

globular domains, two water molecules interrupt hydrogen bonds of the α -helix and form hydrogen bonds to carbonyl oxygens and NH moieties of the backbone in residues 39, 40, 43, and 44. The manner in which the helix is solvated is similar to that found in the apolar peptide despite sequences with quite different properties. In the peptide the hydrophobic sequence Ala-Leu-Aib-Ala is involved, whereas in the protein, the hydrophilic sequence Thr-Lys-Gly-Leu-Gly-Thr is involved.

EXPERIMENTAL

Boc-Ala-Leu-Aib-Ala-Leu-Aib-OMe was synthesized by conventional solution phase procedures. Crystals with quite different external forms were grown from the anhydrous solvent mixture ethyl acetate/petroleum ether and from the polar solvent mixture methanol/water. Nevertheless, each crystal form yielded the same cell parameters and the same structure. The parameters reported in this paper are the result of the least-squares refinement of the x-ray data measured from the crystal grown from the nonpolar solvent mixture. The needle-shaped crystal, 0.70 mm long and 0.13×0.18 mm in cross section, was mounted dry, i.e., not in a capillary with mother liquor. X-ray diffraction data were collected on an automated diffractometer using $\text{CuK}\alpha$ radiation and a graphite monochromator ($\lambda = 1.54178 \text{ \AA}$). The θ - 2θ scan was used with a scan of $2.0^\circ + 2\theta(\alpha_1) - 2\theta(\alpha_2)$, a variable scan rate of 3 – 10° per minute, and $2\theta_{\text{max}} = 120^\circ$, for a total of 3385 unique reflections and 2707 reflections with intensities $> 3\sigma(F)$. Three reflections (402, 060, and 018) that were monitored after every 60 measurements remained constant within 4% during the data collection. Lorentz and polarization corrections were applied to the data. The cell parameters are $a = 12.024(4) \text{ \AA}$, $b = 15.714(6) \text{ \AA}$, $c = 21.411(7) \text{ \AA}$, $V = 4045.4 \text{ \AA}^3$, and $Z = 4$ for space group $P2_12_12_1$. The calculated density is 1.124 g/cm^3 based on a molecular weight of 684.88 for $\text{C}_{32}\text{H}_{58}\text{N}_6\text{O}_9 \cdot \text{H}_2\text{O}$.

The structure was solved using the random tangent option based on the tangent formula⁵ in the SHELX84 package of programs (Micro VAX version of SHELXTL system of programs; Nicolet Analytical Instruments, Madison, WI). Forty atoms were chosen from the initial E-map for input into the partial structure procedure,⁶ which yielded all the nonhydrogen atoms. The six amide H atoms and one H atom of the water molecule were located in difference maps after some preliminary least-squares refinement. The location of the second H atom of the water molecule has not been established. Least-squares refinement of the coordinates of C, N, and O atoms with anisotropic thermal parameters, and of the six amide H atoms and one water H atom with isotropic thermal parameters, resulted in an R factor equal to 6.3% for 2707 reflections measured $\geq 3\sigma(F)$. The 52 H atoms bonded to C atoms were placed in idealized positions and were allowed to ride with the C atoms to which they were bonded during the final cycles of least-squares refinement. The coordinates of these H atoms were not refined independently as were those of the six amide H atoms.

Fractional coordinates for the refined atoms are listed in Table I, bond lengths and angles are shown in Tables II and III, and torsional angles are shown in Table IV.

TABLE I
Atom Coordinates ($\times 10^4$) and Temperature Factors ($\text{\AA}^2 \times 10^3$)

