157. Crystal and Molecular Structure of an Iodo-derivative of the Cyclic Undecapeptide Cyclosporin A

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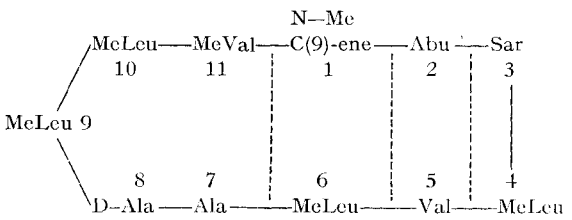
Summary. The crystal structure of an iodo-derivative of cyclosporin A has been determined in order to elucidate the constitution of this cyclic undecapeptide. Crystals of iodocyclosporin A are monoclinic, $a = 10.475(5)$, $b = 19.60(1)$, $c = 21.04(1)$ Å, $\beta = 99.35(2)°$, space group $P2_1$ (C_2^2, No. 4). The structure was solved by the heavy atom method and refined by block-diagonal least-squares analysis to a final R-factor of 0.135 with hydrogen atoms in calculated positions. The cyclic peptide has a conformation which is partly β-pleated sheet and partly open loop. The structure analysis demonstrates for the first time the reality of a new type of dipeptide hydrogen-bonding, predicted by *Pullman* from MO calculations and leading to a conformation described by him as C_7^{eq}.

Cyclosporin A is a cyclic undecapeptide $C_{62}H_{111}N_{11}O_{12}$ which may be isolated from *Trichoderma polysporum* (Link ex Pers.) *Rifai* [1]. The compound is neutral, rich in hydrophobic amino-acids, insoluble in water and *n*-hexane, but very soluble in all other organic solvents, and exceedingly difficult to crystallise. (In fact, it was not until some three months after the completion of this structure analysis, that excellent crystals were serendipitously obtained from acetone solution at $-15°$.) We were, however, able to obtain a crystalline derivative from the reaction of cyclosporin and iodine in the presence of thallium acetate.

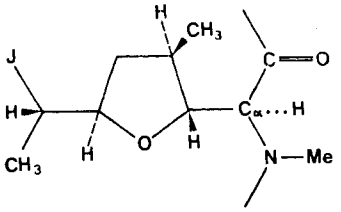
The analysis of iodocyclosporin was undertaken to determine the constitution of the natural product. At the beginning of the analysis the amino-acid composition and part of the sequence was known from chemical and spectroscopic studies [2], as was the presence of a hitherto unknown β-hydroxy, singly unsaturated C_9 amino acid, the site of iodine addition. (We abbreviate this amino-acid as C_9-ene.) The structure analysis showed the constitution of cyclosporin A to be **1**, where all optically active amino-acids are L with the exception of Ala(8) which is D.



1

– – – – = hydrogen bond

The side chain of C_9-ene as found in the crystal structure is **2**, from which the formula of the amino acid is derived to be **3**; namely, 2*S*-methylamino-3*R*-hydroxy-4*R*-methyl-oct-6-en-1-oic acid. ^{1}H-NMR. investigations of cyclosporin A at 360 MHz indicate that the double-bond is *trans* [2].



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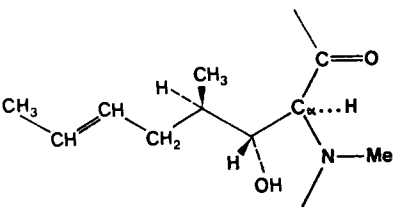

3

Crystal data. – Iodocyclosporin, $C_{62}H_{110}N_{11}O_{12}I$, $M = 1327$, colourless prisms from *n*-heptane, 2.5:1, monoclinic, $a = 10.475(5)$, $b = 19.60(1)$, $c = 21.05(1)$ Å, $\beta = 99.35(2)°$, $U = 4264$ Å^3, $D_c = 1.03$, $Z = 2$, space group $P2_1$, (C_2^2, No. 4). CuK_α radiation, $\lambda = 1.54187$ Å, graphite monochromator.

