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Chemistry

Conformation of a 16-residue zervamicin IIA analog peptide containing three different structural features: 3_{10}-helix, α-helix, and β-bend ribbon

(x-ray structure analysis/hydrogen bonds/parallel packing of helices/peptaibophol antibiotics)

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ABSTRACT **Boc-Trp-Ile-Ala-Aib-Ile-Val-Aib-Leu-Aib-Pro-Ala-Aib-Pro-Aib-Pro-Phe-OMe (where Boc is *t*-butoxycarbonyl and Aib is α-aminoisobutyric acid), a synthetic apolar analog of the membrane-active fungal peptide antibiotic zervamycin IIA, crystallizes in space group *P*1 with $Z = 1$ and cell parameters $a = 9.086 \pm 0.002$ Å, $b = 10.410 \pm 0.002$ Å, $c = 28.188 \pm 0.004$ Å, $\alpha = 86.13 \pm 0.01°$, $\beta = 87.90 \pm 0.01°$, and $\gamma = 89.27 \pm 0.01°$; overall agreement factor $R = 7.3\%$ for 7180 data ($F_0 > 3\sigma$) and 0.91-Å resolution. The peptide backbone makes a continuous spiral that begins as a 3_{10}-helix at the N-terminus, changes to an α-helix for two turns, and ends in a spiral of three β-bends in a ribbon. Each of the β-bends contains a proline residue at one of the corners. The torsion angles ϕ_i range from −51° to −91° (average value −64°), and the torsion angles ψ_i range from −1° to −46° (average value −31°). There are 10 intramolecular NH···OC hydrogen bonds in the helix and two direct head-to-tail hydrogen bonds between successive molecules. Two H_2O and two CH_3OH solvent molecules fill additional space with appropriate hydrogen bonding in the head-to-tail region, and two additional H_2O molecules form hydrogen bonds with carbonyl oxygens near the curve in the helix at Pro-10. Since there is only one peptide molecule per cell in space group *P*1, the molecules repeat only by translation, and consequently the helices pack parallel to each other.**

Transmembrane pore formation by alamethicin and related membrane-modifying peptides containing α-aminoisobutyric acid (Aib) most likely involves lipid phase aggregation of helical peptide molecules, since individual 3_{10}-helices or α-helices have an inner pore much too small for the passage of ions (1). The role of specific side chains in promoting helix aggregation and the mode of aggregation are being studied by means of crystal-structure analyses of membrane-active peptides. The study began with a synthetic apolar analog of zervamicin IIA (2) in which Gln, Thr, and Hyp (Hyp is 4-hydroxyproline) have been replaced with Ala, Val, and Pro, respectively; the N-terminus has been blocked with *t*-butoxycarbonyl (Boc) rather than acetyl; and the C-terminal alcohol function has been replaced by a methyl ester. The structure of the hexadecapeptide Boc-Trp-Ile-Ala-Aib-Ile^{5}-Val-Aib-Leu-Aib-Pro^{10}-Ala-Aib-Pro-Aib-Pro^{15}-Phe-OMe is reported in this paper. The crystal structure of the decapeptide fragment composed of residues 1–10 has been published (3).

A helical conformation (for residues 9–16) containing three β-bend reversals (for Pro^{10}-Ala^{11}, Aib^{12}-Pro^{13}, and Aib^{14}-Pro^{15}; Fig. 1) has been confirmed by crystal-structure analysis. A similar approximate 3_{10}-helix containing a β-bend ribbon was proposed for the conformation of Z-(Aib-Pro)$_4$-OMe (Z, benzyloxycarbonyl) in solution (4, 5). Bent α-helices caused by steric hindrance due to the presence of one central proline residue have been observed in proteins [e.g., see glyceraldehyde-phosphate dehydrogenase residues 146–161 (6) and melittin (7)].

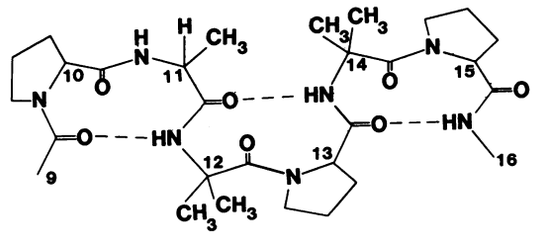

FIG. 1. Schematic representation of β-bend ribbon, with three NH···O=C hydrogen bonds, that winds into an approximate 3_{10}-helix. See Fig. 2 for conformation in the complete molecule drawn by computer using experimentally determined coordinates for the atoms. Note that the proline residues occur either at the $i + 1$ or $i + 2$ position in the β-bends.