Atom	<i>x</i>	<i>y</i>	<i>z</i>	U_{eq}^a
Ob	2972(3)	6387(2)	1104(2)	70(1)
C(1b)	3958(6)	6012(4)	779(3)	68(2)
C(2b)	4933(5)	6615(4)	776(3)	94(3)
C(3b)	4199(7)	5244(4)	1180(3)	119(4)
C(4b)	3630(6)	5762(5)	131(3)	98(3)
C'(0)	2507(5)	7105(3)	889(2)	50(2)
O(0)	2844(3)	7573(3)	496(2)	76(2)
N(1)	1558(4)	7265(3)	1228(2)	48(1)
C ^α (1)	873(4)	7975(3)	1054(2)	49(2)
C'(1)	1336(4)	8847(3)	1229(2)	47(2)
O(1)	977(3)	9491(2)	979(2)	68(1)
C ^β (1)	-299(4)	7881(4)	1343(3)	70(2)
N(2)	2163(3)	8869(2)	1653(2)	42(1)
C ^α (2)	2687(4)	9659(3)	1851(2)	42(2)
C'(2)	3711(4)	9894(3)	1452(2)	41(2)
O(2)	4369(3)	10433(2)	1644(2)	57(1)
C ^β (2)	2982(4)	9653(3)	2537(2)	45(2)
C ^γ (2)	2003(4)	9485(4)	2984(2)	61(2)
C ^δ (21)	2393(5)	9478(4)	3662(2)	84(3)
C ^δ (22)	1052(5)	10088(4)	2889(3)	102(3)
N(3)	3812(3)	9517(3)	901(2)	44(1)
C ^α (3)	4810(4)	9621(3)	497(2)	48(2)
C'(3)	5099(5)	10555(3)	403(2)	48(2)
O(3)	6084(3)	10789(2)	381(2)	65(2)
C ^β (31)	4541(5)	9227(4)	-128(2)	70(2)
C ^β (32)	5786(5)	9157(4)	805(3)	76(3)
N(4)	4266(4)	11110(3)	304(2)	47(1)
C ^α (4)	4502(5)	12007(3)	181(2)	56(2)
C'(4)	5112(4)	12473(3)	700(2)	47(2)
O(4)	5564(3)	13152(2)	597(2)	65(1)
C ^β (4)	3405(6)	12476(5)	38(3)	90(3)
N(5)	5045(3)	12114(2)	1269(2)	39(1)
C ^α (5)	5504(4)	12511(3)	1828(2)	37(1)
C'(5)	6675(4)	12190(3)	1985(2)	37(2)
O(5)	7154(3)	12479(2)	2446(2)	50(1)
C ^β (5)	4748(4)	12349(3)	2383(2)	50(2)
C ^γ (5)	3538(5)	12561(4)	2303(3)	83(3)
C ^δ (51)	2913(5)	12411(6)	2917(3)	126(4)
C ^δ (52)	3374(6)	13412(4)	2098(4)	145(5)
N(6)	7116(3)	11593(2)	1623(2)	44(1)
C ^α (6)	8114(4)	11122(3)	1813(2)	52(2)
C'(6)	9036(5)	11746(4)	1987(3)	55(2)
O(6)	9591(3)	11693(3)	2457(2)	79(2)
C ^β (61)	7840(5)	10552(4)	2373(3)	77(2)
C ^β (62)	8518(5)	10599(4)	1248(3)	70(2)
Om	9243(3)	12303(3)	1539(2)	69(1)
Cm	10203(5)	12830(5)	1632(3)	104(3)
W(1)	1830(5)	10902(4)	333(4)	133(3)
HN(1)	1377(40)	6899(30)	1552(23)	73(16)
HN(2)	2431(31)	8420(22)	1857(16)	29(11)
HN(3)	3553(45)	8911(36)	766(25)	100
HN(4)	3728(47)	11009(36)	341(26)	100
HN(5)	4714(36)	11563(26)	1319(20)	52(13)
HN(6)	6840(45)	11422(34)	1306(25)	100
HW(1)	1580(64)	10496(49)	285(38)	100

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

TABLE II
Bond Lengths^a (Å)