Experimental part

A crystal of approximate dimensions $0.3 \times 0.7 \times 0.4$ mm was sealed in a *Lindemann* capillary and oriented on an *Enraf-Nonius* CAD4-F automatic diffractometer. Intensity data were collected by $\omega - 2\theta$ scans for $1.5 \leqslant \theta \leqslant 50°$ using a scan width $\omega = 1.0 + 0.2 \tan\theta$ and constant count of $\geqslant 6000$ per reflection (or 120 seconds maximum time per measurement). Because of crystal decay, four specimens, all of similar dimensions, had to be used. The composite data were scaled together by an iterative least-squares method. A total of 5658 measurements yielded 4527 symmetry independent reflections of which 2711 were judged to be significantly above background [$I \geqslant 3\sigma(I)$]. Data were corrected for *Lorentz* and polarisation effects, but not for X-ray absorption, and placed on an absolute scale by means of a *Wilson* plot ($\overline{B} = 10.4$ Å, $<|E|> = 0.803$, $<|E^2-1|> = 0.885$, $<|E^2|> = 1.000$).

Structure Analysis. – The iodine position located from a *Patterson* function provided phases for a preliminary *Fourier* synthesis. The interpretation of the resulting density map was complicated by the spurious mirror plane through the I-atom, but it was possible to recognise 14 peaks as forming two fragments of planar peptide units and associated β-carbon atoms. The full structure could then be developed in a sequence of repeated structure factor calculation and *Fourier* syntheses, until an (F_o–F_c)-*Fourier* synthesis revealed no new atomic peaks.

The structure was refined by block-diagonal least-squares analysis employing first isotropic, then anisotropic temperature factors for all atoms. At convergence, hydrogen atoms were introduced in calculated positions (methyl groups *staggered*, N-methyl groups with one C—H bond *trans* to the N—C_α bond) with temperature factors $B = 5.0$ Å^2 and held fixed while the heavier atoms were refined for one further cycle. Positions of hydrogen atoms were then recalculated, the heavier atoms further refined, and the process repeated until convergence at $R = 0.135$. This value is rather high, and partly reflects the poor quality of the data. Firstly, all crystals decayed somewhat in the X-ray beam – we replaced a crystal when the intensity of three check reflections had sunk to 75–80% of the initial value. It is difficult to know how best to correct for this effect. Our chosen method, scaling all reflections by linear interpolation to the starting mean intensity of the check reflections taken as 100% undoubtedly leads to some scaling error between data sets. Secondly, although we collected data out to $\theta = 50°$, giving a theoretical resolution of 1 Å, the resolution of the significant data is less, about 1.1 Å so that the least squares process is somewhat ill-conditioned. Thirdly, there may be some water of crystallization in the structure. We calculated several difference (F_o–F_c) and weighted difference ($2 \cdot F_o$–F_c) *Fourier* maps but were unable to assign any peaks convincingly as localized solvent molecules. Final positional parameters are presented in Table 1. A complete set of parameters and the structure factor tables may be obtained from the authors on request.

Table 1. *Refined atomic co-ordinates for the heavier atoms of iodocyclosporin, together with calculated co-ordinates for hydrogen atoms*[a]). The amino acid residues, each complete in itself, are presented in sequence

