8003 (11)	0·8175 (4)	13·2305
H11*Nc*	−0·1971 (11)	0·8095 (9)	0·7711 (5)	13·1700
H11*a*	−0·3749 (7)	0·6044 (6)	0·7758 (2)	5·1164
H11*b*	−0·1419 (9)	0·6031 (8)	0·7708 (3)	7·1903
H11*C1a*	−0·2925 (13)	0·4658 (8)	0·7526 (4)	8·9984
H11*C1b*	−0·2081 (11)	0·4656 (7)	0·7906 (3)	9·1090
H11*C1c*	−0·1618 (13)	0·4614 (9)	0·7457 (4)	11·0250
H11*C2a*	−0·3058 (12)	0·6100 (13)	0·7112 (3)	10·8065
H11*C2b*	−0·1774 (13)	0·5898 (9)	0·7052 (4)	9·1169
H11*C2c*	−0·2286 (15)	0·6892 (7)	0·7198 (3)	11·7935

C1*CMe* (MeBmt-1) with a shift of 0·10 Å, and O9 (MeLeu-9) with a shift of 0·31 Å.

There are a number of discrepancies between the calculated hydrogen positions for the X-ray structure, and the measured values for the neutron structure. This reflects the somewhat arbitrary choice in the X-ray structure for the hydrogen position from a number of energetically possible options. One of particular interest because of the position in a pharmacologically important region, is the hydrogen atom H1O*B* (MeBmt-1) with a shift of 1·29 Å. There must have been no supplementary information in the X-ray analysis to suggest one of the other options.

Bond lengths and angles for the four intramolecular hydrogen bonds are given in Table 2. Values from the X-ray structures of the $P2_1$, $P4_1$ and $P2_12_12_1$ crystal forms are included for comparison. Despite significant differences in other aspects of the structure (side-chain flexibility, packing geometry, *etc.*), these data indicate that the molecular backbone remains remarkably invariant. The torsional angles φ, ψ and ω for the peptide chain are given in Table 3. The maximum deviation from planarity for the *trans*-peptide bond is 13°.

Hydrogen bonds in protein structures have been studied extensively. A statistical analysis of 500 hydrogen bonds in β-sheet structures gave a mean (O···H) bond length of 1·96 (16) Å and a mean donor bond angle (N—H···O) of 160 (10)° (Baker & Hubbard, 1984). This compares with a mean bond length of 1·95 (3) Å for cyclosporin A hydrogen bonds 1–3, and a mean angle of 158 (8)°. In an apolar environment, hydrogen bonds 1–3 are known to persist unchanged in solution, and hydrogen bond 4 to form a bifurcated bond to O6 (MeLeu-6) and O8 (D-Ala-8) (Loosli *et al.* 1985).

An ordered water molecule was found in a difference Fourier synthesis. It forms an intramolecular bridge between the atoms O10 (MeLeu-10) and H1*OB* (MeBmt-1), and an intermolecular bridge to the atom O9′ (MeLeu-9′) on the next molecule. Bond lengths and angles are given in Table 4. The thermal parameters of the water molecule (Table 1) are high indicating some disorder or large average displacement. Refinement of the site occupancy factors for the water molecule indicated that the site was fully occupied. The precise origin of the water molecule remains to be identified. Because of the extreme hydrophobicity of cyclosporin A, the crystal was grown from a mixture of organic solvents (oil, ethanol and a non-ionic surfactant), using the same procedure that produced the crystals for the X-ray