EXPERIMENTAL PROCEDURES

The 16-residue peptide was synthesized by conventional solution-phase procedures and crystallized from CH_3OH/H_2O. The colorless crystals were fragile and crumbly. An irregular fragment, with maximum dimensions of 0.15 × 0.25 × 0.40 mm, was mounted in a thin glass capillary with some mother liquor. The profiles of the individual x-ray reflections were sharp and symmetric. The data were collected with a four-circle automated diffractometer using Cu Kα radiation and a graphite monochromator. The θ–2θ scan technique was used with a 2.0° scan, 15°/min scan rate, and $2\theta_{max} = 115°$, for a total of 8082 independent reflections and 7180 reflections with intensities $>3\sigma(F)$ to a resolution of 0.91 Å. Three reflections monitored after every 60 measurements remained constant within 4% during the data collection. Lorentz, polarization, and absorption corrections were applied to the data. The space group is *P*1 with the cell dimensions shown in the Abstract. The calculated density was 1.186 g/cm^3, based on a molecular weight of 1763.22 + 136.15 for $C_{90}H_{139}N_{17}O_{19}\cdot 4H_2O\cdot 2CH_3OH$, one formula unit per cell, and cell volume $V = 2658.5$ Å^3.

Since the structure of the peptide fragment containing the first 10 residues was already known (3), the assumption was made, which proved to be correct, that the helical backbone for residues 1–9 is quite similar in the two molecules. Residue

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Abbreviations: Aib, α-aminoisobutyric acid; Boc, *t*-butoxycarbonyl; Hyp, 4-hydroxyproline.

Table 1. Fractional coordinates and thermal factors (Å² × 10²)

