Molecular Structure of *t*-Butyloxycarbonyl-Leu-Aib-Pro-Val-Aib-Methyl Ester, a Fragment of Alamethicin and Suzukacillin: A 3_{10}-Helical Pentapeptide

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Synopsis

The pentapeptide Boc-Leu-Aib-Pro-Val-Aib-OMe, a fragment of alamethicin and suzukacillin, crystallizes in the space group $P2_1$, with a = 11.034 (2), b = 10.894 (2), c = 15.483 (2) Å, β = 104.80 (2)° and Z = 2. The crystal structure has been solved by direct methods and refined to an R value of 0.069. The peptide backbone folds into a right-handed 3_{10}-helical conformation, stabilized by two intramolecular 4 → 1 hydrogen bonds between the Leu(1) CO and Val(4) NH and Aib(2) CO and Aib(5) NH groups. The solid-state conformation is consistent with results of spectroscopic analysis in solution.

INTRODUCTION

Alamethicin[1–3] and suzukacillin[4] (Fig. 1) are membrane-channel-forming polypeptides, containing a high proportion of α-aminoisobutyric acid (Aib). Structural investigations of Aib model peptides[3,5–7] and fragments of the channel-forming ionophores in solution[8–10] and in the solid state[7,11] have suggested that 3_{10}-helical conformations are favored in Aib-containing peptides. A recent structural study of a model 11-residue Aib-containing peptide, Boc-(Ala-Aib)$_2$-Ala-Glu(OBz)-(Ala-Aib)$_2$-Ala-OMe, has established an α-helical conformation for 9 of the 11 residues.[12] While 3_{10}-helical structures have been suggested for considerable lengths of the alamethicin and suzukacillin sequences on the basis of ^{1}H-nmr studies,[8–10] the possibility of conformational flexibility involving the -Gly-Leu-Aib-Pro-Val-Aib- segment (residues 11–16 in alamethicin and 15–20 in suzukacillin) has been considered.[5,8] ^{1}H-nmr and ir studies of the model peptide Boc-Leu-Aib-Pro-Val-Aib-OMe have established the presence of two intramolecular hydrogen bonds involving the Val(4) and Aib(5) NH groups.[5,8] However, the identity of the participating CO groups has not been conclusively established. The presence of Pro at the strategic central position then results in three possible structures having two intramolecular hydrogen bonds, schematically illustrated in Fig. 2. A distinction between these structures is not possible on the basis of ^{1}H-nmr studies. IR results have tentatively favored the mixed structure [Fig. 2(c)].[5] It was therefore desirable that

Biopolymers, Vol. 21, 2461–2472 (1982)

CCC 0006-3525/82/122461-12$02.20

(a) Ac–Aib–Pro–Aib–Ala–Aib–Ala–Gln–Aib–Val–Aib–Gly–Leu–Aib
Pro–Val–Aib–Aib–Glu–Gln–Phol

(b) Ac–Aib–Pro–Val–Aib–Val–Ala–Aib–Ala–Aib–Aib–Gln–Aib–Leu
Aib–Gly–Leu–Aib–Pro–Val–Aib–Aib–Glu–Gln–Phol

Fig. 1. Sequences of alamethicin I (a) and suzukacillin A (b).

the solid-state structure of this fragment be unambiguously established. In this report we describe the crystal structure of Boc-Leu-Aib-Pro-Val-Aib-OMe. The peptide adopts a 3_{10}-helical conformation, stabilized by two 4 → 1 intramolecular hydrogen bonds.

MATERIALS AND METHODS

Boc-Leu-Aib-Pro-Val-Aib-OMe was synthesized from Boc-Leu-Aib-OH and HN-Pro-Val-Aib-OMe using dicyclohexylcarbodiimide-mediated coupling in dichloromethane. Detailed procedures have been reported elsewhere.[13] Single crystals were obtained by slow evaporation of a methanol solution. The peptide crystallized in the space group $P2_1$ with $a = 11.034\ (2)$, $b = 10.894\ (2)$, $c = 15.483\ (2)$ Å, $\beta = 104.80\ (2)°$, and $Z = 2$. Intensities of 2758 reflections were measured on a CAD-4 diffractometer using ω-2θ scan, with CuK_α ($\lambda = 1.5418$ Å) radiation up to a θ limit of 65°. All reflections were used throughout the structure determination and re-

