Type II β-Turn Conformation of Pivaloyl-L-Prolyl-a-Aminoisobutyryl-N-Methylamide: Theoretical, Spectroscopic, and X-Ray Studies

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Synopsis

Pivaloyl-L-Pro-Aib-*N*-methylamide has been shown to possess one intramolecular hydrogen bond in $(CD_3)_2SO$ solution, by ^1H-nmr methods, suggesting the existence of β-turns, with Pro-Aib as the corner residues. Theoretical conformational analysis suggests that Type II β-turn conformations are about 2 kcal mol^{-1} more stable than Type III structures. A crystallographic study has established the Type II β-turn in the solid state. The molecule crystallizes in the space group $P2_1$ with $a = 5.865$ Å, $b = 11.421$Å, $c = 12.966$Å, $\beta = 97.55°$, and $Z = 2$. The structure has been refined to a final R value of 0.061. The Type II β-turn conformation is stabilized by an intramolecular 4 ← 1 hydrogen bond between the methylamide NH and the pivaloyl CO group. The conformational angles are $\phi_{Pro} = -57.8°$, $\psi_{Pro} = 139.3°$, $\phi_{Aib} = 61.4°$, and $\psi_{Aib} = 25.1°$. The Type II β-turn conformation for Pro-Aib in this peptide is compared with the Type III structures observed for the same segment in larger peptides.

Studies on the conformations of peptides containing a-aminoisobutyric acid (Aib) and proline can be useful in establishing stereochemical characteristics of the 0-turn conformations.[1-3] Recent interest in the conformational analysis of Aib peptides stems from their widespread occurrence in transmembrane channel-forming peptides related to alamethicin.[4] Sequences of the type -Aib-Pro-Aib- occur in microbial polypeptides, alamethicin,[5] and hypelcin.[6] Studies of alamethicin fragments in solid state and in solution have established that these sequences adopt consecutive Type III 0-turn or 3_{10} helical conformations.[7-10] In all the cases studied, the sequences have been of the type R-Aib-Pro-Aib-Ala-[7] or R-Pro-Aib-Ala-Aib-,[10] where R is a urethane protecting group. In these oligopeptide structures, the Pro-Aib Type III β-turn is either preceded or succeeded by another Type III 0-turn, resulting in propagation of a 3_{10} helical conformation.

The model peptide pivaloyl-Pro-Aib-NHMe has been studied by ir methods in solution and a Pro-Aib Type II 0-turn has been proposed.[11] The Type III and Type II β-turns differ in the orientation of the central peptide unit, with the former requiring the Aib residue to have ϕ and ψ values[12] in the right-handed 3_{10} or a-helical regions ($\phi \sim -60°$, $\psi \sim -30°$), while in the latter, the ϕ and ψ values fall in the left-handed helical region

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($\phi \sim +60°, \psi \sim +30°$) of the conformational map.[13] The two regions are energetically equivalent and favored for the achiral Aib residue.[14–17] The L-Pro residue requires $\phi = -60°, \psi = -30°$ for Type III structures, while for Type II, $\phi = -60°, \psi = +120°$. Both regions of ψ_{Pro} have been observed in studies of peptides and proteins.[18–20]

It was therefore of interest to determine the conformational preference of the Pro-Aib sequence and to establish whether incorporation into larger peptides influences the stereochemistry of this segment. In this report we describe theoretical calculations, using semiempirical methods, on Ac-Pro-Aib-NHMe, which suggest that Type II conformations are energetically more favorable. We also report ^{1}H-nmr data on pivaloyl-Pro-Aib-NHMe, which establish an intramolecular **4** → 1 hydrogen bond in solution; finally, we demonstrate the occurrence of a Type II β-turn structure in the solid state by x-ray diffraction.