Atom†	Mean value (SD × 10⁴)			U_{eq}‡	Atom†	Mean value (SD × 10⁴)			U_{eq}‡
	x	*y*	*z*			*x*	*y*	*z*	
O(OBu^t)	−0.0913 (9)	−0.4300 (7)	1.2835 (3)	9.1	Cγ(8)	−0.2618 (11)	0.4240 (10)	0.9224 (4)	7.5
C(OBu^t)-2	−0.1330 (18)	−0.5556 (12)	1.2723 (5)	12.3	Cδ1(8)	−0.3797 (14)	0.4837 (16)	0.8976 (5)	13.0
C(OBu^t)-3	−0.2863 (31)	−0.5533 (19)	1.2625 (7)	23.7	Cδ2(8)	−0.2308 (18)	0.4932 (17)	0.9659 (5)	14.7
C(OBu^t)-4	−0.1184 (22)	−0.6362 (14)	1.3187 (5)	15.2	C′(8)	0.0044 (10)	0.3393 (9)	0.8182 (3)	6.1
C(OBu^t)-5	−0.0459 (27)	−0.6069 (15)	1.2324 (6)	19.4	O(8)	0.0132 (8)	0.4348 (6)	0.7911 (2)	7.5
C′(0)	−0.0904 (11)	−0.3328 (9)	1.2518 (4)	7.3	N(9)	0.1083 (8)	0.2485 (7)	0.8217 (2)	5.7
O(0)	−0.1273 (8)	−0.3356 (7)	1.2103 (2)	8.0	Cα(9)	0.2567 (10)	0.2637 (10)	0.8013 (3)	6.2
N(1)	−0.0476 (9)	−0.2232 (8)	1.2694 (3)	7.3	Cβ1(9)	0.3397 (11)	0.1368 (12)	0.8086 (4)	8.4
Cα(1)	−0.0576 (11)	−0.1013 (9)	1.2422 (4)	6.7	Cβ2(9)	0.3356 (13)	0.3677 (12)	0.8242 (4)	9.0
Cβ(1)	−0.0196 (11)	0.0113 (9)	1.2719 (4)	7.2	C′(9)	0.2545 (10)	0.2976 (9)	0.7470 (3)	6.2
Cγ(1)	0.1300 (11)	0.0127 (9)	1.2905 (3)	7.1	O(9)	0.3491 (6)	0.3722 (6)	0.7287 (2)	6.7
Cδ1(1)	0.1700 (13)	−0.0305 (12)	1.3351 (4)	8.8	N(10)	0.1557 (8)	0.2435 (7)	0.7206 (2)	6.1
Cδ2(1)	0.2652 (12)	0.0555 (9)	1.2661 (3)	7.2	Cα(10)	0.1625 (12)	0.2792 (11)	0.6678 (3)	7.8
Cε2(1)	0.3790 (12)	0.0362 (11)	1.2974 (4)	7.9	Cβ(10)	0.0372 (15)	0.1987 (13)	0.6513 (4)	10.4
Cε3(1)	0.2958 (12)	0.1142 (9)	1.2199 (3)	7.2	Cγ(10)	0.0186 (14)	0.0901 (11)	0.6858 (4)	9.6
Cξ2(1)	0.5226 (13)	0.0676 (13)	1.2842 (4)	9.8	Cδ(10)	0.0455 (12)	0.1449 (10)	0.7327 (4)	7.6
Cξ3(1)	0.4407 (12)	0.1446 (10)	1.2076 (4)	8.2	C′(10)	0.1446 (11)	0.4185 (11)	0.6549 (3)	7.9
Cη2(1)	0.5513 (13)	0.1217 (12)	1.2403 (4)	10.0	O(10)	0.1855 (9)	0.4647 (9)	0.6151 (2)	10.4
Nε1(1)	0.3185 (11)	−0.0180 (10)	1.3388 (3)	10.0	N(11)	0.0752 (9)	0.4935 (8)	0.6859 (3)	7.3
C′(1)	0.0389 (10)	−0.0964 (9)	1.1964 (3)	6.1	Cα(11)	0.0571 (11)	0.6293 (11)	0.6755 (4)	8.3
O(1)	0.0071 (8)	−0.0196 (6)	1.1628 (2)	7.5	Cβ(11)	−0.0491 (14)	0.6877 (13)	0.7100 (5)	11.9
N(2)	0.1535 (8)	−0.1772 (7)	1.1931 (2)	5.7	C′(11)	0.2072 (11)	0.7042 (11)	0.6734 (4)	7.9
Cα(2)	0.2439 (10)	−0.1725 (9)	1.1501 (3)	6.3	O(11)	0.2222 (8)	0.8028 (7)	0.6482 (3)	8.3
Cβ(2)	0.3804 (10)	−0.2642 (10)	1.1535 (4)	7.2	N(12)	0.3085 (9)	0.6582 (8)	0.7037 (3)	6.9
Cγ1(2)	0.3401 (11)	−0.4013 (11)	1.1658 (5)	9.9	Cα(12)	0.4416 (11)	0.7325 (10)	0.7112 (3)	7.2
Cγ2(2)	0.4910 (13)	−0.2121 (12)	1.1865 (5)	9.9	Cβ1(12)	0.4000 (14)	0.8594 (13)	0.7337 (5)	10.6
Cδ1(2)	0.4505 (19)	−0.4962 (14)	1.1525 (7)	15.7	Cβ2(12)	0.5384 (12)	0.6498 (12)	0.7462 (4)	8.8
C′(2)	0.1502 (10)	−0.2025 (9)	1.1069 (3)	6.5	C′(12)	0.5292 (11)	0.7636 (10)	0.6636 (4)	7.3
O(2)	0.1855 (7)	−0.1563 (7)	1.0676 (2)	7.7	O(12)	0.5772 (9)	0.8709 (7)	0.6541 (3)	9,9
N(3)	0.0360 (8)	−0.2812 (7)	1.1157 (3)	6.3	N(13)	0.5539 (9)	0.6684 (7)	0.6333 (3)	6.3
Cα(3)	−0.0527 (11)	−0.3165 (9)	1.0774 (3)	7.0	Cα(13)	0.6416 (11)	0.7014 (10)	0.5885 (4)	7.6