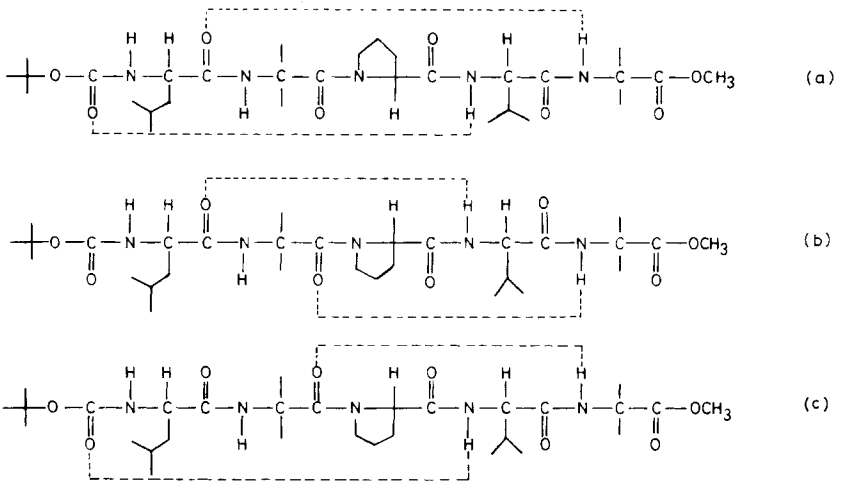


Fig. 2. Hydrogen-bonding schemes in Boc-Leu-Aib-Pro-Val-Aib-OMe, compatible with ^{1}H-nmr studies: (a) two 5 → 1 (13-atom) hydrogen bonds; (b) two 4 → 1 (10-atom) hydrogen bonds; (c) one 5 → 1 and one 4 → 1 hydrogen bond.

TABLE I

Fractional Coordinates ($\times 10^4$) and Anisotropic Temperature Factors[a] ($\times 10^4$) for Nonhydrogen Atoms[b]

Atom	x	y	z	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
C_{B1}	1234(7)	4238(8)	10013(5)	158(8)	171(10)	85(5)	−20(8)	63(5)	−45(6)
C_{B2}	−94(10)	4294(10)	9993(9)	176(10)	195(12)	151(7)	−31(10)	95(7)	−59(9)
C_{B3}	1837(9)	5495(11)	10175(7)	270(14)	277(17)	146(8)	−145(14)	130(9)	−143(10)
C_{B4}	1899(10)	3263(14)	10681(7)	233(13)	318(22)	86(6)	−11(14)	33(7)	−34(9)
C_1	958(6)	2907(6)	8698(4)	131(6)	79(6)	69(4)	1(5)	41(4)	2(4)
C_2^{α}	774(5)	1869(5)	7268(4)	129(6)	65(5)	61(3)	−23(5)	30(4)	−1(3)
C_2^{β}	23(6)	2320(7)	6356(4)	142(7)	124(8)	56(3)	8(6)	16(4)	2(4)
C_2^{γ}	−1214(6)	2958(8)	6367(6)	141(8)	138(9)	92(5)	18(8)	23(5)	3(6)
$C_2^{\delta 1}$	−2123(8)	2138(11)	6636(9)	163(10)	209(14)	159(10)	7(10)	65(8)	−8(10)
$C_2^{\delta 2}$	−1839(9)	3472(14)	5422(7)	211(11)	279(9)	121(7)	65(13)	7(8)	42(11)
C_2	1835(6)	1046(6)	7193(4)	140(7)	67(5)	49(3)	−7(5)	11(4)	−3(3)
C_3^{α}	4106(6)	876(5)	7293(4)	141(7)	57(5)	66(4)	−5(5)	26(4)	−3(3)
$C_3^{\beta 1}$	5227(7)	1680(7)	7707(5)	153(8)	95(7)	85(4)	−24(6)	40(5)	−5(5)
$C_3^{\beta 2}$	4078(8)	569(7)	6316(5)	204(10)	111(8)	76(4)	9(8)	44(5)	−10(5)
C_3	4239(6)	−313(5)	7834(4)	129(7)	62(5)	71(4)	−14(5)	12(4)	−6(4)
C_4^{α}	4280(7)	−1474(7)	9168(5)	189(9)	90(7)	73(4)	−8(7)	−18(5)	14(5)
C_4^{β}	4045(16)	−1151(10)	10045(6)	679(38)	115(10)	68(5)	37(17)	73(11)	14(6)
C_4^{γ}	3438(7)	12(9)	9982(5)	167(9)	182(11)	80(4)	−12(9)	42(5)	18(6)
C_4^{δ}	3523(9)	592(7)	9154(5)	267(12)	105(7)	53(2)	−2(9)	32(5)	−8(4)
C_5	3571(6)	−2615(6)	8767(4)	114(6)	88(6)	69(4)	14(5)	33(4)	19(4)
C_5^{α}	1929(7)	−3588(7)	7631(5)	149(8)	113(7)	83(4)	−32(7)	60(5)	−25(5)

(*continued*)

TABLE I (*continued*)