MATERIALS AND METHODS

Synthesis of Piv-Pro-Aib-NHMe

Boc-Pro was coupled to Aib-OMe in CH_2Cl_2 using DCC to yield Boc-Pro-Aib-OMe, which was converted to Boc-Pro- Aib-NHMe by aminolysis in CH_3OH/CH_3NH_2. Deprotection of the Boc group with HCl/tetrahydrofuran was followed by treatment with triethylamine and pivaloyl chloride in CH_3OH to yield Piv-Pro-Aib-NHMe as a solid, which was recrystallized from ethylacetate. mp 186°C. ^{1}H-nmr (270 MHz): 7.356, m, 1H, N*H*Me; 6.196, s, 1H, Aib NH; 4.206, t, 1H, Pro $C^\alpha H$; 3.756, m, 2H, Pro $C^\delta H_2$; 2.756, d, 3H NH*Me*; 2.26, m, 2H, 2.06, m, 2H, $C^\beta H_2$, $C^\gamma H_2$, Pro; 1.786, s, 3H, 1.636, s, 3H, Aib $C^\beta H_3$; 1.476, s, 9H, Piv CH_3.

Detailed illustrative procedures are described in Refs. 7, 20, and 21.

NMR Studies

^{1}H-nmr spectra were recorded at 270 MHz and ^{13}C spectra at 67.89 MHz on a Bruker WH-270 FT-nmr spectrometer at the Bangalore NMR Facility. All chemical shifts are expressed as δ (ppm) downfield from internal tetramethylsilane. Variable temperature measurements were carried out over the range of 20–70°C at a peptide concentration of 10 mg/mL in $(CD_3)_2SO$.

Theoretical Conformation Analysis of Ac-Pro- Aib-NHMe

Standard Pauling-Corey geometries were adopted for the secondary amide units, while a modified geometry was used for the tertiary amide unit.[22] Methyl hydrogens were fixed in the staggered positions. In the pyrrolidine ring, hydrogens were fixed, bisecting the C-C-N or C-C-C angles. All H-C-N bond angles were fixed at 109.5°, C-H bond lengths at 1.1Å, and

τ(N-C$^{\alpha}$-C) at both a-carbons at 110'. Five representative puckerings of the proline ring were chosen from the list of low-energy conformations given in Table II of Ref. 23, so as to account for the following: (i) a sufficiently wide range of ring conformational angles is explored, (ii) the ϕ values lie in the range -50" to $-70°$, and (iii) there is a minimum variation in the bond angles. The five puckerings chosen are denoted as A_1, A_2, A_3 (C$^{\gamma}$-*exo*) and B_1, B_2 (C$^{\gamma}$-*endo*).[23]

The molecular conformation **of** Ac-Pro-Aib-NHMe can be defined using the conformational angles ϕ_{Pro}, ψ_{Pro}, ϕ_{Aib}, ψ_{Aib}, assuming planar amide units. ϕ_{Pro} is fixed for a given pyrrolidine puckering and is determined by the relation $\phi_{Pro} = \theta - 60°$.[24,25] The other three angles were varied in the sterically allowed regions for Aib[14–17] and Pro[18] residues and conformations examined for specific intramolecular 4 ← 1 hydrogen bonds. Conformations with satisfactory N---O distances (2.7–3.2 Å) and H-N-O angle (<40°) were selected for energy calculations. Total conformational energy was estimated using standard procedures.[13] The Buckingham potential was used for computation of nonbonded energy,[26] while the monopole charges on the C, O, and N atoms attached to the pyrrolidine ring are from Holzwarth and Chandrasekaran[17]; the hydrogen-bond energy is calculated according to Balasubramanian et al.[28]

X-Ray Diffraction

Piv-Pro-Aib-NHMe crystallized from ethylacetate in the space group $P2_1$, with a = 5.865 (1), b = 11.421(1), c = 12.966(1) Å, β = 97.55(1)°, Z = 2. Intensity data were collected on a crystal of dimensions 0.8 × 0.3 × 0.1 mm on a CAD-4 diffractometer employed the ω-2θ scan, up to a maximum Bragg angle of 60°, using CuK_{α} radiation (λ = 1.5418Å). Of the 1360 reflections collected in this range, 1250 having $I > 3\sigma(I)$ were used for structure determination and refinement. Intensities were corrected for Lorentz and polarization factors but not for absorption.