Bond	Boc(0)	Ala(1)	Leu(2)	Aib(3)	Ala(4)	Leu(5)	Aib(6)
N(<i>i</i>)-C ^α (<i>i</i>)		1.437	1.455	1.489	1.461	1.459	1.468
C ^α (<i>i</i>)-C'(<i>i</i>)		1.526	1.544	1.520	1.519	1.533	1.526
C'(i)-O(<i>i</i>)	1.188	1.224	1.230	1.241	1.218	1.230	1.211
C'(i)-N(<i>i</i> + 1)	1.376	1.348	1.324	1.345	1.346	1.327	1.323 ^b
C ^α (<i>i</i>)-C ^β (<i>i</i>)		1.546	1.510	1.510	1.542	1.518	1.532
				1.531			1.541
C ^β (<i>i</i>)-C ^γ (<i>i</i>)			1.539			1.503	
C ^γ (<i>i</i>)-C ^δ (<i>i</i>)			1.527			1.532	
			1.499			1.420 ^c	

^aEstimated standards deviations (ESDs) for backbone atoms ~ 0.007 Å; for side chains ~ 0.010 Å.

^bC'(6)-O(OMe).

^cThermal factor has a large component; therefore the value of the bond length has a large error.

TABLE III
Bond Angles^a (deg)

Angle	Boc(0)	Ala(1)	Leu(2)	Aib(3)	Ala(4)	Leu(5)	Aib(6)
C'(i - 1)N(i)C ^α (i)		118.7	122.6	122.8	120.7	122.8	121.4
N(i)C ^α (i)C'(i)		115.1	112.8	111.6	115.2	112.7	109.8
C ^α (i)C'(i)N(i + 1)	108.6 ^b	117.3	117.4	118.5	115.6	118.1	112.6 ^c
C ^α (i)C'(i)O(i)	128.4 ^d	120.4	119.4	120.6	120.4	118.9	124.1
N(i + 1)C'(i)O(i)	122.9	122.2	123.1	120.8	123.9	122.9	123.0 ^e
C'(i)C ^α (i)C ^β (i)		108.7	110.7	109.1	109.2	108.9	109.8
				110.0			107.8
N(i)C ^α (i)C ^β (i)		110.1	112.3	107.3	109.3	110.1	109.6
				108.4			108.0
C ^α (i)C ^β (i)C ^γ (i)			115.2			116.9	
C ^β (i)C ^γ (i)C ^δ (i)			108.3			110.1	
			105.0			112.2	

^aESDs for backbone atoms ~ 0.4°-0.5°.

^bObC'(0)N(1).

^cC^α(6)C'(6)O(OMe).

^dObC'(0)O(0).

^eO(6)C'(6)O(OMe).

RESULTS

Conformation of Molecule

A diagram of the molecule, drawn by computer using the experimentally determined coordinates, is shown in Fig. 1. A distorted 3_{10} -helix is formed containing three $4 \rightarrow 1$ hydrogen bonds, N(3) \cdots O(0), N(5) \cdots O(2), and N(6) \cdots O(3) (see Table V). The fourth $4 \rightarrow 1$ hydrogen bond that should be formed between N(4) and O(1) for a more perfect helix cannot exist since a water molecule W(1) has been inserted between N(4) and O(1). As a result, the N(4) \cdots O(1) distance has been enlarged to 4.92 Å, hydrogen bonds N(4) \cdots W(1) and W(1) \cdots O(1) have been formed (see Table V), and the helix

TABLE IV
Torsion Angles^a (deg)

Angle	Ala(1)	Leu(2)	Aib(3)	Ala(4)	Leu(5)	Aib(6)
$\phi(N-C^\alpha)$	-74.4 ^b	-90.9	-51.1	-60.9	-95.3	-53.1
$\psi(C^\alpha-C')$	-16.2	17.9	-41.4	-20.7	-1.8	-53.9 ^c
$\omega(C'-N)$	178.8	-173.9	-177.1	-175.0	-166.6	-171.4 ^d
$\chi^1(C^\alpha-C^\beta)$		-56.8			-51.4	
$\chi^2(C^\beta-C^\gamma)$		179.1			-176.6	
		-54.8			-53.8	

^aThe torsion angles for rotation about bonds of the peptide backbone (ϕ , ψ , ω) and about bonds of the amino acid side chains (χ) are described in Ref. 7. For a right-handed α -helix, ideal values of ϕ and ψ are -65° and -41° (Ref. 8). For a right-handed 3_{10} -helix, ideal values of ϕ and ψ are -60° and -30° . ESDs $\sim 0.7^\circ$.