	X	Y	Z
N(1)	4172(20)	4701(10)	6461(9)
C(N1)	5455(27)	4390(16)	6488(16)
C(1A)	4012(26)	5434(15)	6358(12)
C(1)	3575(27)	5547(14)	5639(13)
O(1)	4064(20)	5246(11)	5248(9)
C(1B)	5226(33)	5839(16)	6541(13)
C(1C)	5059(30)	6626(19)	6317(17)
C(1D)	5689(38)	6755(18)	5770(19)
C(1E)	5739(42)	6957(20)	6976(18)
C(1F)	5666(47)	6402(27)	7460(17)
O(1B)	5607(22)	5799(10)	7202(10)
C(1G)	6355(61)	6449(25)	8076(35)
C(1H)	6645(36)	5853(30)	8539(20)
I(1)	6823(6)	7500(0)	8428(2)
H(1CN1)	612	461	687
H(2CN1)	580	447	603
H(3CN1)	538	384	656
H(1A)	329	560	663
H(1B)	597	563	630
H(1C)	404	674	619
H(1D1)	526	643	537
H(1D2)	670	664	589
H(1D3)	556	728	563
H(1E1)	521	740	707
H(1E2)	672	709	693
H(1F)	474	642	762
H(1G)	730	665	805
H(1H1)	719	547	833
H(1H2)	574	563	863
H(1H3)	719	603	898
N(2)	2630(20)	5970(12)	5471(9)
C(2A)	2145(30)	6147(16)	4821(15)
C(2)	1760(45)	6810(23)	4803(16)
O(2)	1345(25)	7134(13)	5259(15)
C(2B)	877(41)	5786(23)	4547(15)
C(2C)	185(52)	5934(19)	3953(23)
H(2)	219	618	585
H(2A)	287	602	453
H(2B1)	18	585	487
H(2B2)	105	524	449
H(2C1)	77	583	358
H(2C2)	– 10	646	393
H(2C3)	– 66	561	386
N(3)	2211(37)	7340(16)	4419(15)
C(3A)	2269(71)	8092(31)	4646(32)
C(N3)	3185(57)	7110(26)	3956(21)
C(3)	2950(42)	8310(18)	5236(14)
O(3)	4070(29)	8049(18)	5333(17)
H(1CN3)	416	718	419
H(2CN3)	301	741	352
H(3CN3)	303	657	383
H(3A1)	263	834	425
H(3A2)	125	821	462
N(4)	2447(37)	8743(16)	5579(17)
C(N4)	1095(45)	9026(26)	5455(26)
C(4A)	3277(37)	8874(19)	6232(26)
C(4)	2256(61)	8503(28)	6671(22)
O(4)	2568(44)	8881(17)	7135(25)
C(4B)	3711(80)	9668(25)	6375(27)
C(4C)	4442(59)	9877(22)	6070(30)
C(4D)	5803(49)	9534(25)	6269(22)
C(4E)	4699(82)	10614(32)	6152(43)
H(1CN4)	113	957	550
H(2CN4)	54	881	580
H(3CN4)	63	889	497
H(4A)	418	860	630
H(4B1)	287	999	627
H(4B2)	414	972	687
H(4C)	408	978	556
H(4D1)	571	899	619
H(4D2)	615	963	677
H(4D3)	647	973	597
H(4E1)	508	1071	665
H(4E2)	380	1089	601
H(4E3)	535	1100	604
N(5)	1858(31)	7972(14)	6586(14)
C(5A)	1218(32)	7754(17)	7093(15)
C(5)	1743(31)	6975(17)	7265(19)
O(5)	2106(21)	6650(10)	6817(8)
C(5B)	– 103(39)	7752(21)	6894(21)
C(5C)	– 654(53)	8514(30)	6878(25)
C(5D)	– 655(42)	7396(24)	6314(26)
H(5)	191	763	618
H(5A)	145	807	751
H(5B)	– 57	752	726
H(5C1)	– 20	881	654
H(5C2)	– 44	873	735
H(5C3)	– 168	850	672
H(5D1)	– 40	686	636
H(5D2)	– 27	760	590
H(5D3)	– 169	745	623
N(6)	1606(30)	6674(16)	7831(11)
C(N6)	1358(55)	7117(22)	8355(18)
C(6A)	1715(36)	5928(15)	7889(14)
C(6)	428(21)	5623(16)	7662(13)
O(6)	– 389(17)	5726(12)	8000(9)
C(6B)	2464(34)	5740(22)	8584(14)