The structure was solved using the direct methods program, MULTAN.[29] A 11-atom fragment could be identified in the E map corresponding to the best set of phases generated by MULTAN. The remaining 10 nonhydrogen atoms were located by the Karle recycling process.[30] The structure was refined using a block diagonal least-squares method. Initial refinement of all nonhydrogens atoms with isotropic temperature factors and overall scale factor yielded an R value of 0.099. The difference Fourier computed at this stage revealed the positions of 21 out of 27 hydrogen atoms. The remaining hydrogens were fixed using stereochemical considerations as described.[31] Refinement using anisotropic and isotropic temperature factors for the nonhydrogen and hydrogen atoms, respectively, with a u-weighting scheme resulted in a final R value of 0.061. The final difference Fourier was featureless. The scattering factors used were those of Cromer and Waber[32] for nonhydrogen atoms and of Stewart et al.[33] for hydrogen atoms. The atomic and thermal parameters with their standard deviations

are given in Tables I and II. A listing of the observed and calculated structure factors is available on request.

RESULTS AND DISCUSSION

NMR Studies

The pivaloyl group was chosen to restrict the Piv-Pro bond to the *trans* geometry.[34,35] Only a single conformer is observed in both 270-MHz ^1H and 67.89-MHz ^{13}C spectra. The Pro C^β and C^γ resonances appear at 27.56 and 26.36, respectively, in $CDCl_3$—confirming that the *trans* X-Pro geometry is obtained.[36] Solution studies with other blocking groups like *t*-butyloxycarbonyl (Boc) or acetyl are complicated by the population of both cis and *trans* states in solution. For example, in Boc-Pro-Aib-NHMe, two conformations could be detected by ^1H- and ^{13}C-nmr, with the degree of cis-form present being 37% in $(CD_3)_2SO$.

The presence of an intramolecularly hydrogen-bonded NH group in Piv-Pro-Aib-NHMe was established by determining the solvent and temperature dependences of the NH chemical shifts.[36] The two NH resonances can be unambiguously identified with the Aib NH appearing as a singlet [6.19δ, $CDCl_3$; 8.176, $(CD_3)_2SO$], while the methylamide NH occurs as a broad quartet [7.35δ, $CDCl_3$; 7.526, $(CD_3)_2SO$]. The Aib NH moves 1.986 downfield on going from $CDCl_3$ to the more strongly hydrogen-bonding solvent $(CD_3)_2SO$, while the methylamide NH is relatively less affected (0.176). The temperature coefficients determined in $(CD_3)_2SO$ are 6.23×10^{-3} and 2.66×10^{-3} ppm/°C for the Aib NH and methylamide NH groups, respectively. These results suggest the involvement of the methylamide NH in an intramolecular hydrogen bond, while the Aib NH is exposed to solvent. The nmr data are fully consistent with an earlier study, which provided evidence for intramolecular hydrogen-bonded structures from ir studies.[11]

The known stereochemical preferences of Aib and Pro residues[2-10] lead us to conclude that the preferred conformation in solution is a **4** → **1** hydrogen-bond-stabilized β-turn. Three categories of β-turns, viz., Type I ($\phi_{Pro} = -60°, \psi_{Pro} = -30°; \phi_{Aib} = -90°, \psi_{Aib} = 0°$), Type II ($\phi_{Pro} = -60°, \psi_{Pro} = 120°; \phi_{Aib} = 80°, \psi_{Aib} = 0°$), and Type III ($\phi_{Pro} = -60°, \psi_{Pro} = -30''; \phi_{Aib} = -60°, \psi_{Aib} = -30''$) must be considered for L-Pro-Aib sequences. Type I 0-turns are not considered separately, since they form only a small variant of the Type III structure. The two most frequently observed conformations for L-Pro residues lie in the region $\phi \sim -60'' \pm 10°, \psi \sim -30° \pm 20°$, and $\phi \sim -60° \pm 10°, \psi \sim 120'' \pm 20°$.[18,20] For Aib residues, the region $\$ \pm 60° \mathbf{f} 20°, \$ \pm 30° \mathbf{f} 20''$ is almost exclusively observed in peptide crystal structures.[4] It is thus seen that both Type II and Type III 0-turns are stereochemically feasible for Pro-Aib sequences.