^bC'(0), N(1), C $^\alpha$ (1), C'(1).

^cN(6), C $^\alpha$ (6), C'(6), O(OMe).

^dC $^\alpha$ (6), C'(6), O(OMe), C(OMe).

axis has been bent in the vicinity of C $^\alpha$ (2) mostly by rotational changes at C $^\alpha$ (2) where $\phi = -91^\circ$ and $\psi = 18^\circ$. The water molecule W(1) forms a third hydrogen bond to carbonyl O(4) from a neighboring molecule.

The portion of the molecule containing the solvation of the helix in Boc-(Ala-Leu-Aib)₂-OMe and in Boc-Aib-(Ala-Leu-Aib)₃-OMe³ is entirely similar in the two molecules. The conformations of the two molecules as deter-

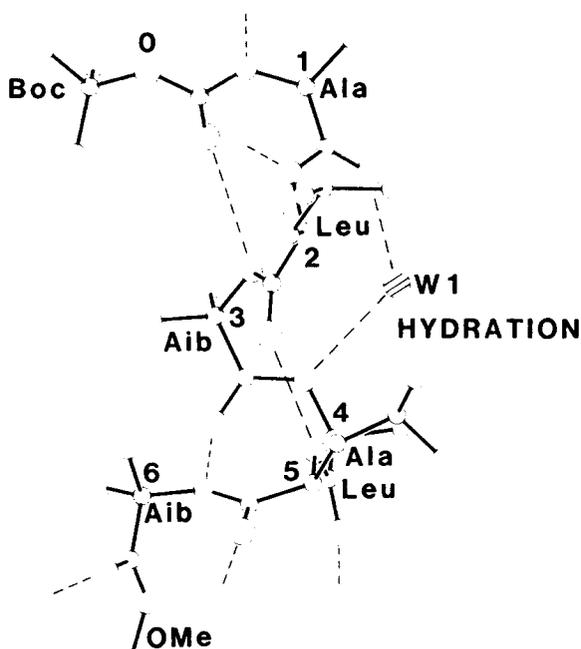


Fig. 1. A view of Boc-(Ala-Leu-Aib)₂-OMe drawn along the approximate helical axis. Water W(1) is inserted into the helical backbone. The C $^\alpha$ atoms are labeled 1-6. The number 0 is at the position of an O atom in the Boc group attached to the amino terminus. Intra- and intermolecular hydrogen bonds are indicated by dashed lines.

TABLE V
 Hydrogen Bonds

Type	Donor	Acceptor	Length (Å)	Angle (deg)	
				C = O ··· N	H ··· O (Å)
Intramolecular					
4 → 1	N(3)	O(0)	3.383	120	2.34
	N(5)	O(2)	2.878	136	1.95
	N(6)	O(3)	3.194	117	2.40
Insertion	N(4)	W(1)	2.949		2.29
	W(1)	O(1)	2.807		2.29
Intermolecular	N(1)	O(6) ^a	3.262	118	2.44
	N(2)	O(5) ^a	3.027	155	2.16
	W(1)	O(4) ^b	2.915		

^aSymmetry equivalent atoms obtained from O(5) and O(6) by $1 - x, -1/2 + y, 1/2 - z$.

^bSymmetry equivalent atom obtained from O(4) by $-1/2 + x, 5/2 - y, -z$.

mined by crystal structure analyses are superimposed in Fig. 2, where the molecules have been oriented with respect to each other by a least-squares fit of the four residues in the vicinity of the water molecule, i.e., the Ala-Leu-Aib-Ala segment. The rms fit of the 23 atoms in this segment in each of the two molecules is 0.23 Å. The backbones and the side chains adopt the same conformation near the water molecule. In each peptide, even the hydrogen-bond lengths to the water molecule are very similar.³ For the N ··· W(1) bond, the lengths are 2.95 and 2.93 Å, respectively, for the hexapeptide and the decapeptide; for the W(1) ··· O bond, the lengths are 2.81 and 2.86 Å.