Cβ(3)	−0.1704 (14)	−0.4089 (12)	1.0946 (4)	9.4	Cβ(13)	0.7140 (12)	0.5702 (12)	0.5769 (4)	9.2
C′(3)	−0.1279 (10)	−0.1924 (11)	1.0532 (4)	7.8	Cγ(13)	0.6114 (20)	0.4763 (14)	0.5996 (6)	15.0
O(3)	−0.1407 (8)	−0.1832 (8)	1.0100 (2)	8.4	Cδ(13)	0.5369 (12)	0.5310 (11)	0.6398 (4)	8.4
N(4)	−0.1743 (9)	−0.1020 (9)	1.0818 (3)	7.6	C′(13)	0.5450 (11)	0.7477 (11)	0.5466 (3)	7.5
Cα(4)	−0.2460 (11)	0.0178 (12)	1.0636 (4)	8.6	O(13)	0.6081 (9)	0.7784 (8)	0.5091 (3)	9.3
Cβ1(4)	−0.2700 (15)	0.1075 (14)	1.1039 (4)	11.6	N(14)	0.4018 (10)	0.7508 (10)	0.5542 (3)	9.0
Cβ2(4)	−0.3923 (12)	−0.0119 (15)	1.0425 (5)	12.1	Cα(14)	0.2954 (13)	0.7652 (13)	0.5142 (4)	10.1
C′(4)	−0.1436 (11)	0.0874 (11)	1.0251 (4)	7.9	Cβ1(14)	0.1419 (15)	0.7749 (18)	0.5364 (5)	14.1
O(4)	−0.1869 (8)	0.1405 (9)	0.9891 (3)	9.8	Cβ2(14)	0.3045 (19)	0.6470 (16)	0.4851 (6)	14.9
N(5)	−0.0012 (8)	0.0891 (8)	1.0369 (3)	6.9	C′(14)	0.3273 (11)	0.8914 (13)	0.4832 (4)	8.8
Cα(5)	0.1059 (10)	0.1580 (9)	1.0058 (3)	6.4	O(14)	0.3084 (9)	0.8898 (9)	0.4406 (3)	10.5
Cβ(5)	0.2484 (11)	0.1812 (10)	1.0311 (4)	7.5	N(15)	0.3729 (10)	0.9977 (9)	0.5017 (3)	8.0
Cγ1(5)	0.2209 (16)	0.2383 (14)	1.0795 (5)	11.7	Cα(15)	0.4024 (12)	1.1137 (12)	0.4695 (4)	9.2
Cγ2(5)	0.3530 (13)	0.2626 (13)	0.9984 (5)	10.5	Cβ(15)	0.4224 (15)	1.2218 (13)	0.5028 (5)	11.2
Cδ1(5)	0.1365 (20)	0.3550 (17)	1.0803 (6)	16.6	Cγ(15)	0.3519 (18)	1.1730 (15)	0.5494 (5)	12.6
C′(5)	0.1330 (10)	0.0920 (9)	0.9595 (3)	5.9	Cδ(15)	0.3800 (14)	1.0314 (14)	0.5516 (4)	10.2
O(5)	0.1549 (8)	0.1538 (6)	0.9217 (2)	7.4	C′(15)	0.5357 (13)	1.0997 (12)	0.4358 (4)	8.7
N(6)	0.1368 (8)	−0.0371 (7)	0.9626 (2)	5.7	O(15)	0.5532 (10)	1.1812 (8)	0.4021 (3)	10.8
Cα(6)	0.1585 (10)	−0.1079 (9)	0.9207 (3)	6.4	N(16)	0.6271 (10)	1.0034 (9)	0.4454 (3)	8.0
Cβ(6)	0.1716 (11)	−0.2530 (10)	0.9314 (4)	7.6	Cα(16)	0.7635 (13)	0.9825 (11)	0.4193 (4)	8.4
Cγ1(6)	0.1824 (14)	−0.3285 (11)	0.8877 (5)	10.5	Cβ(16)	0.8932 (14)	0.9752 (14)	0.4525 (4)	10.5
Cγ2(6)	0.3014 (13)	−0.2854 (11)	0.9623 (4)	8.7	Cγ(16)	0.9255 (13)	1.1045 (14)	0.4726 (4)	10.6
C′(6)	0.0325 (11)	−0.0731 (9)	0.8844 (3)	6.6	Cδ1(16)	1.0062 (16)	1.1991 (17)	0.4468 (5)	13.6
O(6)	0.0641 (8)	−0.0519 (7)	0.8423 (2)	7.7	Cδ2(16)	0.8679 (17)	1.1372 (20)	0.5163 (5)	15.7
N(7)	−0.1049 (8)	−0.0695 (8)	0.9031 (3)	6.7	Cε1(16)	1.0328 (19)	1.3176 (17)	0.4638 (8)	18.7
Cα(7)	−0.2303 (11)	−0.0376 (10)	0.8740 (4)	7.5	Cε2(16)	0.8925 (20)	1.2549 (22)	0.5340 (5)	20.6
Cβ1(7)	−0.2532 (16)	−0.1384 (12)	0.8379 (5)	11.5	Cξ(16)	0.9775 (24)	1.3424 (22)	0.5043 (9)	25.1
Cβ2(7)	−0.3642 (12)	−0.0332 (14)	0.9066 (5)	11.3	C′(16)	0.7558 (12)	0.8725 (12)	0.3904 (4)	9.1
C′(7)	−0.2152 (9)	0.0981 (9)	0.8470 (3)	6.1	O(16)	0.8600 (11)	0.8145 (10)	0.3743 (4)	15.5
O(7)	−0.2625 (8)	0.1204 (7)	0.8074 (2)	7.9	O(OMe)	0.6249 (11)	0.8445 (10)	0.3790 (4)	13.1
N(8)	−0.1522 (8)	0.1886 (7)	0.8707 (2)	5.5	C(OMe)	0.6028 (18)	0.7371 (16)	0.3476 (6)	14.8
Cα(8)	−0.1325 (10)	0.3198 (9)	0.8516 (3)	5.9	W(1)§	0.2738 (15)	0.6728 (13)	0.2846 (5)	18.8¶
Cβ(8)	−0.1213 (11)	0.4112 (10)	0.8910 (3)	6.9	W(2)§	0.1616 (49)	0.6484 (43)	0.3754 (16)	25.7¶