The Pro-Aib segments in two tetrapeptides, benzyloxycarbonyl-Aib-Pro-Aib-Ala-methyl ester7 and **t-butyloxycarbonyl-Pro-Aib-Ala-Aib-**

TABLE I

Final Atomic Coordinates and Thermal Parameters for the Nonhydrogen Atoms[a]

Atom	x	y	z	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
C_{P1}	0.5923(18)	0.5067(7)	0.3384(7)	0.0858(56)	0.0091(8)	0.0142(9)	−0.0124(18)	−0.0092(19)	−0.0009(8)
C_{P2}	0.2151(15)	0.5574(8)	0.2424(8)	0.0437(33)	0.0085(8)	0.0218(12)	0.0006(14)	0.0064(16)	−0.0022(8)
C_{P3}	0.5487(15)	0.5069(8)	0.1439(7)	0.0502(34)	0.0117(9)	0.0130(7)	−0.0070(15)	0.0089(13)	0.0005(7)
C_1^{α}	0.4326(11)	0.4798(6)	0.2389(5)	0.0320(22)	0.0068(5)	0.0077(5)	−0.0013(10)	0.0006(8)	0.0005(4)
C_1	0.3586(10)	0.3496(5)	0.2474(4)	0.0252(18)	0.0061(5)	0.0060(4)	0.0000(8)	0.0023(7)	0.0001(4)
O_1	0.4052(7)	0.2967(4)	0.3285(3)	0.0368(16)	0.0076(4)	0.0058(2)	−0.0014(7)	0.0003(5)	0.0005(3)
N_2	0.2394(8)	0.2957(4)	0.1633(3)	0.0309(17)	0.0062(4)	0.0049(3)	−0.0011(7)	−0.0002(5)	−0.0012(3)
C_2^{α}	0.1921(10)	0.1697(5)	0.1747(4)	0.0300(21)	0.0066(5)	0.0057(4)	−0.0017(9)	0.0020(7)	−0.0004(4)
C_2^{β}	0.0759(11)	0.1353(7)	0.0649(4)	0.0332(22)	0.0104(7)	0.0065(4)	−0.0023(11)	−0.0004(7)	−0.0008(5)
C_2^{γ}	−0.0316(15)	0.2490(8)	0.0203(6)	0.0528(33)	0.0118(8)	0.0081(6)	−0.0044(14)	−0.0045(11)	0.0008(6)
C_2^{δ}	0.1587(14)	0.3360(7)	0.0587(5)	0.0546(33)	0.0086(7)	0.0072(5)	−0.0024(13)	−0.0030(10)	0.0016(5)
C_2	0.4183(10)	0.1005(6)	0.2023(4)	0.0250(19)	0.0075(6)	0.0062(4)	−0.0003(8)	0.0009(7)	−0.0009(4)
O_2	0.5871(7)	0.1249(5)	0.1588(3)	0.0278(14)	0.0106(5)	0.0093(3)	−0.0000(7)	0.0068(5)	0.0018(4)
N_3	0.4097(8)	0.0174(4)	0.2731(4)	0.0217(15)	0.0063(4)	0.0066(3)	−0.0008(7)	0.0022(5)	0.0006(3)
C_3^{α}	0.6089(11)	−0.0572(0)	0.3106(5)	0.0274(20)	0.0068(6)	0.0072(4)	0.0009(8)	0.0034(9)	−0.0003(4)
$C_3^{\beta 2}$	0.6881(14)	−0.1268(7)	0.2213(6)	0.0465(31)	0.0096(7)	0.0094(5)	0.0034(12)	0.0054(10)	−0.0025(5)
$C_3^{\beta 1}$	0.5335(11)	−0.1376(6)	0.3931(5)	0.0332(24)	0.0078(6)	0.0095(5)	0.0005(10)	0.0038(9)	0.0028(5)
C_3	0.8115(10)	0.0172(6)	0.3637(5)	0.0262(21)	0.0096(6)	0.0069(4)	−0.0007(10)	0.0027(8)	0.0016(5)
O_3	1.0068(7)	−0.0264(5)	0.3753(4)	0.0199(13)	0.0111(5)	0.0134(4)	0.0028(7)	0.0047(6)	0.0021(4)
N_4	0.7619(8)	0.1191(5)	0.4038(4)	0.0267(17)	0.0086(5)	0.0079(4)	−0.0013(8)	0.0003(6)	−0.0009(4)
C_4^{α}	0.9419(12)	0.1940(8)	0.4609(5)	0.0342(26)	0.0130(8)	0.0083(5)	−0.0044(12)	−0.0022(9)	−0.0012(6)