The N(3) ··· O(0) distance of 3.383 Å exceeds the values usually observed for hydrogen bonds in 3_{10} -helices. The proximity of the helix distortion at C^α(2) by the insertion of W(1) may be responsible for enlarging the N(3) ··· O(0) separation. However, the equivalent hydrogen-bond distance N(4) ··· O(1) in Boc-Aib-(Ala-Leu-Aib)₃-OMe³ is only 3.065 Å (Fig. 2). Since the geometry about the N(3) and O(1) atoms in the present molecule is appropriate for a 4 → 1 hydrogen bond, and since there is no other possibility for hydrogen-bond formation with these atoms, a weak hydrogen bond is assumed. Another deviation from the norm for peptide residues is the nonplanarity of the C'(5)-N(6) amide bond where $\omega_5 = 166.6^\circ$ instead of the more usually observed values of 174–180°. No such distortion is observed in the decapeptide. The leucyl side chains in the present molecule have $\chi^1 \approx -60^\circ$ and $\chi^2 \approx 180^\circ$ and -60° , i.e., g^+ , tg^+ , the conformation normally found in peptides.⁹

The bulky leucyl side chains of Boc-(Ala-Leu-Aib)₂-OMe are on the left side of the molecule, as drawn in Fig. 2, while the water molecule with the attendant hydrogen bonds to C=O and NH moieties of the backbone is on the right side. Thus, amphiphilic character is imparted to the hexapeptide helix in a manner similar to the decapeptide helix.³ However, the aggregation of the molecules in the crystal is quite different in each case. Head-to-tail hydrogen bonding of helices with the formation of infinite helical columns, as has been found in apolar peptides with 10–16 residues,^{1–3} does not occur in the hexapeptide (Fig. 3). In this crystal, lateral hydrogen bonds are formed

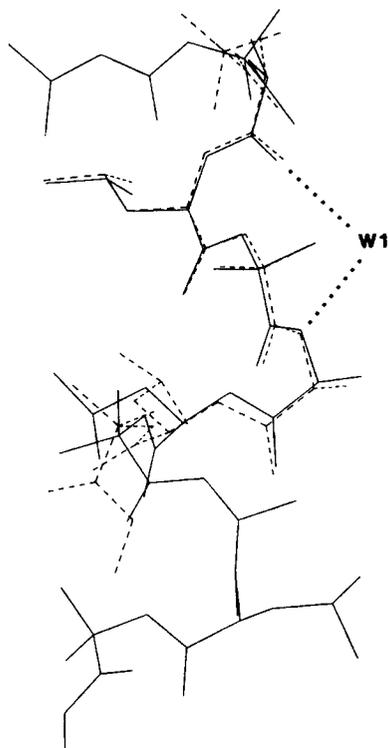


Fig. 2. Superposition of molecules Boc-Aib-(Ala-Leu-Aib)₃-OMe³ (solid lines) and Boc-(Ala-Leu-Aib)₂-OMe (dashed lines). Residues Ala-Leu-Aib-Ala, in the vicinity of water molecule W(1), have almost identical conformations in the two peptides.

INTERMOLECULAR HYDROGEN BONDING

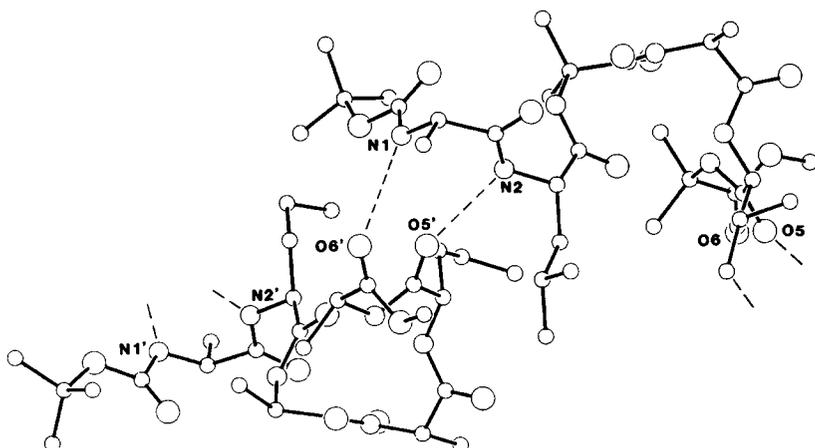


Fig. 3. Intermolecular NH \cdots O=C bonds between adjacent peptide molecules related by a twofold screw parallel to the *b* axis (horizontal direction).