	X	Y	Z
C(6C)	2777(42)	4992(18)	8661(15)
C(6D)	3672(49)	4713(23)	8350(21)
C(6E)	3156(59)	4849(25)	9337(20)
H(1CN6)	212	705	876
H(2CN6)	44	698	849
H(3CN6)	132	764	819
H(6A)	238	576	757
H(6B1)	337	601	867
H(6B2)	188	587	894
H(6C)	191	469	850
H(6D1)	459	496	849
H(6D2)	338	477	783
H(6D3)	376	417	846
H(6E1)	237	497	959
H(6E2)	399	515	952
H(6E3)	338	431	940
N(7)	175(21)	5302(11)	7132(8)
C(7A)	– 987(3)	5137(15)	6850(12)
C(7)	– 1374(26)	4417(18)	7058(13)
O(7)	– 1576(20)	3912(12)	6740(10)
C(7B)	– 1160(30)	5142(19)	6103(14)
H(7)	100	516	691
H(7A)	– 164	551	698
H(7B1)	– 49	478	594
H(7B2)	– 96	564	593
H(7B3)	– 214	500	590
N(8)	– 1458(22)	4369(13)	7726(12)
C(8A)	– 1793(35)	3740(17)	8010(17)
C(8)	– 562(27)	3300(17)	8226(12)
O(8)	378(17)	3649(10)	8458(8)
C(8B)	– 2363(46)	3912(28)	8608(23)
H(8)	– 126	482	802
H(8A)	– 247	345	766
H(8B1)	– 321	422	847
H(8B2)	– 165	418	894
H(8B3)	– 262	344	883
N(9)	– 552(24)	2629(13)	8175(10)
C(N9)	– 1693(37)	2280(20)	7911(16)
C(9A)	626(28)	2262(16)	8449(13)
C(9)	1313(32)	1866(16)	7967(17)
O(9)	583(25)	1426(13)	7742(11)
C(9B)	319(50)	1772(24)	8975(18)
C(9C)	– 277(55)	2060(28)	9455(26)
C(9D)	– 854(75)	1628(53)	9822(41)
C(9E)	853(114)	2459(52)	9851(22)
H(1CN9)	– 200	196	827
H(2CN9)	– 150	196	751
H(3CN9)	– 244	264	773
H(9A)	126	265	867
H(9B1)	– 32	137	875
H(9B2)	120	154	921
H(9C)	– 104	239	924
H(9D1)	– 14	127	1006
H(9D2)	– 160	134	951
H(9D3)	– 127	191	1017
H(9E1)	121	283	954
H(9E2)	162	211	1003
H(9E3)	51	271	1024
N(10)	2361(24)	2023(13)	7857(13)
C(N10)	2935(54)	1546(18)	7377(20)
C(10A)	3164(29)	2596(17)	8027(12)
C(10)	3633(27)	2930(18)	7418(13)
O(10)	4768(22)	2951(12)	7379(10)
C(10B)	4396(31)	2448(21)	8513(13)
C(10C)	4130(46)	2235(22)	9136(22)
C(10D)	5365(46)	1937(29)	9538(21)
C(10E)	3663(39)	2738(34)	9454(21)
H(1CN10)	387	136	760
H(2CN10)	302	182	694
H(3CN10)	230	111	725
H(10A)	258	295	824
H(10B1)	494	204	832
H(10B2)	497	290	858
H(10C)	339	184	907
H(10D1)	611	232	960
H(10D2)	568	150	929
H(10D3)	516	177	1000
H(10E1)	277	292	917
H(10E2)	435	315	952
H(10E3)	348	255	991
N(11)	2647(18)	3238(11)	6989(9)
C(N11)	1330(26)	3310(16)	7068(14)
C(11A)	3119(25)	3523(15)	6397(10)
C(11)	3088(23)	4317(14)	6432(9)
O(11)	2056(15)	4608(9)	6392(7)
C(11B)	2193(29)	3249(17)	5766(11)
C(11C)	2221(43)	2508(19)	5786(16)
C(11D)	2876(35)	3581(19)	5211(13)
H(1CN11)	71	306	667
H(2CN11)	108	384	707
H(3CN11)	118	307	751
H(11A)	409	335	638
H(11B)	121	343	576
H(11C1)	187	233	621
H(11C2)	320	233	579
H(11C3)	160	230	536
H(11D1)	385	339	525
H(11D2)	287	412	525
H(11D3)	235	343	474

a) For the heavier atoms, x/a, y/b, z/c are $\times 10^4$, for hydrogen atoms, 10^3. The numbers in brackets are estimated standard deviations derived from the block-diagonal least squares refinement and are probably underestimates.

Description and Discussion of the Structure. – A stereo view of the molecule is shown in Fig. 1, in which the antiparallel β-pleated sheet conformation of residues 1–6, the open loop of residues 7–11, and the rather large thermal vibrations of some of the side-chain atoms, particularly those of MeLeu-9 are apparent. The absolute configuration was not determined as part of the structure analysis, since sufficient of the hydrolysis products could reliably be identified as L-amino-acids. It became apparent during the analysis, however, that Ala-8 has the D-configuration. The molecular geometry at termination of refinement is presented in Fig. 2–10, where the backbone torsion angles, θ, ψ and ω are also given. Table 2 collects these values and Fig. 11 is the *Ramachandran* diagram of iodocyclosporin. Even from this diagram it is clear that Ala-8 cannot be an L-amino acid, falling as it does, along with Sar-3, in the allowed region for β-structure of D-amino-acids. There is one *cis*-N-methyl peptide linkage, namely that between MeLeu-9 and MeLeu-10. The peptide torsion angles (ω) show some deviation from strict planarity, particularly $\omega_{2,3}$ where this deviation is 30°.