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

TABLE II
Positional **Coordinates** for **Hydrogen** Atoms

Atom	x	y	z
H_{P11}	0.616	0.570	0.344
H_{P12}	0.693	0.491	0.328
H_{P13}	0.444	0.477	0.439
H_{P21}	0.223	0.639	0.269
H_{P22}	0.078	0.567	0.218
H_{P23}	0.155	0.542	0.347
H_{P31}	0.609	0.588	0.123
H_{P32}	0.498	0.521	0.044
H_{P33}	0.672	0.465	0.147
$H_2^{\beta 1}$	0.321	0.385	−0.019
$H_2^{\beta 2}$	0.120	0.395	0.059
$H_2^{\gamma 1}$	0.052	0.274	−0.085
$H_2^{\gamma 2}$	−0.274	0.275	0.045
$H_2^{\beta 1}$	0.196	0.114	0.020
$H_2^{\beta 2}$	−0.040	0.091	0.073
H_2^{α}	0.086	0.160	0.242
H_3	0.310	0.013	0.329
$H_3^{\beta 11}$	0.744	−0.084	0.153
$H_3^{\beta 12}$	0.603	−0.165	0.194
$H_3^{\beta 13}$	0.832	−0.169	0.233
$H_3^{\beta 21}$	0.601	−0.176	0.430
$H_3^{\beta 22}$	0.512	−0.105	0.475
$H_3^{\beta 23}$	0.406	−0.193	0.384
H_4	0.601	0.161	0.411
$H_4^{\alpha 1}$	0.829	0.221	0.531
$H_4^{\alpha 2}$	1.042	0.214	0.419
$H_4^{\alpha 3}$	0.950	0.151	0.541

benzyl ester[10] adopt Type III β-turn conformations in the crystalline state. The Pro-L-Ala and Pro-D-Ala sequences in blocked dipeptide alkylamides, however, occur in the Type II conformation.[37,38] The Aib residue, in principle, could adopt a conformation that is compatible for both L- and D-Ala residues. Aubry et al. have suggested a Type II conformation for Piv-Pro-Aib-NHMe on the basis of the similarity of its ir spectra with that of the corresponding Pro-D-Ala peptide.[11] However, an unequivocal assignment of the p-turn type cannot be made either from ir or the present nmr data.

Theoretical Analysis

Energy calculations using semiempirical methods have been carried out to evaluate the relative stabilities of the Type II and Type III β-turn conformations for the Pro-Aib sequence. Calculations were carried out in a restricted region of conformational space, requiring maintenance of the 4 ← 1 hydrogen bond, for different pyrrolidine ring conformations (see Materials and Methods). The results of these calculations are summarized

TABLE III
Conformational Parameters and Energies of Low-Energy β-Turn Conformations for Ac-Pro-Aib-NHMe

Puckering[a]	Conformational Angles (deg) ϕ_{Pro}	ψ_{Pro}	ϕ_{Aib}	ψ_{Aib}	Hydrogen-Bond Parameters N- - -O (Å)	H—N—O (deg)	Total Energy (kcal/mol)
Type II[b]							
A_1	−50	110	40	50	2.93	24.9	−17.35
A_2	−60	110	50	40	3.05	16.7	−18.88
A_3	−70	120	60	30	3.18	18.2	−17.10
B_2	−60	110	50	40	3.05	16.7	−19.04
B_3	−70	120	60	30	3.18	18.2	−17.48
Type III[c]							
A_1	−50	−40	−40	−50	2.89	33.8	−14.92
A_2	−60	−30	−50	−40	3.00	26.7	−16.60
A_3	−70	−20	−60	−30	3.17	19.3	−14.50
B_2	−60	−30	−50	−40	3.00	26.7	−17.04
B_3	−70	−20	−60	−30	3.17	19.3	−15.00

[a] A_1 corresponds to the third conformation of Table II of Ref. 23 and similarly A_2 to 2, A_3 to 13, B_2 to 21, and B_3 to 18.