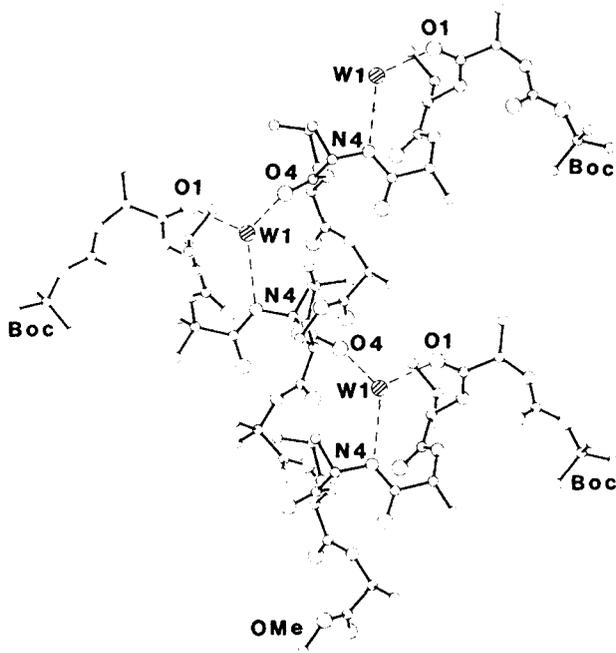


Fig. 4. Packing of three molecules of Boc-(Ala-Leu-Aib)₂-OMe · H₂O that are related by a twofold screw parallel to the α axis (vertical direction). Hydrogen bonds to the water molecules are indicated by dashed lines.

between molecules related by a twofold screw symmetry. Furthermore, the packing of the hexapeptide molecules around the water molecule, (Fig. 4) does not create polar channels such as occur in the crystal of the decapeptide containing the same sequence repetitions.³

DISCUSSION

The insertion of a water molecule into a helical backbone of an apolar peptide has been demonstrated for two peptide molecules, one a hexapeptide and another a decapeptide, with repetitions of the same sequence. The sequence Ala-Leu-Aib-Ala captures a water molecule by hydrogen bonding with the C=O of the first Ala residue and the NH of the second Ala residue. However, the presence of the sequence Ala-Leu-Aib-Ala is not sufficient to guarantee an attraction of a water molecule, since the second such sequence in Boc-Aib-(Ala-Leu-Aib)₃-OMe forms an almost ideal α -helix without any invasion by water molecules. Other apolar sequences, such as Boc-Aib-Val-Ala-Leu-Aib-Val-Ala-Leu-Aib-OMe¹⁰ and Boc-Aib-Val-Aib-Aib-Val-Val-Val-Aib-Val-Aib-OMe,¹¹ as well as Boc-Trp-Ile-Ala-Aib-Ile-Val-Aib-Leu-Aib-Pro-OMe¹ form helices with predominantly α -type hydrogen bonds, although some 3_{10} -type hydrogen bonds may be present at either terminus. If water is cocrystallized in crystals of these latter peptides, it is found only in the head-to-tail hydrogen-bonding region and not in the body of the helix.

The solvation of the helix is not obviously related to the nature of the solvent. Structures of crystals of the present peptide are identical whether the

crystals were grown from the polar mixture methanol/water or the nonpolar mixture ethyl acetate/petroleum ether.

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Supplementary material consisting of coordinates for hydrogen atoms in idealized positions and anisotropic thermal parameters have been deposited with the Cambridge Structural Database or may be obtained from I. L. Karle.

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