Table 1. (*Continues on opposite page*)

Table 1. *(Continued)*

Atom†	Mean value (SD × 10^4) x	y	z	U_{eq}‡	Atom†	Mean value (SD × 10^4) x	y	z	U_{eq}‡
W(3)§	0.6533 (11)	0.2747 (10)	0.7236 (4)	14.0¶	O(M1)‖	0.3314 (38)	0.4477 (33)	0.3503 (12)	24.5¶
W(4A)§	0.6446 (17)	0.1351 (15)	0.6393 (5)	14.5¶	C(M2)‖	0.6563 (33)	0.4829 (28)	0.4354 (10)	12.3¶
W(4B)§	0.5535 (31)	0.1511 (26)	0.6530 (10)	10.6¶	O(M2)‖	0.5433 (23)	0.4436 (20)	0.4059 (7)	13.9¶
C(M1)‖	0.3319 (36)	0.3431 (31)	0.3323 (12)	13.9¶					

†The three equivalent C atoms of the *t*-butoxy (OBu^t) moiety of the Boc blocking group are numbered 3, 4, and 5; the central atom is numbered 2. Atoms of the carbonyl moiety of Boc (residue 0) and of amino acid residues 1–16 are identified according to conventions recommended by the IUPAC–IUB Commission on Biochemical Nomenclature (10); for instance, Cε3(1) is the ε3 carbon of Trp-1.

‡$U_{eq} = \frac{1}{3}\Sigma\Sigma U_{ij} a_i^* a_j^* (\mathbf{a}_i \cdot \mathbf{a}_j)$.

§W = oxygen of water molecule.

¶Isotropic value.

‖Atoms in methanol molecules 1 and 2.

Pro-10 was not included, since the backbone in the decapeptide undergoes a helix reversal at that position. The conformation of 36 atoms from the decapeptide backbone was used as a model for the vector search procedure using the diffraction data from the hexadecapeptide in the rotation function contained in the computer program DIRDIF (8). The orientation of the fragment in the new cell was found in this manner. A translation search was not needed because the hexadecapeptide crystallizes in the triclinic space group $P1$. The remainder of the structure was derived in a number of cycles by phase extension with the use of the tangent formula (9). The close fit of the model to the structure of the unknown, where the root-mean-square deviation was 0.25 Å for the 36 atoms in common, no doubt greatly facilitated the search procedure.

Least-squares refinement with anisotropic thermal factors for all the C, N, and O atoms in the peptide molecule, isotropic thermal parameters for the atoms in the solvent molecules, and 139 H atoms in the peptide kept fixed in idealized positions was performed in block-diagonal fashion.‡ The number of variables was 1172. The final agreement factor was $R = 0.073$ for 7180 reflections measured $>3\sigma(F)$, for a resolution of 0.91 Å. Fractional coordinates for the C, N, and O atoms are listed in Table 1. Bond lengths and angles (estimated SD 0.014 Å for bonds and 0.8° for angles) do not show significant or systematic deviations from expected values.§

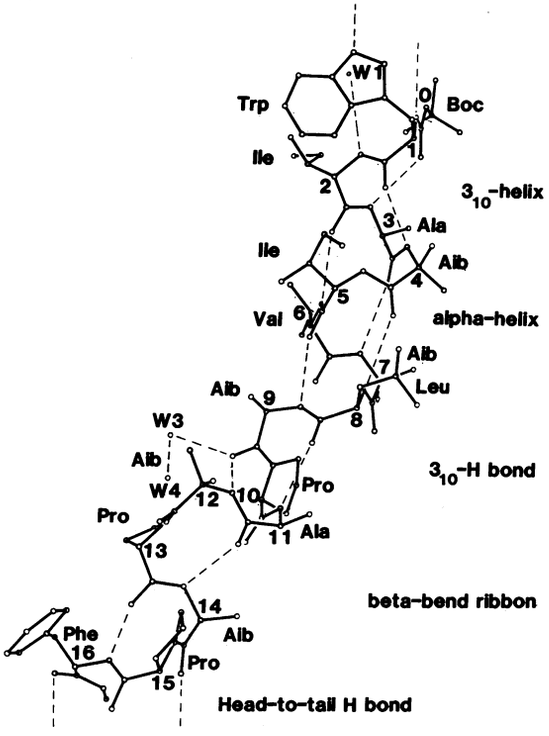

FIG. 2. View along the helix of the hexadecapeptide, one of two water molecules (W) in the head-to-tail region, and two water molecules near the bend in the helix. The Cα atoms are labeled 1–16. The number 0 is at the position of an oxygen atom in the Boc group attached to the N-terminus. Hydrogen bonds are indicated by dashed lines.

RESULTS

Conformation of Helix. The backbone of the peptide molecule winds into a continuous helix with characteristics of a 3_{10}-helix, α-helix, and β-bend ribbon. The helix is initiated by the Trp-1 residue at the N-terminus. A significant bend in the helix occurs near residue Pro-10. A view of the molecule, perpendicular to the helix, is shown in Fig. 2. The conformational angles for the backbone and side chains are listed in Table 2 and the hydrogen bonds are listed in Table 3.

