The Crystal Structure of the Amino-terminal Pentapeptide of Suzukacillin. Occurrence of a Four-fold Peptide Helix

By **Athappilly K. Francis, Mohammed Iqbal, Padmanabhan Balaram,* and Mamannamana Vijayan,***
Molecular Biophysics Unit, Indian Institute of Science, Bangalore 560 012, India

The monohydrate of the protected amino-terminal pentapeptide of suzukacillin, t-butoxycarbonyl-α-aminoisobutyryl-L-prolyl-L-valyl-α-aminoisobutyryl-L-valine methyl ester, $C_{29}H_{51}N_5O_8$, crystallizes in the orthorhombic space group $P2_12_12_1$ with a = 10.192, b = 10.440, c = 32.959 Å, and Z = 4. The structure has been solved by direct methods and refined to an R value of 0.101 for 1 827 observed reflections. The molecule exists as a four-fold helix with a pitch of 5.58 Å. The helix is stabilised by N–H · · · O hydrogen bonds, two of the 5→1 type (corresponding to the α-helix) and the third of the 4→1 type (3_{10} helix). The carbonyl oxygen of the amino-protecting group accepts two hydrogen bonds, one each from the amide NH groups of the third (4→1) and fourth (5→1) residues. The remaining 5→1 hydrogen bond is between the two terminal residues. The lone water molecule in the structure is hydrogen bonded to carbonyl oxygens of the prolyl residue in one molecule and the non-terminal valyl residue in a symmetry-related molecule.

ACYCLIC polypeptides like alamethicin,[1-3] suzukacillin,[4] antiamoebins,[3] emerimicins,[3] trichotoxin A-40,[5] and hypelcins [6] form a class of ionophores which form transmembrane channels for ion transport.[7] The distinguishing characteristic structural feature of these polypeptides is that they contain a large proportion of the unusual amino-acid α-aminoisobutyric acid (Aib). The X-ray analyses of several fragments of alamethicin, perhaps the best characterised member of the family of Aib-containing channel formers,[8] have already been reported.[9-12] The crystal structures of several other acyclic Aib-containing peptides are also available.[12-19] Here we report the X-ray structure analysis of the N-terminal end-protected pentapeptide of suzukacillin, Boc-Aib-L-Pro-L-Val-Aib-L-Val-OMe (I).

EXPERIMENTAL

The peptide was synthesized by solution-phase procedures as described for alamethicin.[20] Platy elongated crystals of the compound were grown from an ethyl acetate solution containing a few drops of methanol and water The space group and the unit-cell dimensions were determined from oscillation and Weissenberg photographs and the density was measured by flotation in an aqueous potassium iodide solution. The cell dimensions were subsequently refined on a four-circle diffractometer.

Crystal Data.—t-Butoxycarbonyl-α-aminoisobutyryl-L-prolyl-L-valyl-α-aminoisobutyryl-L-valine methyl ester (I) monohydrate, $C_{29}H_{51}N_5O_8 \cdot H_2O$, M = 615.8. Orthorhombic, $P2_12_12_1$, a = 10.192(6), b = 10.440(5), c = 32.959(16) Å, U = 3507.0 Å^3, D_m = 1.158(5) g cm^{-3}, D_c = 1.165 g cm^{-3}, Z = 4. Cu-K_α radiation, μ(Cu-K_α) = 7.17 cm^{-1}.

The X-ray intensity data were collected on a CAD-4 four-circle diffractometer employing a ω—2θ scan up to a maximum Bragg angle of 60° using graphite-monochromated Cu-K_α radiation from a specimen of approximate dimensions 0.60 × 0.25 × 10 mm^3. Of the 2 978 reflections measured in this range, 1 827 reflections having $I > 2\sigma(I)$ were used in the refinement cycles. The intensities were corrected for Lorentz and polarisation factors but not for absorption.

The solution of the structure using direct methods turned out to be far from straightforward. The structure was eventually developed by the successive application of the Karle recycling procedure and trial calculations from an eight-membered fragment identified in the E map corresponding to the second highest probable set of phase angles obtained from a MULTAN [21] run using spherically symmetric group scattering factors for the five-membered prolyl ring. The structure was refined on a DEC-1090 computer by the block-diagonal SFLS method with anisotropic and isotropic temperature factors for non-hydrogen and hydrogen atoms, respectively. Refinement converged at R 0.101 when all the least-squares shifts were less than the corresponding estimated standard deviations. The scattering factors for the non-hydrogen atoms and the hydrogen atoms were taken from refs. 22 and 23, respectively. The final positional parameters and the equivalent isotropic temperature factors [24] of the non-hydrogen atoms are given in Table 1. The anisotropic thermal parameters of the non-hydrogen atoms, the positional and the isotropic thermal parameters of the hydrogen atoms, and the observed and the calculated structure factors are given in Supplementary Publication No. SUP 23370 (17 pp.).†

It may be mentioned that the final R factor and the estimated standard deviations of the positional parameters are rather high, presumably due to the high temperature factors of most of the atoms in the structure and the consequent weakness of the high-angle data.