The β-pleated sheet, residues 1–6, which is quite markedly twisted [3], is held together by the following hydrogen bonds: NH of α-aminobutyric acid to C=O of Val-5 (N–O 326 pm); NH of Val-5 to C=O of α-aminobutyric acid (N–O 321 pm);

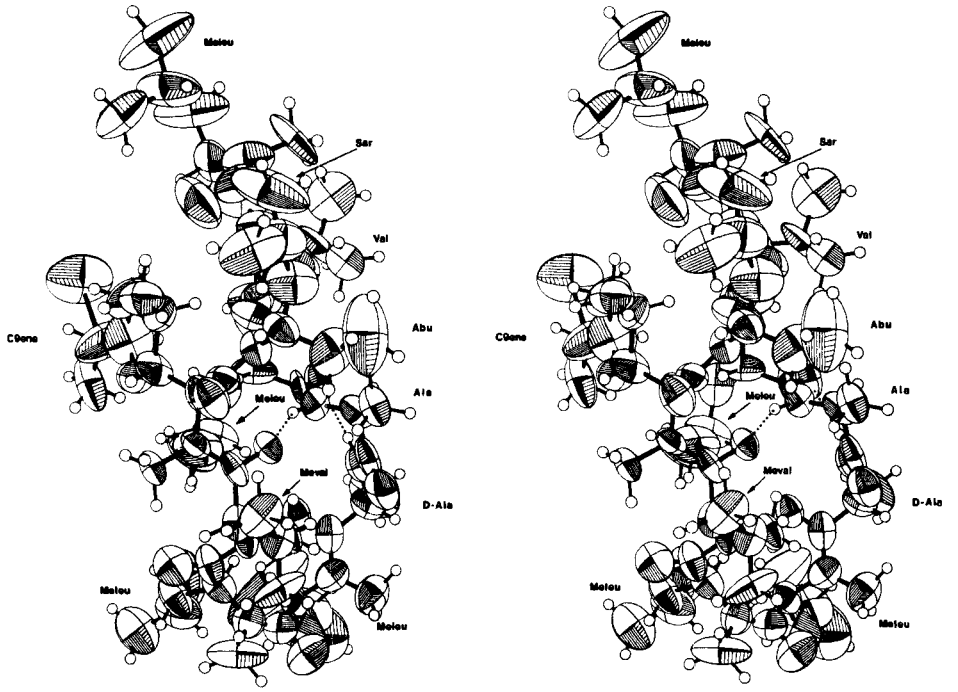

Fig. 1. A stereoscopic view of the iodocyclosporin molecule showing 50% probability ellipsoids of thermal vibration. The C_9 amino acid is at centre left on the forward side of the molecule and the conventional sequence then extends upwards at the front through α-aminobutyric acid, glycine *etc.* One of the hydrogen bonds of the β-pleated sheet is indicated (dotted) in the centre and the interesting hydrogen bond Ala-8 → MeLeu-6 appears at centre right.

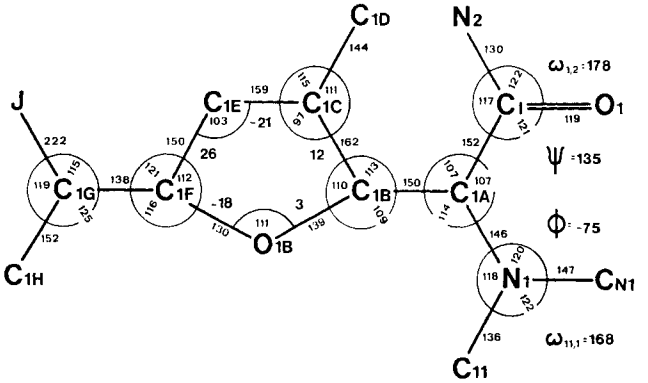

Fig. 2. *Molecular geometry* (picometers and degrees) *of the iodinated C_9 amino acid, residue 1.* Backbone torsion angles are indicated and the heavy numbers inside the five-membered ring are also torsion angles

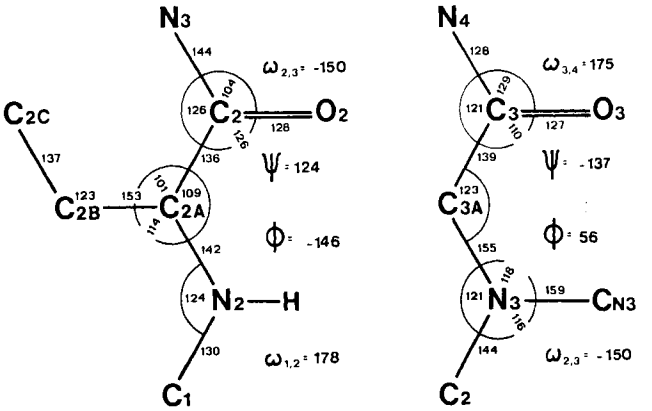

Fig. 3. *Geometry of residues 2 and 3* (α-aminobutyric acid and glycine, respectively)