Atom	x	y	z	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
C_5^{β}	723(7)	−3198(12)	6901(9)	100(8)	290(19)	204(11)	−40(10)	31(7)	−150(13)
$C_5^{\gamma 1}$	−227(8)	−2736(18)	7442(8)	142(9)	453(33)	136(8)	32(15)	53(7)	−52(14)
$C_5^{\gamma 2}$	176(14)	−3874(25)	6185(10)	370(24)	499(40)	132(10)	104(28)	8(13)	−37(19)
C_5	2853(6)	−4328(6)	7231(5)	154(8)	80(6)	74(4)	−23(6)	46(4)	−7(4)
C_6^{α}	4634(6)	−4263(6)	6556(4)	172(8)	67(5)	75(4)	−3(5)	65(5)	−0(4)
$C_6^{\beta 1}$	5648(7)	−4730(8)	7327(5)	182(9)	121(8)	84(5)	8(8)	40(5)	4(5)
$C_6^{\beta 2}$	5132(7)	−3279(7)	6014(5)	174(9)	111(7)	98(5)	−10(7)	79(6)	−0(5)
C_6	4087(7)	−5291(6)	5914(5)	190(9)	67(6)	74(4)	2(6)	58(5)	3(4)
C_M	2397(9)	−5962(9)	4727(6)	240(12)	135(10)	107(6)	−27(9)	46(7)	−40(7)
O_1^1	466(5)	2090(4)	9011(3)	221(6)	100(5)	83(3)	−44(5)	73(4)	1(3)
O_1^2	1400(4)	3954(4)	9127(3)	184(6)	106(5)	77(3)	−38(4)	64(3)	−31(3)
O_2	1648(4)	−52(4)	7031(3)	169(5)	56(4)	82(3)	−16(4)	9(3)	−7(3)
O_3	4690(4)	−1222(4)	7557(3)	159(5)	63(4)	101(3)	7(4)	44(3)	−9(3)
O_4	3963(4)	−3611(4)	9062(4)	169(6)	86(5)	105(3)	21(4)	25(4)	41(3)
O_5	2732(5)	−5454(4)	7150(3)	215(6)	68(4)	92(3)	−36(4)	75(4)	−12(3)
O_6	4603(5)	−6240(5)	5879(4)	252(7)	96(5)	101(3)	36(6)	55(4)	−8(4)
O_M	3006(5)	−5003(4)	5349(3)	211(7)	80(4)	82(3)	4(5)	40(4)	−6(3)
N_2	1160(5)	2893(5)	7884(3)	153(6)	70(4)	57(3)	−24(4)	40(3)	−6(3)
N_3	2967(4)	1554(4)	7317(3)	121(5)	63(4)	66(3)	−12(4)	17(3)	−4(3)
N_4	3962(5)	−357(4)	8636(3)	156(6)	68(5)	54(3)	3(4)	2(3)	−3(3)
N_5	2538(4)	−2504(5)	8087(3)	113(5)	75(4)	64(3)	3(4)	27(3)	−3(3)
N_6	3633(5)	−3689(5)	6887(4)	167(6)	68(5)	90(4)	−20(5)	75(4)	−7(3)

[a] $T = \exp[-(\beta_{11}h^2 + \beta_{22}k^2 + \beta_{33}l^2 + 2\beta_{12}hk + 2\beta_{13}hl + 2\beta_{23}kl)]$.

[b] Standard deviations are given in parentheses.

TABLE II
Fractional Coordinates ($\times 10^3$) and Isotropic Temperature Factors of Hydrogen Atoms[a]

Atom	x	y	z	B (Å^2)
H^1_{B2}	−41(6)	342(10)	1001(5)	11.0(2.4)
H^2_{B2}	−7(7)	475(10)	1060(6)	11.5(2.6)
H^3_{B2}	−41(7)	474(9)	944(6)	8.2(2.3)
H^1_{B3}	135(9)	603(11)	1046(7)	17.0(3.0)
H^2_{B3}	263(5)	556(7)	1006(4)	6.9(1.5)
H^3_{B3}	180(8)	552(10)	1098(6)	14.6(2.8)
H^1_{B4}	129(9)	247(12)	1047(7)	15.7(3.2)
H^2_{B4}	279(6)	332(8)	1039(5)	12.3(2.0)
H^3_{B4}	218(8)	385(11)	1141(6)	15.4(2.7)
H^α_2	32(5)	133(6)	759(3)	5.3(1.2)
$H^{\beta 1}_2$	−7(6)	151(7)	599(4)	6.0(1.6)
$H^{\beta 2}_2$	73(5)	286(7)	618(4)	4.6(1.5)
H^1_2	382(6)	59(8)	1060(5)	7.7(2.0)
$H^{\delta 11}_2$	−178(8)	173(10)	712(6)	13.3(2.5)
$H^{\delta 12}_2$	−227(7)	125(9)	640(5)	6.8(2.1)
$H^{\delta 13}_2$	−292(7)	233(10)	655(6)	11.0(2.7)
$H^{\delta 21}_2$	−259(8)	−272(12)	500(6)	12.9(2.8)
$H^{\delta 22}_2$	−285(9)	382(12)	539(7)	11.1(2.9)
$H^{\delta 23}_2$	−125(7)	422(9)	532(5)	11.8(2.3)
$H^{\beta 11}_3$	535(5)	178(7)	842(4)	6.3(1.5)
$H^{\beta 12}_3$	602(5)	119(7)	778(4)	7.6(1.5)
$H^{\beta 13}_3$	517(6)	239(8)	737(5)	4.4(1.7)
$H^{\beta 21}_3$	341(6)	4(8)	587(4)	6.3(1.7)
$H^{\beta 22}_3$	393(7)	131(8)	589(5)	8.1(1.9)
$H^{\beta 23}_3$	483(5)	16(7)	629(4)	6.9(1.4)

(*continued*)

finement except for the last cycle, where 2546 reflections with $I > 3\sigma(I)$ were used. Intensities were corrected for Lorentz and polarization factors but not for absorption.