RESULTS AND DISCUSSION

Bond Lengths and Valency Angles.—The bond lengths and valency angles in the structure are given in Figure 1. The dimensions of the protecting groups deviate substantially from the expected values presumably due to the high thermal vibration amplitude of these groups resulting from their location at the two extremities of the molecule. With regard to the other dimensions, the shortening of the C^β–C^γ [C(17)–C(16)] bond is particularly noteworthy. As has been noted earlier,[25] the displacements of these atoms from the plane of the pyrrolidine ring can assume a continuous range of values leading to possible static disorder which, along with thermal vibrations, often results in highly anisotropic thermal parameters for C^β and C^γ, and the consequent reduction in the C^β–C^γ bond length. In the present structure, the

† For details of Supplementary Publications see Notice to Authors No. 7 in *J. Chem. Soc., Perkin Trans. 2*, 1981, Index Issue.

atoms vibrate highly anisotropically, the B values along the principal axes of the thermal vibration ellipsoid being 17.1, 6.8, and 1.1 Å^2 for C$^\beta$ and 21.1, 5.5, and 2.9 Å^2 for C$^\gamma$. The angle between the major axis and the normal to the mean plane of the pyrrolidine ring is 35.4° for C$^\beta$ and 7.2° for C$^\gamma$. Thus, it is interesting to note that the major axis is nearly perpendicular to the plane of the ring in both the cases and particularly so for C$^\gamma$.

TABLE 1

Final positional co-ordinates ($\times 10^4$) and equivalent isotropic thermal parameters [17] of non-hydrogen atoms, with standard deviations in parentheses

Atom	x	y	z	B(Å^2)
C(1)	−1 640(19)	282(21)	4 005(7)	8.7(1.3)
C(2)	−466(21)	1 225(20)	3 430(6)	7.8(1.2)
C(3)	−1 088(18)	2 551(21)	4 010(6)	7.7(1.2)
C(4)	−667(16)	1 206(16)	3 859(5)	5.4(0.9)
O(5)	556(10)	818(9)	4 074(3)	4.8(0.5)
C(6)	1 671(14)	1 518(14)	4 060(4)	3.9(0.7)
O(7)	1 736(9)	2 596(8)	3 915(3)	3.8(0.4)
N(8)	2 633(10)	905(10)	4 245(3)	2.8(0.5)
C(9)	3 887(15)	1 514(11)	4 334(4)	3.3(0.6)
C(10)	4 886(15)	533(12)	4 491(4)	3.8(0.7)
C(11)	3 690(16)	2 500(15)	4 672(4)	4.5(0.8)
C(12)	4 462(15)	2 169(14)	3 950(4)	4.2(0.7)
C(13)	4 949(10)	3 248(9)	3 987(3)	4.5(0.5)
N(14)	4 473(11)	1 573(10)	3 600(3)	3.1(0.5)
C(15)	4 028(19)	199(14)	3 486(4)	5.1(0.8)
C(16)	4 905(28)	−117(18)	3 134(6)	9.9(1.4)
C(17)	5 254(24)	1 057(17)	2 939(5)	8.3(1.2)
C(18)	5 199(14)	2 158(15)	3 266(5)	4.3(0.8)
C(19)	4 552(15)	3 310(15)	3 076(4)	4.0(0.7)
O(20)	5 157(11)	3 950(10)	2 817(3)	5.7(0.6)
N(21)	3 306(11)	3 570(10)	3 177(3)	3.0(0.5)
C(22)	2 584(13)	4 656(11)	3 012(4)	2.6(0.6)
C(23)	1 261(15)	4 271(12)	2 865(4)	3.7(0.7)
C(24)	1 391(18)	3 351(16)	2 500(5)	5.6(0.9)
C(25)	432(17)	5 381(18)	2 737(5)	6.0(1.0)
C(26)	2 456(13)	5 736(14)	3 346(4)	3.5(0.6)
O(27)	2 796(10)	6 816(9)	3 264(3)	4.9(0.5)
N(28)	1 980(9)	5 390(9)	3 702(3)	2.2(0.4)
C(29)	1 730(13)	6 328(13)	4 029(4)	3.1(0.6)
C(30)	1 257(14)	5 567(14)	4 401(4)	4.1(0.7)
C(31)	711(17)	7 331(16)	3 898(6)	5.9(0.9)
C(32)	3 025(16)	6 955(14)	4 139(4)	4.2(0.7)
O(33)	3 088(10)	8 046(9)	4 290(3)	4.7(0.5)
N(34)	4 135(11)	6 238(9)	4 137(3)	3.1(0.5)
C(35)	5 437(16)	6 688(15)	4 258(4)	4.7(0.8)
C(36)	6 068(15)	5 829(16)	4 572(5)	5.1(0.8)
C(37)	7 468(22)	6 361(27)	4 653(6)	10.0(1.5)
C(38)	5 256(19)	5 769(20)	4 952(5)	6.6(1.0)
C(39)	6 190(21)	6 886(21)	3 875(6)	8.3(1.3)
O(40)	6 671(23)	7 902(16)	3 789(6)	14.7(1.4)
O(41)	6 287(16)	5 964(14)	3 648(4)	10.1(0.9)
C(42)	7 011(28)	6 076(30)	3 257(7)	14.0(2.1)
W(43)	7 630(13)	3 312(18)	2 499(5)	12.0(1.1)