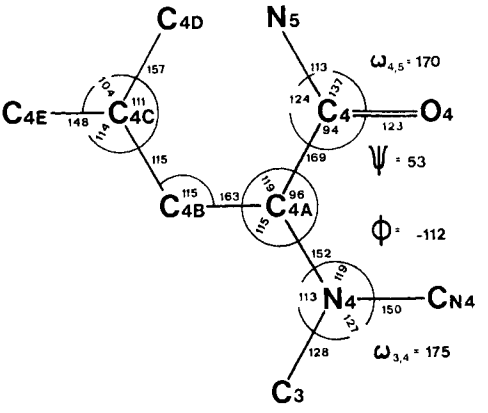

Fig. 4. *Geometry of residue 4* (Leucine)

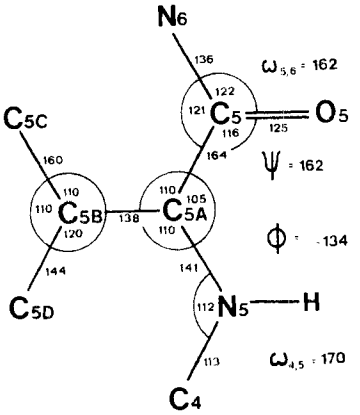

Fig. 5. *Geometry of residue 5* (Valine)

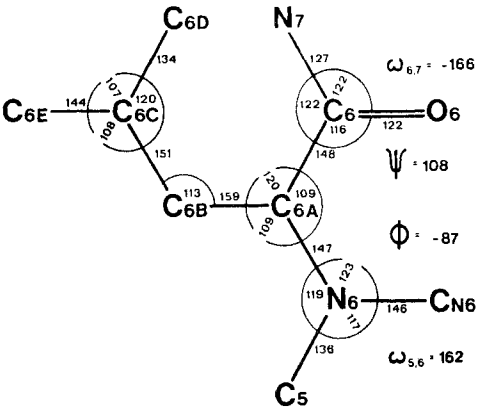

Fig. 6. *Geometry of residue 6* (Leucine)

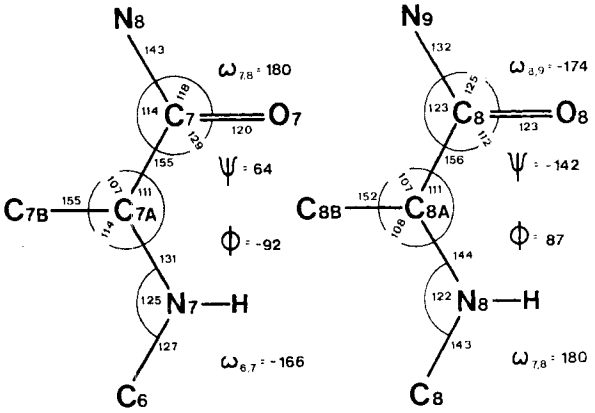

Fig. 7. *Geometry of residues 7 and 8* (L-alanine and D-alanine, respectively)

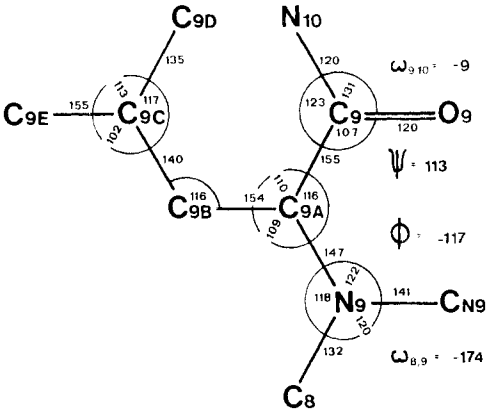

Fig. 8. *Geometry of residue 9* (Leucine)

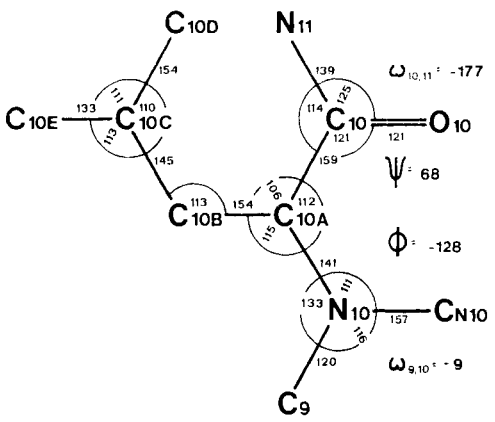

Fig. 9. *Geometry of residue 10* (Leucine)