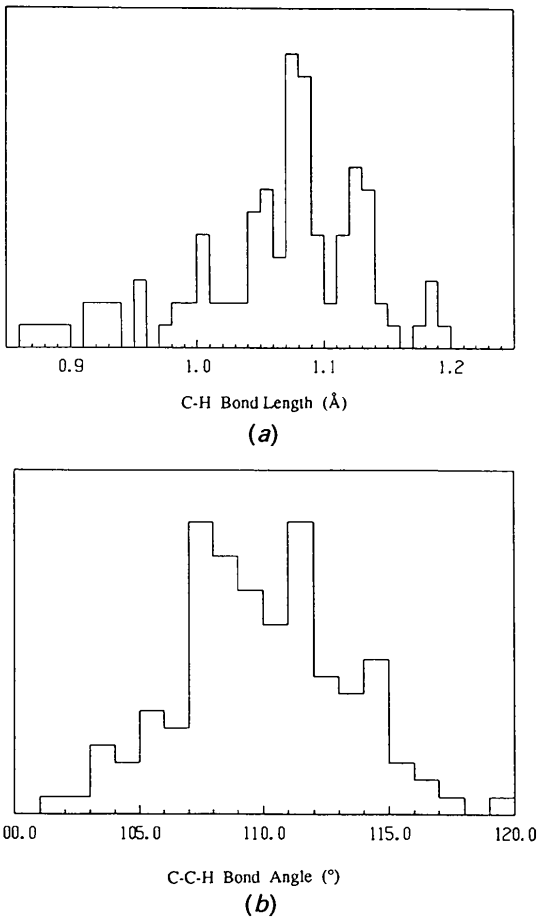

Fig. 2. Histograms of the geometric data for the 111 hydrogen atoms in cyclosporin A: (*a*) the C—H bond length distribution (uncorrected for thermal motion) at 0·01 Å resolution; (*b*) the C—C—H bond angle distribution at 1° resolution.

Table 2. *Bond lengths* (Å) *and angles* (°) *for the four intramolecular hydrogen bonds in cyclosporin A*

Interatomic distances are denoted N···O for nitrogen–oxygen, H···O for hydrogen–oxygen and N—H for the nitrogen–hydrogen bond. The angle between the N—H bond and the H···O (hydrogen) bond is denoted by N—H···O.

	$P2_12_12_1$		$P4_1$	$P2_1$
Bond	(Neutron)	(X-ray)	(X-ray)	(X-ray)
N···O				
1	2·978	2·957	3·02	3·21
2	2·942	2·967	2·85	3·26
3	2·970	2·994	2·89	3·03
4	2·948	2·911	2·96	2·91
H···O				
1	1·979	2·005*	2·06*	2·16*
2	1·925	1·966*	1·84*	2·15*
3	1·940	1·994*	1·98*	1·99*
4	2·019	2·002*	1·95*	2·00*
N—H				
1	1·079	1·021*	1·27*	1·094*
2	1·030	1·020*	1·29*	1·067*
3	1·054	1·029*	1·31*	1·083*
4	1·056	1·020*	1·28*	1·080*
N—H···O				
1	155·8	154·1*		
2	167·2	166·4*		
3	151·1	163·2*		
4	149·0	147·0*		

* Calculated hydrogen position.

Table 3. *Torsional angles* φ, ψ *and* ω (°) *for the cyclic peptide chain that forms the molecular backbone of cyclosporin A*

Residue	φ	ψ	ω
MeBmt-1	−99	103	−169
Abu-2	−108	103	−175
Sar-3	68	−136	173
MeLeu-4	−106	34	177
Val-5	−110	119	167
MeLeu-6	−86	107	−173
Ala-7	−88	51	−180
D-Ala-8	83	−127	−170
MeLeu-9	−122	102	3
MeLeu-10	−145	66	−176
MeVal-11	−98	121	178

Table 4. *Bond lengths* (Å) *and angles* (°) *for the water molecule*

The angle between the O—H bond and the H···O (hydrogen) bond is denoted by O—H···O.

HS1—OSOL	0·965*
HS2—OSOL	0·965*
OSOL···H1*OB*	2·043
HS1···O10	2·118
HS2···O9′	2·344
HS1—OSOL—HS2	104·6*
O1*B*—H1*OB*···*OSOL*	169·7
OSOL—HS1···O10	157·2
OSOL—HS2···O9′	173·8

Symmetry code: $-1-x, \frac{1}{2}+y, 1\frac{1}{2}-z$.

* Fixed atom position.

analysis. The crystal was stored in an air- and light-tight container until mounted on the neutron diffractometer. As outlined above, the only notable differences in the atomic coordinates from the X-ray and neutron analyses are in the region occupied by the water molecule.

The interaction of water with protein structures is of fundamental importance and has been studied extensively. At present, statistical analysis of observed geometries provides the best indication of the energetics of the hydrogen bonds involved in the water interaction. Many questions remain unanswered but a number of general trends are emerging.

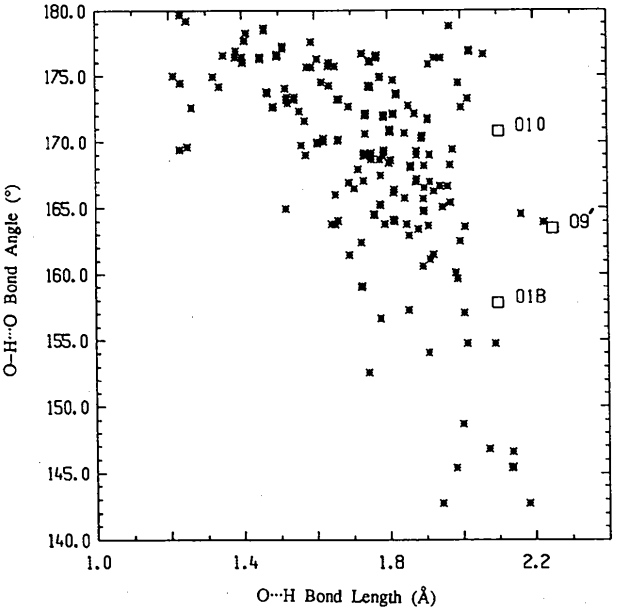

Fig. 3. Plot of the donor angle (O—H···O) against the O···H bond length for hydrogen bonds involved in water interactions in small hydrate structures (after Savage & Finney, 1986). The three water hydrogen bonds for the cyclosporin A molecule are labelled with the O···H oxygen atom.