Another noteworthy, though anticipated, feature involves the pyrrolidine ring and the peptide group preceding the prolyl residue. The valency angles C_1^α–C_1–N_2 [C(9)–C(12)–N(14)] and C_1–N_2–C_2^δ [C(12)–N(14)–C(15)] have values greater than those normally observed [26] in proline-containing peptides. This enhancement can be accounted for by the steric interactions between the side chains of the conformationally restrictive prolyl and Aib residues.[27]

Main Chain Conformation.—Conformation of peptides containing Aib residues has been studied extensively in recent years in order to investigate the steric constraints imposed by the presence of these residues. The presence of two β-methyl groups restricts them to regions in conformational space corresponding to the left- or right-handed 3_{10} and α-helices.[28,29] Indeed, the average values of ϕ, ψ angles [30] of the Aib residue obtained from several crystal-structure determinations work out to be around ±(50°, 42°).[12,31] These values are in between those appropriate for an α-helix and a 3_{10} helix. However, the intramolecular hydrogen bonding pattern in tetra- and penta-peptides containing Aib residues was indicated from *X*-ray,[9,11-13] n.m.r.,[9,16,32] and i.r.[33,34] studies to be appropriate to that for the 3_{10} helix. Electron diffraction data on poly-Aib were also interpreted in terms of a 3_{10} helix.[35] However, the hydrogen bonding pattern appropriate for an α-helix has been reported recently in the crystal structure of an eleven-membered linear peptide containing four Aib residues.[17] Theoretical studies [12,29,31,36] have indicated several possible related helical conformations for Aib-containing peptides. Thus, experimental and theoretical investigations indicate some, albeit small, conformational flexibility for Aib-containing peptides in the helical region. It is in this context that the observed conformation in the present structure becomes interesting.

The torsional angles which define the main chain conformation [30] of the pentapeptide are listed in Table 2.

TABLE 2

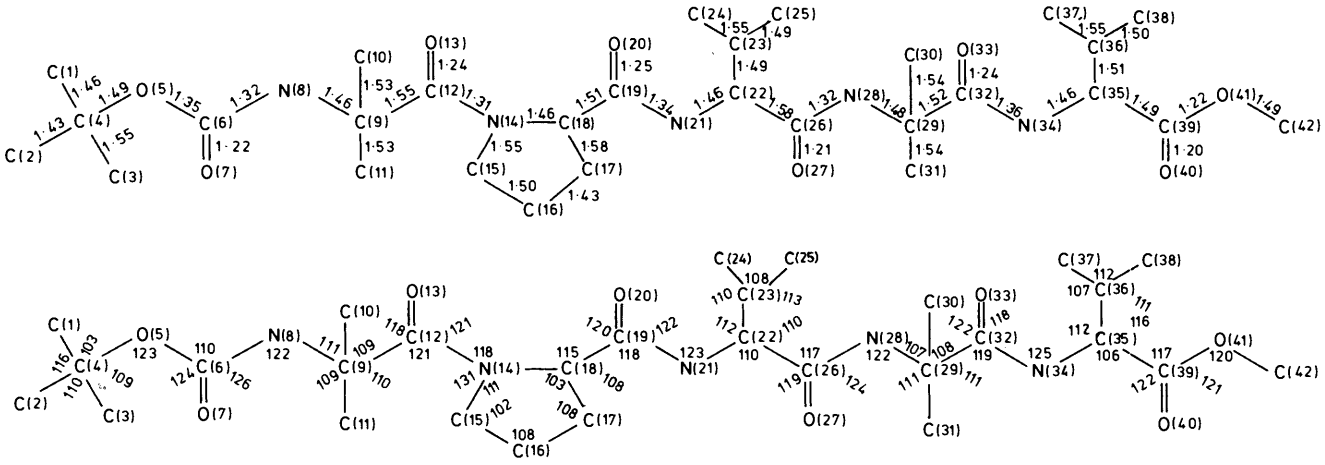
Main chain torsion angles (°), with standard deviations in parentheses