Structure Determination and Refinement

The structure was solved using the direct methods program, MULTAN.[14] An E map with the highest figure of merit revealed a 21-atom fragment. Successive Karle recycling[15] allowed the location of 39 out of 41 nonhydrogen atoms. The structure was refined isotropically using a block diagonal least-squares method to an R value of 0.216. The two remaining atoms (Leu C^δ) were located on a difference Fourier map. Further refinement using anisotropic temperature factors yielded an R value of 0.097. All 53 hydrogen atoms were fixed at this stage using stereochemical considerations as described.[6] Further refinement, with anisotropic temperature factors for nonhydrogen atoms, isotropic factors for hydrogens, and a σ-weighting scheme, converged at an R value of 0.069. The shifts in the parameters in the final refinement were less than $\frac{1}{5}$ of the estimated

TABLE II (*continued*)

Atom	x	y	z	B (Å^2)
H_4^{α}	555(7)	−131(11)	943(6)	13.3(2.5)
$H_4^{\beta 1}$	491(10)	−119(17)	1103(8)	18.8(4.0)
$H_4^{\beta 2}$	374(9)	−186(11)	1038(7)	8.7(3.1)
$H_4^{\gamma 1}$	−104(6)	373(9)	672(5)	9.2(2.1)
$H_4^{\gamma 2}$	301(6)	51(8)	1036(5)	10.4(1.8)
$H_4^{\delta 1}$	373(6)	137(8)	922(4)	7.5(1.8)
$H_4^{\delta 2}$	248(6)	91(7)	885(4)	8.9(1.7)
H_5^{α}	184(5)	−409(6)	814(4)	4.7(1.5)
H_5^{β}	84(10)	−267(14)	633(7)	8.3(3.2)
$H_5^{\gamma 11}$	−11(8)	−334(10)	797(6)	9.7(2.4)
$H_5^{\gamma 12}$	−98(8)	−324(11)	742(6)	13.0(2.9)
$H_5^{\gamma 13}$	−63(8)	−203(11)	758(6)	9.2(2.8)
$H_5^{\gamma 21}$	−77(7)	−422(10)	633(6)	11.2(2.1)
$H_5^{\gamma 22}$	−50(6)	−375(8)	680(7)	9.2(2.2)
$H_5^{\gamma 23}$	72(8)	−467(9)	614(8)	11.0(2.2)
$H_6^{\beta 11}$	599(6)	−385(8)	783(5)	8.6(1.8)
$H_6^{\beta 12}$	520(6)	−535(7)	764(4)	6.5(1.8)
$H_6^{\beta 13}$	652(6)	−489(9)	717(5)	12.5(1.9)
$H_6^{\beta 21}$	419(5)	−293(6)	547(4)	6.8(1.5)
$H_6^{\beta 22}$	544(6)	−253(8)	642(5)	8.9(1.8)
$H_6^{\beta 23}$	580(6)	−358(7)	574(4)	7.1(1.6)
H_M^1	131(6)	−579(8)	439(5)	10.0(1.9)
H_M^2	280(8)	−588(11)	420(6)	11.1(2.7)
H_M^3	217(6)	−660(8)	507(5)	11.9(2.0)
H_{N2}	161(6)	356(7)	767(4)	4.9(1.7)
H_{N3}	310(5)	230(7)	751(4)	6.1(1.5)
H_{N5}	222(5)	−182(6)	783(4)	4.7(1.3)
H_{N6}	374(5)	−291(7)	702(4)	5.6(1.5)

[a] Estimated standard deviations are given in parentheses.

standard deviations. The scattering factors used were those of Cromer and Waber[16] for nonhydrogen atoms and of Stewart et al.[17] for hydrogen atoms. The atomic and thermal parameters with their standard deviations are given in Tables I and II. The bond lengths and bond angles are listed in Tables III and IV. A listing of the observed and calculated structure factors is available on request.

RESULTS AND DISCUSSION

Peptide Backbone Conformation

A perspective view of the molecule is shown in Fig. 3(a). The pentapeptide folds into a right-handed helical conformation, stabilized by two intramolecular 4 → 1 hydrogen bonds between the Val(4) NH and Leu(1) CO ($N_5 \cdots O_2$, 3.154 Å; $H\hat{N}_5O_2$, 7.1°) and Aib(5) NH and Aib(2) CO ($N_6 \cdots O_3$, 3.007 Å; $H\hat{N}_6O_3$, 15°) groups. The conformational angles[18] for the peptide backbone and side chains are listed in Table V. All the peptide bonds in the molecule are nearly planar, with the largest deviation ($\Delta\omega$ =

TABLE III
Bond Lengths (Å) Involving Nonhydrogen Atoms[a]