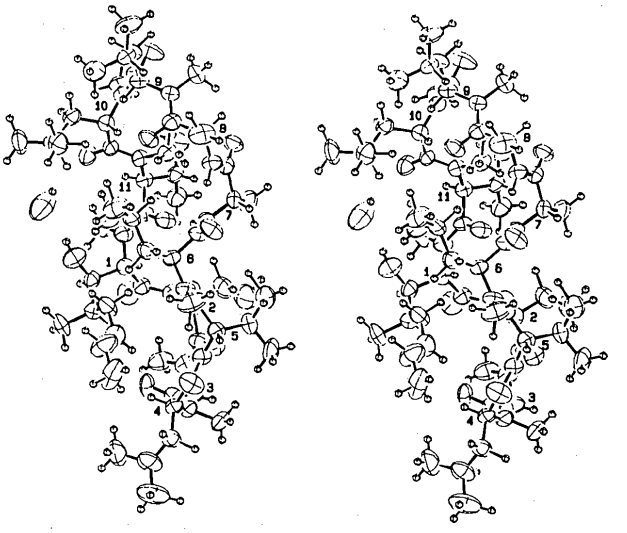

Fig. 4. Stereoview (Johnson, 1976) of the cyclosporin A molecule. Thermal ellipsoids are drawn at the 50% probability level for the non-hydrogen atoms, and hydrogen atoms are drawn as spheres of arbitrary radius. Residues are numbered as close as possible to the α-carbon atom.

The dependence of donor angle (O—H···O) on O···H bond length provides a semi-quantitative indication of the bond strength. Fig. 3 is a comparison between data obtained from an analysis of water structures in small hydrate crystal structures (Savage & Finney, 1986) and that from this study. The hydrogen-bond lengths tend toward the upper limit of values documented in the Savage & Finney survey. In an apolar environment, the MeBmt-1 side chain has been observed to rotate out of the cleft of the β-sheet and locate proboscis-like in the solvent (Loosli *et al.*, 1985).

No other ordered solvent molecules of significance were found in the final difference Fourier synthesis, where maximum positive and negative residuals were 3·1% of the height of an N-atom peak. All intermolecular distances are in the range of normal van der Waals values.

The *ORTEP*II molecular graphics program (Johnson, 1976) was used to generate the stereoview of the cyclosporin A molecule given in Fig. 4. The water molecule is shown hydrogen bonding the MeBmt-1 side chain to the molecular backbone.

The addition of the geometric parameters for the hydrogen atoms completes the high-resolution structure of cyclosporin A in the single-crystal environment. The geometric parameters of a single bound water molecule has provided evidence for an ordered solvent interaction. What contribution this structural information makes to the understanding of the highly specific pharmacological function of cyclosporin A is under investigation.

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Structure and Stereochemistry of an Acetate Derivative of Cacospongionolide, a New Antitumoral Sesterterpenoid from Marine Sponge *Cacospongia mollior*

BY RAFFAELLA PULITI AND SALVATORE DE ROSA

Istituto per la Chimica di Molecole di Interesse Biologico CNR, Via Toiano 6, 80072 *Arco Felice, Napoli, Italy*

AND CARLO ANDREA MATTIA AND LELIO MAZZARELLA

Dipartimento di Chimica, Università Federico II, *Via Mezzocannone* 4, 80134 *Napoli, Italy*

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Abstract. 2,5-Dihydro-3-{3,6-dihydro-5-[2-(perhydro-1,2,3-trimethyl-4a,5-methano-1-naphthyl)ethyl]-2*H*-pyran-2-yl}-5-oxo-2-furyl acetate, $C_{27}H_{38}O_5$, M_r = 442·6, monoclinic, $P2_1$, a = 9·717 (4), b = 7·064 (3), c = 18·751 (6) Å, β = 96·94 (3)°, V = 1278 (2) Å^3, Z = 2, D_x = 1·150 Mg m^{-3}, λ(Cu $K\alpha$) =

0108-2701/90/081533-04$03.00