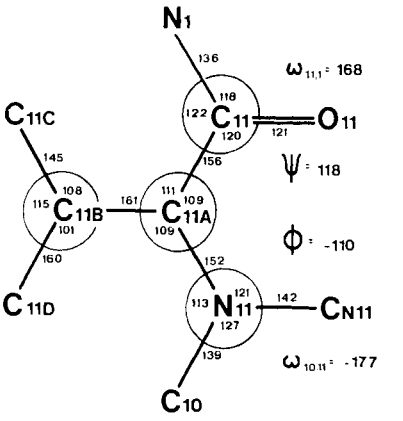

Fig. 10. *Geometry of residue 11* (Valine)

Table 2. *Collected backbone torsion angles for iodocyclosporin*

n	Residue	ϕ_n	ψ_n	$\omega_{n,n+1}$	Configuration
1	Me-C_9	– 75	135	178	L
2	α-AB	–146	124	–150	L
3	Me-Gly	56	–137	175	–
4	Me-Leu	–112	53	170	L
5	Val	–134	162	162	L
6	Me-Leu	– 87	108	–166	L
7	Ala	– 92	64	180	L
8	Ala	87	–142	–174	D
9	Me-Leu	–116	113	– 9	L
10	Me-Leu	–128	68	–177	L
11	Me-Val	–110	118	168	L

and NH of Ala-7 to C=O of MeVal-11 (N–O 303 pm). The β-turn formed by residues Sar-3 and MeLeu-4 is of the common Type I with the carboxyl oxygen atom under the plane of the molecule as drawn in (**1**).

The remaining hydrogen bond is of a type which to our knowledge has not previously been observed although predicted from theoretical considerations by *Pullman* [4]. This hydrogen bond is from NH of Ala-8 back to the C=O of MeLeu-6 (N–O 291 pm), resulting in a conformation for Ala-7 which *Pullman* has named C_7^{eq}. This hydrogen bond is visible (dotted) in the stereodiagram (Fig. 1); it is the rightmost of the two hydrogen bonds shown there. It partially stabilises the conformation of the open loop formed by residues 7–11.

In conclusion, since the purpose of the structure analysis was not only to determine the sequence of the native peptide, but also to give some insight into its shape, we have built a *Beevers* model of the native molecule in a conformation based on that observed for the iodo-derivative. Two views of this molecular model are presented in the colour plate. We suggest that the β-hydroxy group in the C_9-ene side-chain