Atoms	Bond Length	Atoms	Bond Length
C_{B1}-C_{B2}	1.459(14)	C_3^{α}-C_3	1.529(9)
C_{B1}-C_{B3}	1.515(15)	C_3-O_3	1.233(7)
C_{B1}-C_{B4}	1.533(15)	C_3-N_4	1.353(8)
C_{B1}-O_1^2	1.463(9)	N_4-C_4^{δ}	1.465(9)
O_1^2-C_1	1.347(8)	C_4^{δ}-C_4^{γ}	1.454(11)
C_1-O_1^1	1.208(8)	C_4^{γ}-C_4^{β}	1.425(16)
C_1-N_2	1.334(8)	C_4^{β}-C_4^{α}	1.488(13)
N_2-C_2^{α}	1.458(9)	N_4-C_4^{α}	1.460(9)
C_2^{α}-C_2^{β}	1.524(9)	C_4^{α}-C_4	1.514(10)
C_2^{β}-C_2^{γ}	1.536(10)	C_4-O_4	1.213(8)
C_2^{γ}-$C_2^{\delta 1}$	1.480(14)	C_4-N_5	1.346(8)
C_2^{γ}-$C_2^{\delta 2}$	1.553(14)	N_5-C_5^{α}	1.450(9)
C_2^{α}-C_2	1.503(9)	C_5^{α}-C_5^{β}	1.567(14)
C_2-O_2	1.228(7)	C_5^{β}-$C_5^{\gamma 1}$	1.583(16)
C_2-N_3	1.335(8)	C_5^{β}-$C_5^{\gamma 2}$	1.339(23)
N_3-C_3^{α}	1.466(8)	C_5^{α}-C_5	1.548(10)
C_3^{α}-$C_3^{\beta 1}$	1.517(10)	C_5-O_5	1.236(8)
C_3^{α}-$C_3^{\beta 2}$	1.542(10)		
C_5-N_6	1.320(9)	C_6^{α}-C_6	1.517(9)
N_6-C_6^{α}	1.470(9)	C_6-O_6	1.188(9)
C_6^{α}-$C_6^{\beta 1}$	1.501(10)	C_6-O_M	1.323(9)
C_6^{α}-$C_6^{\beta 2}$	1.547(10)	O_M-C_M	1.463(11)

[a] Standard deviations given in parentheses.

8.5°) being observed for the Aib(2)-Pro(3) bond. The ϕ,ψ values for the Aib(2), Pro(3), and Val(4) residues are reasonably compatible with right-handed 3_{10}- or α-helical conformations (α-helix $\phi \sim -55°$, $\psi \sim -45°$; 3_{10}-helix $\phi \sim -60°$, $\psi \sim -30°$).[19] The two helical structures can be readily distinguished on the basis of their intramolecular hydrogen-bonding patterns, viz., $4 \rightarrow 1$ for a 3_{10}-helix[20] and $5 \rightarrow 1$ for an α-helix.[21] Thus, the peptide backbone in Boc-Leu-Aib-Pro-Val-Aib-OMe describes a 3_{10}-helical structure, generated by two consecutive type III β-turns[22] having Aib(2)-Pro(3) and Pro(3)-Val(4) as the corner residues. The helical folding of the peptide is clearly illustrated in Fig. 3(b). A projection of the backbone atoms down the helix axis is shown in Fig. 3(c), demonstrating the approximately threefold nature of the helix. The C_2-C_5 and C_3-C_6 distances are 6.08 and 5.77 Å, respectively, yielding a pitch of ~6 Å for the peptide helix. The O_1^1---N_5 and O_2---N_6 distances are 5.83 and 4.56 Å, conclusively eliminating the possibility of intramolecular $5 \rightarrow 1$ hydrogen bonds.

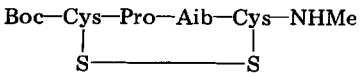
The 3_{10}-helical or consecutive type III β-turn conformation of the pentapeptide provides further evidence for the strong tendency of Aib residues to promote helical conformations. While the structures of Z-Aib-Pro-Aib-Ala-OMe,[7] Tosyl-$(Aib)_5$-OMe,[23] Z-Aib-Pro-NHMe,[6] Z-Aib-Aib-Ala-OMe,[24] Boc-Pro-Aib-Ala-Aib-OBz,[11] Z-$(Aib)_4$-O*t*Bu,[25] Z-$(Aib)_5$-O*t*Bu[25]

TABLE IV
Valence Angles (deg) Involving Nonhydrogen Atoms[a]