The entire structure can be described as a mixed $3_{10}/\alpha$-helix with the three proline residues being incorporated into the helix with the loss of three hydrogen bonds. The distortions from idealized values reflect mainly the positioning of the proline residues. To prevent too close proximity between the $C\delta H_2$ group of proline residue i and the carbonyl of residue $i - 3$ [$CO(i - 3)$], distortions in the helix must occur to relieve these short contacts. In this structure, appreciable distortions (>14°) occur in the values of ϕ, ψ, and ω in two residues at $i - 2$ with respect to the locations of proline residues—i.e., in Leu-8 and Pro-13. Only the ω value of Ala-11 is affected. All the distortions in ω (of magnitude greater than 1–2°) are in the negative direction. The conformation determined for the C-terminal octapeptide segment (residues 9–16) may be of relevance in developing structural models for related fungal peptides, like antiamoebin, that have a similar disposition of proline/4-hydroxyproline and α-aminoisobutyric acid/isovaline residues (11).

The curved helix can be approximated by two straight segments with a bend of ≈30° in the vicinity of residues 8 and 9. A view of the molecule projected down the line passing through Cα(1) and Cα(8) shows that the backbone of the first nine residues winds around an imaginary axis perpendicular to the paper (Fig. 3). The view in Fig. 4 has been rotated with respect to Fig. 3 to show that the backbone of residues 8–15 winds around an imaginary axis in this segment of the helix.

‡Refined with program CRYLSQ by R. Olthof-Hazekamp from the XTAL System of Crystallographic Programs, Technical Report 1364.2, University of Maryland, Computer Science Center, College Park, MD 20742, 3rd Ed., March 1986.

§Supplementary material consisting of observed and calculated structure factors, anisotropic thermal factors, coordinates for H atoms, and bond lengths and bond angles is available from I.L.K.

Table 2. Torsion angles

Residue	Value,* degrees ϕ	ψ	ω	$\chi 1$	$\chi 2$
Trp-1	−62.7†	−22.2	179.9	61.4	−97.1
					81.0
Ile-2	−60.7	−30.3	−178.0	57.3	157.0
				−71.0	
Ala-3	−61.8	−37.7	180.0		
Aib-4	−55.8	−43.6	−176.0		
Ile-5	−69.0	−38.7	178.6	−49.2	−55.5
				−175.6	
Val-6	−58.9	−44.9	179.3	−60.3	
				175.5	
Aib-7	−58.2	−36.6	−178.6		
Leu-8	−82.3	−19.4	−165.8	−65.5	−66.8
					168.0
Aib-9	−57.0	−37.7	−178.8		
Pro-10	−58.8	−23.0	178.9	−25.4	37.1
Ala-11	−67.4	−34.5	−169.1		
Aib-12	−56.6	−46.2	−178.5		
Pro-13	−91.3	−1.3	−166.0	26.2	−28.1
Aib-14	−58.0	−34.0	179.4		
Pro-15	−68.4	−14.9	−171.3	−19.4	33.6
Phe-16	−108.6	24.5‡	177.3§	−68.7	−81.2
					94.6

The torsion angles for rotation about bonds of the peptide backbone (ϕ, ψ, and ω) and about bonds of the amino acid side chains (χ) are described in ref. 10. For a right-handed α-helix, idealized values of ϕ and ψ are −57° and −47°. For a right-handed 3_{10}-helix, idealized values of ϕ and ψ are −60° and −30°.
*Estimated SD ≈ 0.9°.
†C′(0), N(1), Cα(1), C′(1).
‡N(16), Cα(16), C′(16), O(OMe).
§Cα(16), C′(16), O(Me), C(OMe).

Table 3. Hydrogen bonds

Type	Donor (D)	Acceptor (A)	Length, Å	Angle, degrees (C=O···D)
Intramolecular				
3_{10}-Helix (4→1)	N(3)	O(0)	3.027	133
	N(4)	O(1)	3.040	121
α-Helix (5→1)	N(6)	O(2)	3.175	157
	N(7)	O(3)	3.170	159
	N(8)	O(4)	3.346	148
	N(9)	O(5)	2.965	158
3_{10}-Helix	N(11)	O(8)	3.021	137
β-Bend ribbon*	N(12)	O(9)	3.034	126
	N(14)	O(11)	3.137	111
	N(16)	O(13)	2.856	137
Intermolecular				
Head-to-tail	Nε1(1)	O(14)	2.963	158
region	N(1)	O(16)	3.095	130
	N(2)	W(1)	3.147	
	W(2)	O(16)	3.222	157
	W(1)	W(2)	2.718	
	O(M2)	O(15)	2.741	127
	O(M1)	O(M2)	2.524	
	W(2)	O(M1)	2.699	
Interhelical	W(3)	O(7)	2.887	157
(Pro region)	W(3)	O(9)	2.935	117
	W(4A) or W(4B)†	W(3)	2.871	
			2.635	
	W(4A) or W(4B)†	O(12)	2.826	170
			2.920	152