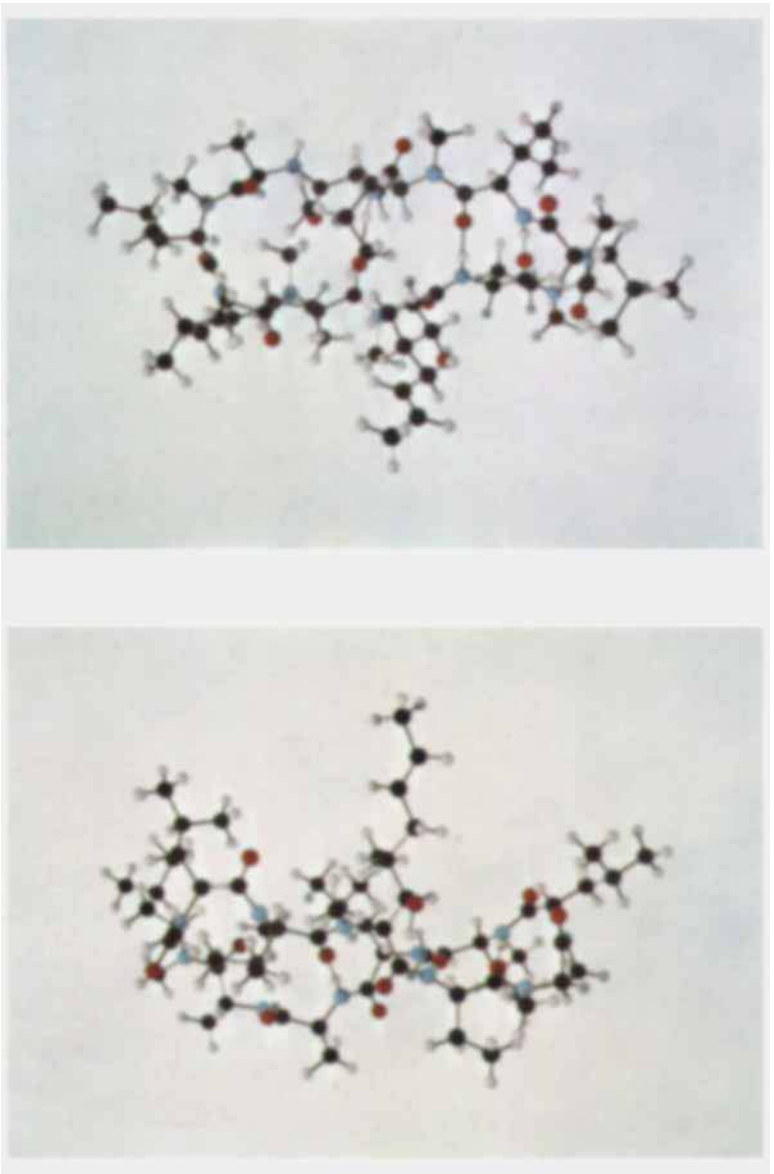

Fig. 12. *Two roughly orthogonal views of a model of native cyclosporin in the conformation we suggest as probable.* The backbone conformation corresponds to that of iodocyclosporin. We have idealized the staggered conformation of some side-chains and inserted the natural amino acid side-chain at position 1

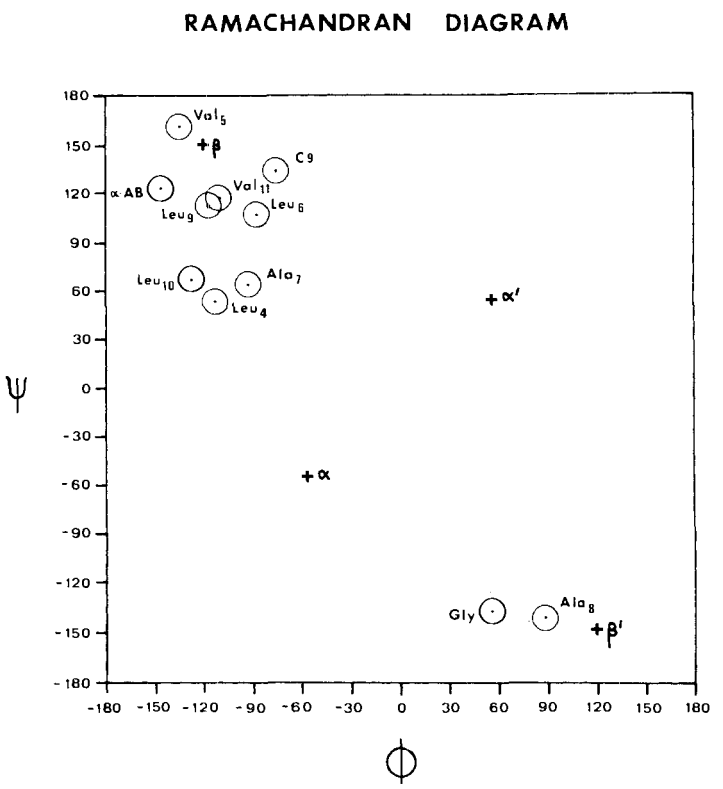

Fig. 11. Ramachandran *diagram for the backbone of iodocyclosporin.* The angles ϕ and ψ given are torsion angles and thus correspond to the 1969 IUPAC-IUB convention. ϕ, ψ pairs are marked for right-handed (α, β) and left-handed (α', β') α-helix and β-pleated sheet secondary structure

forms a hydrogen bond to the carbonyl oxygen atom of the same amino-acid. The molecule has then something of a butterfly shape, carrying an extended proboscis in the form of the C_9-ene side-chain. It is known that this unsaturated side-chain is intimately associated with the immuno-suppressive activity [5] and the way in which this aliphatic chain extends outwards from the mass of the molecule is certainly suggestive of some special function.

REFERENCES

[1] *M. Dreyfuss, E. Härri, A. Hofmann, W. Pache & H. Tscherter,* European Journ. of appl. Microbiol. *1976,* in press.

[2] *A. Rüegger, M. Kuhn, H. Lichti, H. R. Loosli, R. Huguenin, C. Quiquerez & A. von Wartburg,* Helv. *59,* 1075 (1976).

[3] *C. Chothia,* J. mol. Biol. *75,* 295–302 (1973).

[4] *B. Pullman & B. Maigret* in 'The Jerusalem Symposia on Quantum Chemistry and Biochemistry', Vol. 5, Eds. E. D. Bergmann and B. Pullman, The Israel Academy of Sciences and Humanities, Jerusalem 1973, pp. 13–39, esp. p. 17.

[5] *J. F. Borel, C. Feurer, H. U. Gubler & H. Stähelin,* Agents and Actions, *6,* Heft 4, in press (1976).