Atoms	Valence Angle	Atoms	Valence Angle
C_{B2}-C_{B1}-C_{B3}	111.2(8)	$C_2^{\delta 1}$-C_2^{γ}-$C_2^{\delta 2}$	108.3(8)
C_{B2}-C_{B1}-C_{B4}	110.4(8)	C_2^{β}-C_2^{α}-C_2	111.8(5)
C_{B2}-C_{B1}-O_1^2	110.8(7)	C_2^{α}-C_2-O_2	120.2(5)
C_{B3}-C_{B1}-C_{B4}	113.6(8)	C_2^{α}-C_2-N_3	117.4(5)
C_{B3}-C_{B1}-O_1^2	101.1(7)	O_2-C_2-N_3	122.4(6)
C_{B4}-C_{B1}-O_1^2	109.5(7)	C_2-N_3-C_3^{α}	124.3(5)
C_{B1}-O_1^2-C_1	121.7(5)	N_3-C_3^{α}-$C_3^{\beta 1}$	108.0(5)
O_1^2-C_1-O_1^1	124.7(6)	N_3-C_3^{α}-$C_3^{\beta 2}$	109.4(5)
O_1^2-C_1-N_2	110.4(5)	$C_3^{\beta 1}$-C_3^{α}-$C_3^{\beta 2}$	110.5(6)
O_1^1-C_1-N_2	124.8(6)	N_3-C_3^{α}-C_3	112.1(5)
C_1-N_2-C_2^{α}	122.4(5)	$C_3^{\beta 1}$-C_3^{α}-C_3	107.7(5)
N_2-C_2^{α}-C_2	113.8(5)	$C_3^{\beta 2}$-C_3^{α}-C_3	109.2(5)
N_2-C_2^{α}-C_2^{β}	111.0(5)	C_3^{α}-C_3-O_3	118.7(6)
C_2^{α}-C_2^{β}-C_2^{γ}	114.3(6)	C_3^{α}-C_3-N_4	121.3(5)
C_2^{β}-C_2^{γ}-$C_2^{\delta 1}$	113.4(7)	O_3-C_3-N_4	119.7(6)
C_2^{β}-C_2^{γ}-$C_2^{\delta 2}$	109.6(7)	C_3-N_4-C_4^{δ}	131.4(6)
C_3-N_4-C_4^{α}	118.0(5)	C_5^{β}-C_5^{α}-C_5	112.1(7)
C_4^{δ}-N_4-C_4^{α}	110.2(5)	N_5-C_5^{α}-C_5	110.4(6)
N_4-C_4^{δ}-C_4^{γ}	106.3(6)	C_5^{α}-C_5-O_5	119.4(6)
C_4^{δ}-C_4^{γ}-C_4^{β}	108.1(8)	C_5^{α}-C_5-N_6	116.8(6)
C_4^{γ}-C_4^{β}-C_4^{α}	109.7(9)	O_5-C_5-N_6	123.2(6)
C_4^{β}-C_4^{α}-N_4	104.2(7)	C_5-N_6-C_6^{α}	122.7(6)
C_4^{β}-C_4^{α}-C_4	112.1(7)	N_6-C_6^{α}-$C_6^{\beta 1}$	110.0(6)
N_4-C_4^{α}-C_4	115.4(6)	N_6-C_6^{α}-$C_6^{\beta 2}$	107.3(5)
C_4^{α}-C_4-O_4	118.8(6)	$C_6^{\beta 1}$-C_6^{α}-$C_6^{\beta 2}$	111.7(6)
C_4^{α}-C_4-N_5	119.5(6)	$C_6^{\beta 1}$-C_6^{α}-C_6	111.2(6)
O_4-C_4-N_5	121.6(6)	$C_6^{\beta 2}$-C_6^{α}-C_6	107.0(6)
C_4-N_5-C_5^{α}	120.2(5)	N_6-C_6^{α}-C_6	109.5(5)
N_5-C_5^{α}-C_5^{β}	109.4(7)	C_6^{α}-C_6-O_6	123.9(6)
C_5^{α}-C_5^{β}-$C_5^{\gamma 1}$	105.0(9)	C_6^{α}-C_6-O_M	113.5(6)
C_5^{α}-C_5^{β}-$C_5^{\gamma 2}$	124.6(12)	O_6-C_6-O_M	112.5(7)
$C_5^{\gamma 1}$-C_5^{β}-$C_5^{\gamma 2}$	113.2(13)	C_6-O_M-C_M	116.8(6)

[a] Standard deviations given in parentheses.

and



(Ref. 26) yield type III β-turns stabilized by 4 → 1 hydrogen bonds, recent structure determinations of Boc-(Ala-Aib)$_2$-Glu(OBz)-(Ala-Aib)$_2$-Ala-OMe[12] and Boc-Aib-Pro-Val-Aib-Val-OMe·½H_2O (A. K. Francis, M. Iqbal, P. Balaram, and M. Vijayan, unpublished) have provided evidence for helical folding accompanied by formation of 5 → 1 hydrogen bonds. Early theoretical analysis[27] correctly predicted that Aib residues are conformationally restricted to adopt helical conformations. Recent theoretical studies on the conformational preferences of Aib oligopeptides appear to rationalize the preference for 3_{10}-helical structures.[28] For poly(Aib),

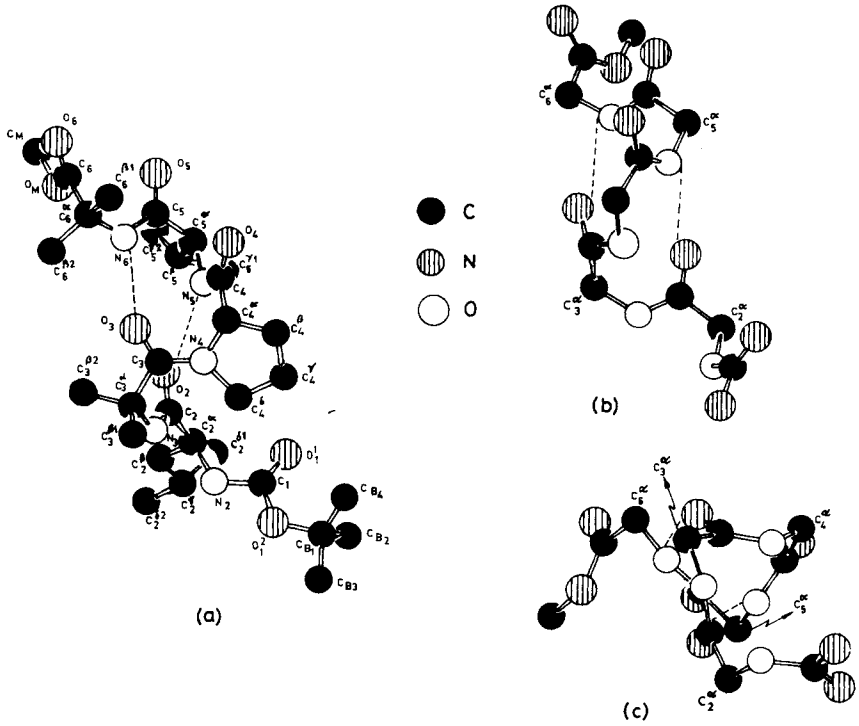


Fig. 3. (a) Perspective view of the molecular structure of Boc-Leu-Aib-Pro-Val-Aib-OMe. (b) View of peptide backbone to illustrate 3_{10}-helical folding. (c) Projection of backbone atoms viewed down the helix axis.