The N(5)–O(2) and N(9)–O(6) separations are 3.139 Å and 3.170 Å, respectively; however, the geometries are unfavorable for 4→1 hydrogen bonds. Atoms N(10), N(13), and N(15) occur in proline residues and cannot participate in hydrogen bonding. Atom O(10) does not participate in any hydrogen bonding.
*In the β-bend ribbon, two of the three hydrogen bonds have ϕ and ψ values closer to 3_{10}-helix type than to type I β-bend.
†Atoms W(4A) and W(4B) are mutually exclusive. Each one occupies at random only one-half of the unit cells.

Residue 16 does not continue the helix, as shown in Fig. 4, but is folded back underneath residue 10.

Side Chains. The aromatic rings in Trp-1 and Phe-16 contribute to a compact structure by folding against the helix rather than extending outward. In general, the smaller side chains, in residues Ala-3, Aib-4, Aib-7, Aib-11, and Aib-14, occur on the convex side of the helix (Figs. 2 and 3), whereas the bulky side chains, in residues Trp-1, Ile-2, Ile-5, Val-6, and Phe-16, occur on the concave side. The side chains in Ile-2 and Ile-5 have different conformations. The pyrrolidine ring in Pro-13 has Cγ flipped in the opposite direction to that found in Pro-10 and Pro-15, as shown by the reversal of the signs of χ values in Table 2.

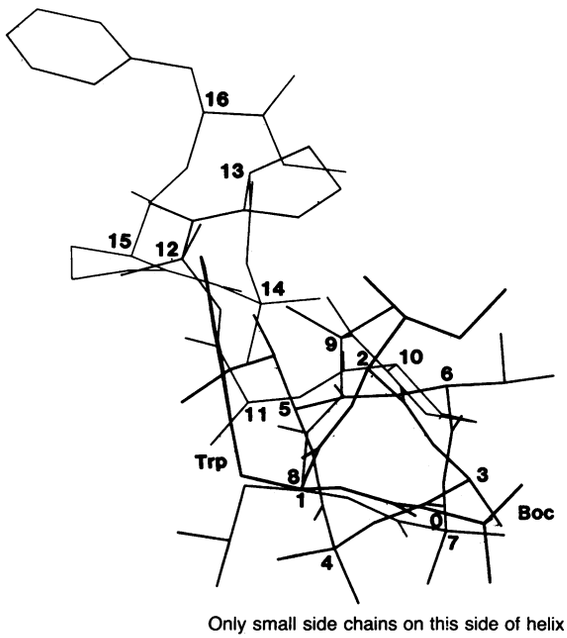

FIG. 3. View down the upper part of the helix of the hexadecapeptide. The Cα atoms are labeled 1–16.

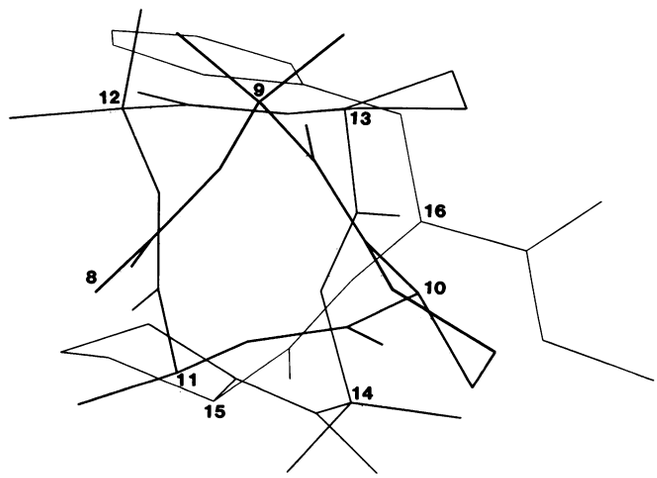

FIG. 4. View down the lower part of the helix. Only residues 8–16 are shown.