[b] Scan for Type II was done by varying ϕ_{Aib}, ψ_{Aib} from 0° to 100° and ψ_{Pro} from 60° to 180° at 5" intervals.

[c] Scan for Type III was done by varying ϕ_{Aib}, ψ_{Aib} from −100° to 0° and ψ_{Pro} from −70° to 0° at 5" intervals.

in Table III. Only the lowest-energy structures for each pyrrolidine ring conformation chosen are listed. The minimum-energy conformer among the C^{γ}_{exo} (A_1, A_2, A_3) family is not significantly different in energy from the lowest conformer in the C^{γ}_{endo} class (B_1, B_2) for both Type II and Type III 0-turns. It is seen from Table III that the lowest Type II conformation is approximately 2 kcal mol^{-1} more stable than the lowest Type III structure. The perspective diagrams for the most stable Type II and Type III 0-turns are shown in Fig. **1.** In order to further establish the stereochemical preferences of the Pro-Aib sequence, we have determined the solid-state conformation of Piv-Pro-Aib-NHMe by x-ray diffraction.

TABLE IV
Conformational Angles (deg)

Peptide Backbone		Pyrrolidine Ring	
$\omega_1(C^{\alpha}_1\text{-}C_1\text{-}N_2\text{-}C^{\alpha}_2)$	175.1(3)	$\theta(C^{\delta}_2\text{-}N_2\text{-}C^{\alpha}_2\text{-}C^{\beta}_2)$	0.8(6)
$\phi_2(C_1\text{-}N_2\text{-}C^{\alpha}_2\text{-}C_2)$	−57.8(6)	$\chi^1_2(N_2\text{-}C^{\alpha}_2\text{-}C^{\beta}_2\text{-}C^{\gamma}_2)$	−26.2(6)
$\psi_2(N_2\text{-}C^{\alpha}_2\text{-}C_2\text{-}N_3)$	139.3(5)	$\chi^2_2(C^{\alpha}_2\text{-}C^{\beta}_2\text{-}C^{\gamma}_2\text{-}C^{\delta}_2)$	40.5(7)
$\omega_2(C^{\alpha}_2\text{-}C_2\text{-}N_3\text{-}C^{\alpha}_3)$	−179.3(5)	$\chi^3_2(C^{\beta}_2\text{-}C^{\gamma}_2\text{-}C^{\delta}_2\text{-}N_2)$	−39.7(7)
$\phi_3(C_2\text{-}N_3\text{-}C^{\alpha}_3\text{-}C_3)$	61.4(7)	$\chi^4_2(C^{\beta}_2\text{-}C^{\delta}_2\text{-}N_2\text{-}C^{\alpha}_2)$	24.8(7)
$\psi_3(N_3\text{-}C^{\alpha}_3\text{-}C_3\text{-}N_4)$	25.1(7)		
$\omega_3(C^{\alpha}_3\text{-}C_3\text{-}N_4\text{-}C^{\alpha}_4)$	176.9(5)		