electron diffraction data support a 3_{10}-helical model,[29] while theoretical calculations favor α-helical[30] or fourfold helical[31] structures. It thus appears that the choice between $4 \rightarrow 1$ (3_{10}) and $5 \rightarrow 1$ (α) hydrogen-bonded conformations may be controlled by a number of factors—like peptide chain length, sequence, and presence of solvents of crystallization—whose precise role needs to be established. It should be noted that only relatively small changes in ϕ,ψ values are necessary to interconvert 3_{10}- and α-helical structures.[19]

Bond Lengths, Angles, and Side-Chain Conformation

The bond lengths and valence angles listed in Tables III and IV are largely unexceptional. Bond angles in the Aib(2)-Pro(3) segment deviate from normal values. The angle C_3^{α}-C_3-N_4 (121.3°) is large compared to average values in proline peptides (114° or 115.5°),[32,33] while the angle O_3-C_3-N_4 (119.7°) is smaller than the average reported values (125° or 123.2°).[32,33] The angles C_3-N_4-C_4^{δ} (131.4°) and C_3^{α}-C_3-N_4 (121.3°) are also larger than the reported values of 126.3° and 118°, respectively. These distortions presumably relieve unfavorable steric interactions between the Pro $C^{\delta}H_2$ and Aib $C^{\beta}H_3$ groups. Similar features have been noted in the

TABLE V
Backbone and Side-Chain Torsional Angles[a]

Dihedral Angle Backbone	(deg)	Dihedral Angle Side Chain	(deg)
$\omega_1(O_1^2\text{-}C_1\text{-}N_2\text{-}C_2^\alpha)$	−179.8	$\chi_2^1(N_2\text{-}C_2^\alpha\text{-}C_2^\beta\text{-}C_2^\gamma)$	−62.9
$\phi_2(C_1\text{-}N_2\text{-}C_2^\alpha\text{-}C_2)$	−103.9	$\chi_2^{2,1}(C_2^\alpha\text{-}C_2^\beta\text{-}C_2^\gamma\text{-}C_2^{\delta 1})$	−63.6
$\psi_2(N_2\text{-}C_2^\alpha\text{-}C_2\text{-}N_3)$	−30.0	$\chi_2^{2,2}(C_2^\alpha\text{-}C_2^\beta\text{-}C_2^\gamma\text{-}C_2^{\delta 2})$	175.3
$\omega_2(C_2^\alpha\text{-}C_2\text{-}C_3\text{-}C_3^\alpha)$	178.2	$\theta_4(C_4^\delta\text{-}N_4\text{-}C_4^\alpha\text{-}C_4^\beta)$	−1.4
$\phi_3(C_2\text{-}N_3\text{-}C_3^\alpha\text{-}C_3)$	−46.3	$\chi_4^1(N_4\text{-}C_4^\alpha\text{-}C_4^\beta\text{-}C_4^\gamma)$	8.7
$\psi_3(N_3\text{-}C_3^\alpha\text{-}C_3\text{-}N_4)$	−41.1	$\chi_4^2(C_4^\alpha\text{-}C_4^\beta\text{-}C_4^\gamma\text{-}C_4^\delta)$	−12.8
$\omega_3(C_3^\alpha\text{-}C_3\text{-}N_4\text{-}C_4^\alpha)$	−171.5	$\chi_4^3(C_4^\beta\text{-}C_4^\gamma\text{-}C_4^\delta\text{-}N_4)$	11.5
$\phi_4(C_3\text{-}N_4\text{-}C_4^\alpha\text{-}C_4)$	−65.3	$\chi_4^4(C_4^\gamma\text{-}C_4^\delta\text{-}N_4\text{-}C_4^\alpha)$	−6.1
$\psi_4(N_4\text{-}C_4^\alpha\text{-}C_4\text{-}N_5)$	−14.8	$\chi_5^{1,1}(N_5\text{-}C_5^\alpha\text{-}C_5^\beta\text{-}C_5^{\gamma 1})$	−69.7
$\omega_4(C_4^\alpha\text{-}C_4\text{-}N_5\text{-}C_5^\alpha)$	173.1	$\chi_5^{1,2}(N_5\text{-}C_5^\alpha\text{-}C_5^\beta\text{-}C_5^{\gamma 2})$	157.4
$\phi_5(C_4\text{-}N_5\text{-}C_5^\alpha\text{-}C_5)$	−58.7		
$\psi_5(N_5\text{-}C_5^\alpha\text{-}C_5\text{-}N_6)$	−38.1		
$\omega_5(C_5^\alpha\text{-}C_5\text{-}N_6\text{-}C_6^\alpha)$	174.4		
$\phi_6(C_5\text{-}N_6\text{-}C_6^\alpha\text{-}C_6)$	51.3		
$\psi_6(N_6\text{-}C_6^\alpha\text{-}C_6\text{-}O_M)$	42.8		
$\omega_6(C_6^\alpha\text{-}C_6\text{-}O_M\text{-}C_M)$	−178.0		