	Aib(1)	Pro(2)	Val(3)	Aib(4)	Val(5)
ω (°)	−171(1)	−180(2)	−176(1)	−176(1)	179(2) *
ϕ (°)	−51(2)	−74(2)	−106(1)	−61(2)	−104(2)
ψ (°)	−46(2)	−11(2)	−52(1)	−37(2)	−56(2) *

* The ester oxygen atom in the protecting group has been treated as geometrically equivalent to an amide nitrogen atom in the calculation of these torsion angles.

The conformation is best described as a right-handed four-fold helix, with a pitch of 5.58 Å, similar to the α′-helix suggested for poly-Aib by Prasad and Sasisekharan.[36] Not unexpectedly, differences in detail, however, exist between the observed and the α′-helices. Two views of the backbone of the molecule, one along the helix axis and the other perpendicular to it, are shown in Figure 2. Considering only the α-carbon atoms, the average unit twist works out to be 88.3° with a maximum deviation of 4.0° for any individual value. The height per residue, however, varies between 1.01 and 1.78 Å with an average value of 1.39 Å. Although the values of ϕ and ψ differ considerably from residue to residue, it is interesting to note that those for the Aib residues are close to the average values for Aib residues obtained from other crystal structures. It is also interesting that the torsional angles for the valyl residues deviate maximally from those for helical structures as it is known that valine has a low propensity to be in a helix.[37]

As can be seen from Table 3, which lists the hydrogen bond parameters in the structure, there exist three



(I)

C(1) - C(4) - C(3) 108°; N(8) - C(9) - C(12) 111°; N(28) - C(29) - C(32) 108°
C(2) - C(4) - O(5) 111°; C(10) - C(9) - C(11) 107°; C(30) - C(29) - C(31) 111°

FIGURE 1 Bond lengths (Å) and angles (°) in the molecule of (I). The standard deviations of lengths vary between 0.016 and 0.028 Å and those of angles between 1 and 2°

intramolecular N–H . . . O hydrogen bonds. Two of them are of the 5→1 type (corresponding to the α-helix) whereas the third is of the 4→1 type (3_{10} helix). While there are three separate hydrogen bond donors, all amide nitrogens, there are only two carbonyl oxygen acceptors. The carbonyl oxygen of the amino-protecting group accepts two hydrogen bonds. The carbonyl oxygen atom of the prolyl residue, which could have taken part in a 4→1 hydrogen bond with the terminal residue, forms a hydrogen bond with the lone water molecule in the structure.

In a recent ^{1}H n.m.r. study [38] of this peptide in $CDCl_3$ and $(CD_3)_2SO$ solution, the presence of three intramolecularly hydrogen bonded NH groups was established using solvent and temperature dependences of NH chemical shifts. These results, together with the known stereochemical preferences of Aib residues, were used to postulate a 3_{10} helical conformation for the peptide, stabilized by three intramolecular 4→1 hydrogen bonds, involving the Val(3), Aib(4), and Val(5) NH groups. This conformation differs from that observed in the solid state. However, it may be noted that ^{1}H n.m.r. methods establish only the nature of the NH groups involved in hydrogen bonding and do not permit identification of the corresponding CO groups. The presence of three solvent-shielded NH groups is a point of similarity between the solution and solid-state results. Further, the intramolecular hydrogen bonding

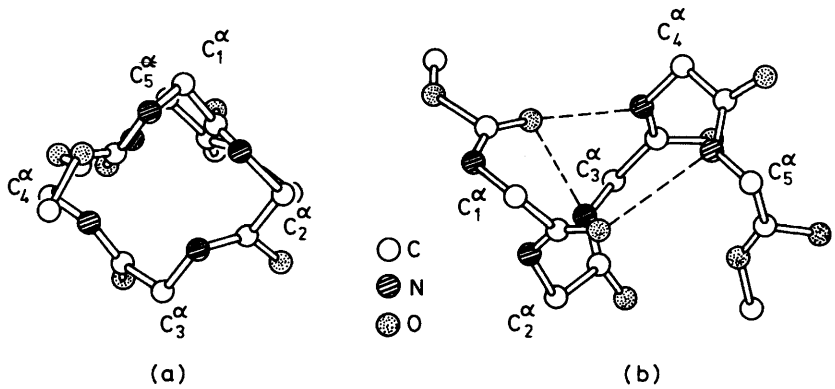


FIGURE 2 The main chain conformation of the molecule as viewed along (a) the helix axis and (b) in a direction perpendicular to the helix axis. The hydrogen bonds are indicated by broken lines in (b)