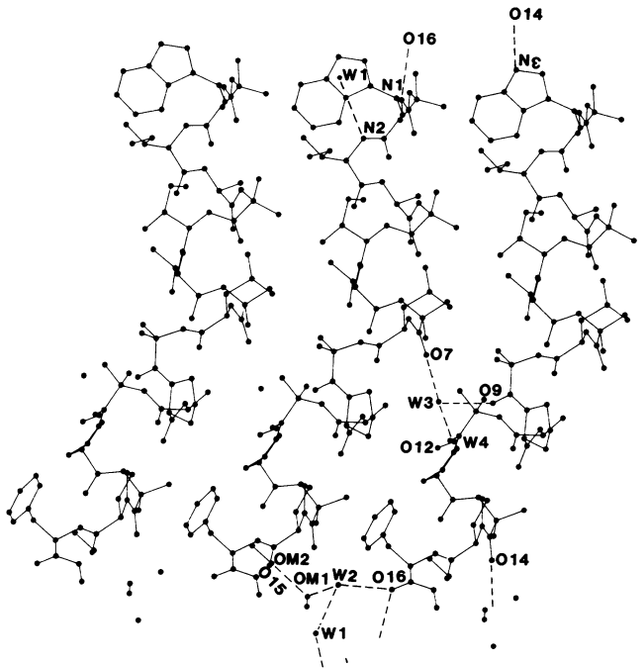

FIG. 5. Packing of molecules in three adjacent cells viewed down the *b* axis. Some of the intermolecular hydrogen bonds are indicated by dashed lines.

Packing of Parallel Helices. *Intermolecular hydrogen bonding.* In the direction of the long axis of the cell, the helical molecules are connected by head-to-tail hydrogen bonding. At the top of the molecule there are three NH groups, N(1)H, N(2)H, and the Nε1(1)H in the Trp-1 side chain, with the N—H bonds directed upward. At the bottom of the molecule, carbonyl groups CO(14) and CO(15) have the C=O bond directed downward, and CO(16) is directed to the side. Two direct hydrogen bonds are formed between peptide molecules: Nε1(1)H···O(14) and N(1)H···O(16). Indirect head-to-tail bonds via water molecules are formed between N(2) and O(16): N(2)H···W(1)···W(2)···O(16). In the head-to-tail region there also are voids that are occupied by two CH_3OH molecules that form hydrogen bonds to each other, W(2), and O(15) (see Table 3).

The bend in the helix near Pro-10 causes the carbonyl oxygens O(7), O(9), O(10), and O(12) to be exposed to the exterior. Except for O(9), these atoms do not participate in any intramolecular hydrogen bonding but are available for bonding to adjacent molecules. Two additional water molecules cocrystallize in the vicinity of these atoms: W(3) and the disordered W(4A) and W(4B) distributed between two adjacent sites at one-half occupancies. These water molecules serve to bond adjacent peptide molecules sideways and front-to-back (in the view shown in Figs. 2 and 5) by forming bridging hydrogen bonds: CO(7)···W(3)···CO(9) and W(3)··· W(4A) or W(4B)···CO(12). Carbonyl oxygen O(10) does not participate in any hydrogen bonding. Under different packing conditions O(10) could be involved in interhelix hydrogen bonding.

Aggregation of helices. Since the peptide crystallizes with one molecule in the triclinic cell, the only relationship between molecules is one of translation. The helices must pack in a parallel fashion. As is shown in Fig. 5, the curved helices nestle within each other quite efficiently. The nearest approach between side chains in neighboring molecules is 3.64 Å between Cδ1(8) and Cβ2(9). There are many interhelical C–C approaches less than 3.9 Å. The α-helices in the crystal of the decapeptide (3) having the same sequence as the first 10 residues of the present peptide also pack in a parallel fashion.

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1. Mathew, M. K. & Balaram, P. (1983) *FEBS Lett.* **157,** 1–5.
2. Rinehart, K. L., Gaudiso, L. A., Moore, M. L., Pandey, R. C., Cook, J. C., Barber, M., Sedgwick, R. D., Bordoli, R. S., Tyler, A. N. & Green, B. N. (1981) *J. Am. Chem. Soc.* **103,** 6517–6520.
3. Karle, I. L., Sukumar, M. & Balaram, P. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 9284–9288.
4. Venkatachalapathi, Y. V. & Balaram, P. (1981) *Biopolymers* **20,** 1137–1145.
5. Prasad, B. V. V. & Balaram, P. (1982) *Int. J. Biol. Macromol.* **4,** 99–102.
6. Richardson, J. S. (1981) in *Advances in Protein Chemistry*, eds. Anfinson, C. B., Anson, M. L., Edsall, J. T. & Richards, F. M. (Academic, New York), pp. 169–339.
7. Terwilliger, T. C. & Eisenberg, D. (1981) *J. Biol. Chem.* **257,** 6016–6022.
8. Beurskens, P. T., Bosman, W. P., Doesburg, H. M., van den Hark, Th., Prick, P. A. J., Noordik, J. H., Beurskens, G., Gould, R. O. & Parthasarathi, V. (1982) in *Conformation in Biology*, eds. Srinivasan, R. & Sarma, R. H. (Adenine, New York), pp. 389–406.
9. Karle, J. & Hauptman, H. (1956) *Acta Crystallogr.* **9,** 635–651.
10. IUPAC–IUB Commission on Biochemical Nomenclature (1970) *Eur. J. Biochem.* **17,** 193–201.
11. Das, M. K., Raghothama, S. & Balaram, P. (1986) *Biochemistry* **25,** 7110–7177.