[a] The average standard deviation is 0.8°.

structure of Z-Aib-Pro-NHMe.[6] The large temperature factor for C_4^β Pro (B = 11.1 Å^2) results in an uncertainity in its atomic position, with consequent shortening of the observed C^α-C^β and C^β-C^γ distances. Such effects have also been noted in other analyses of proline peptides.[6,32]

The pyrrolidine ring conformational angles are presented in Table V. A C^γ-*endo* conformation is observed with the C_4^γ and C_4 atoms showing deviations of 0.148 and 1.163 Å on the same side of the plane defined by $N_4C_4^\alpha C_4^\delta$. C_4^β is displaced by 0.036 Å on the side opposite to C_4. It is interesting to note that in the structures Z-Aib-Pro-NHMe[6] and Z-Aib-Pro-Aib-Ala-OMe,[34] which also involve Aib-Pro type III β-turns, the Pro ring adopts a C^γ-*exo* conformation. These results suggest that there may not be any meaningful correlation between pyrrolidine ring puckering and backbone conformation in Aib-Pro sequences.[35] The Leu sidechain ($C_2\text{-}C_2^\alpha\text{-}C_2^\beta\text{-}C_2^\gamma\text{-}C_2^\delta$) adopts a *trans* zigzag conformation. In the Val sidechain the C^α-H bond is *gauche* with respect to both $C^\gamma H_3$ groups. These sidechain conformations are generally observed for Leu and Val residues in peptides.

Molecular Packing

The packing of pentapeptide molecules in the *bc* plane is shown in Fig. 4. Peptide helixes are arranged in columns in head-to-tail fashion. The NH groups of Leu(1) and Aib(2) appear to be simultaneously hydrogen-bonded to the Val CO of a neighboring molecule. The hydrogen-bond parameters are $N_2\text{---}O_5$, 2.924 Å; $H\text{-}N_2\text{-}O_5$, 9.9° and $N_3\text{---}O_5$, 3.275 Å; $H\text{-}N_3\text{-}O_5$, 25.4°.

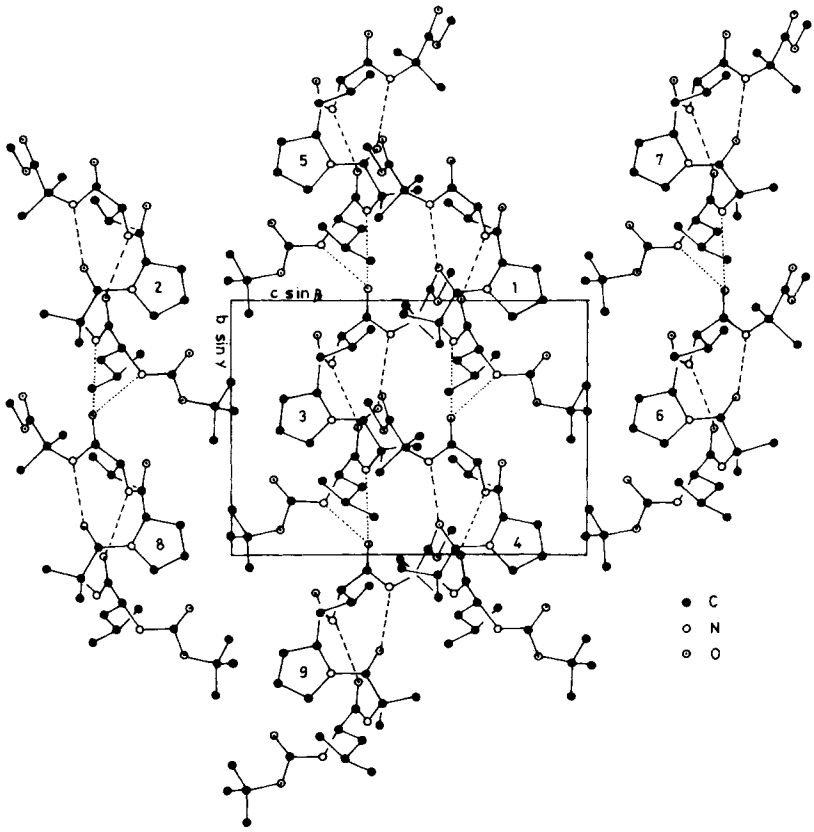


Fig. 4. Packing of pentapeptide molecules in the crystal viewed along the *a* axis. Inter- and intramolecular hydrogen bonds are indicated.

CONCLUSIONS

The 3_{10}-helical conformation of Boc-Leu-Aib-Pro-Val-Aib-OMe established above suggests that helical folding is likely to be maintained in the central segment of alamethicin (11–16) and suzukacillin (15–20). Together with earlier reports on the conformational analysis of Aib-containing fragments of membrane-channel-forming polypeptides, the results of the present study provide further evidence for the preferred helical conformations of these sequences.

This research was supported by the Department of Science and Technology, Government of India. P.B. is the recipient of a UGC Career Award. We thank Dr. R. Nagaraj for providing a sample of the pentapeptide, Dr. N. Shamala for assistance in the early part of this work and Prof. C. N. R. Rao for his interest.

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Received February 2, 1982
Accepted June 30, 1982