TABLE 3
Hydrogen bond parameters, with standard deviations in parentheses

Hydrogen bond	l(N · · · O)	θ(H–N · · · O)
N(21) · · · O(7)	3.08(1)	14(7)
N(28) · · · O(7)	3.01(1)	20(8)
N(34) · · · O(13)	3.27(1)	21(8)
W(43) · · · O(20)	2.81(2)	14(15)
W(43) · · · O(27) *	2.99(2)	9(15)

* Related to O(27) in the reference molecule by $-x + 1$, $\frac{1}{2} + y - 1$, $\frac{1}{2} - z$.

pattern in the crystal may reflect the perturbing effect of the water molecule. This peptide is the first example of an end-protected apolar Aib-containing oligopeptide crystallizing in a hydrated form.

Side Chain Conformation.—The lone prolyl residue in the molecule exists in the C_s–C^γ exo conformation [39] with C^γ deviating from the mean plane of the other four atoms in the pyrrolidine ring by 0.40 Å. The torsion angles χ^{11} and χ^{12}, which define the side chain conformation of valyl residues, are −65 and 173° for Val(3) and −61 and 177° for Val(5).

Thus, in both the valyl residues, one of the γ-carbon atoms is staggered between the hydrogen atom attached to C^α and the amide nitrogen atom while the other is between the hydrogen atom and the carbonyl carbon. This is indeed sterically the most favourable conformation for the valyl side chain.[40]

Crystal Structure.—The crystal structure as viewed along the *a* axis is shown in Figure 3. The helix axis of the molecule is roughly parallel to the crystallographic *b* axis, the angle between the two axes being 22°. Thus the molecules are stacked in a head-to-tail

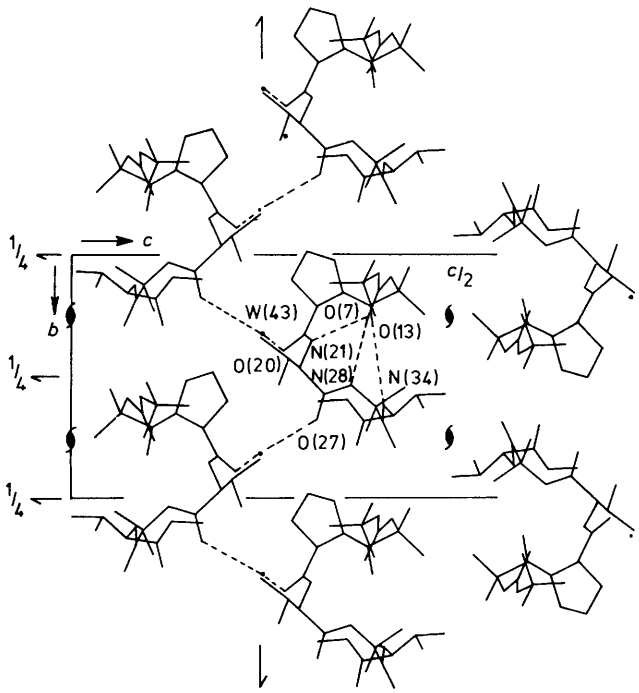


FIGURE 3 The crystal structure viewed along the crystallographic *a* axis. The broken lines indicate hydrogen bonds. Only atoms involved in hydrogen bonds are numbered

fashion along a direction roughly parallel to the helix axis. Each such column is bridged through water molecules to a column related to it by a 2_1 screw axis parallel to the *b* axis. Each water molecule is hydrogen bonded to the carbonyl oxygen of the prolyl residue and the carbonyl oxygen of the non-terminal valyl residue of the symmetry-related molecule. The columns bridged by water molecules can also be looked upon as a two-fold helix centred around a 2_1 screw axis parallel to the *b* axis with the pentapeptide (along with a water molecule) as the repeating unit. The two-fold helix and its equivalents generated by translation along the *a* axis form a layer in the *bc* plane. Such layers stack along the *c* axis, adjacent layers having helices pointing in opposite directions, to form the crystal. It is interesting to note that the crystal structure provides an example of a water molecule trapped in a relatively hydrophobic environment.

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