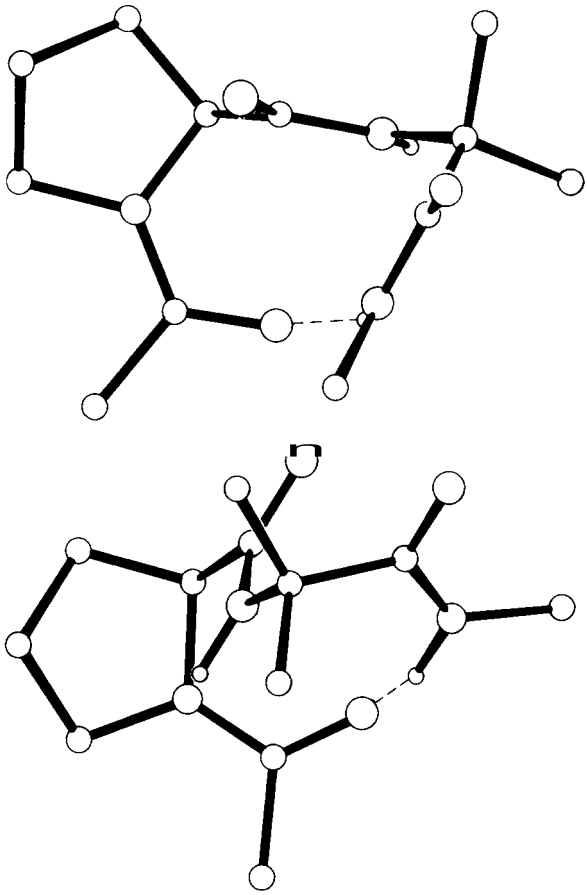

Fig. 1. Perspective diagrams for the theoretically calculated minimum-energy β-turn conformations of Ac-Pro-Aib-NHMe (top) Type II and (bottom) Type III. Only amide hydrogens are shown.

Molecular Structure of Piv-Pro-Aib-NHMe

A perspective diagram of the molecular structure of Piv-Pro-Aib-NHMe viewed down the a axis is shown in Fig. 2. The peptide backbone adopts a Type II 0-turn, stabilized by a **4 ← 1** intramolecular hydrogen bond, between the pivaloyl CO and methylamide NH groups. The N---O distance is 2.98 Å and the H-N-O angle is **30.5".** The conformational angles $\phi_{Pro} = -58°, \psi_{Pro} = +139°, \phi_{Aib} = 61°$, and $\psi_{Aib} = 25"$ are very close to the values expected for an ideal Type II β-turn. The bond lengths and bond angles are largely unexceptional and are summarized in Figs. **3** and **4,** and the conformational angles in Table IV. A view of the molecular packing in the crystal is shown in Fig. 5.

The molecules are held together by intermolecular hydrogen bonds involving the NH and CO groups of the Aib residue. The observed N---O distance is 2.90 Å and H-N-O angle is 20.5". The conformational angles

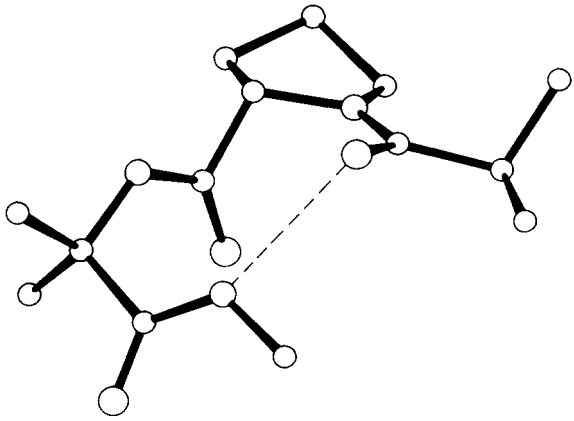

Fig. 2. Perspective view of the molecular structure of Piv-Pro-Aib-NHMe in the solid state.

obtained for Piv-Pro-Aib-NHMe for the peptide backbone and the pyrrolidine ring are compared in Table **V** with values determined earlier in related sequences and with the theoretically predicted minimum-energy structure. The Type II β-turn conformation in Piv-Pro-Aib-NHMe is very similar to those established earlier for *N*-isobutyl-L-prolyl-L-alanyl-isopropylamide and *N*-isobutyl-L-prolyl-D-alanylisopropylamide.[37] Furthermore, the Pro ring adopts the C^{γ} ***exo*** conformation in **all** three peptides. However, the related structure Ac-Pro-D-Ala-NHMe[38] has been shown to adopt a Type II 0-turn conformation with the Pro ring in the C^{γ}-*endo* state. These results are fully consistent with the theoretical calculations, which suggest that Pro ring puckering does not significantly affect backbone geometry. There is good agreement between the theoretically predicted conformation and the solid-state structure of Piv-Pro-Aib-NHMe.

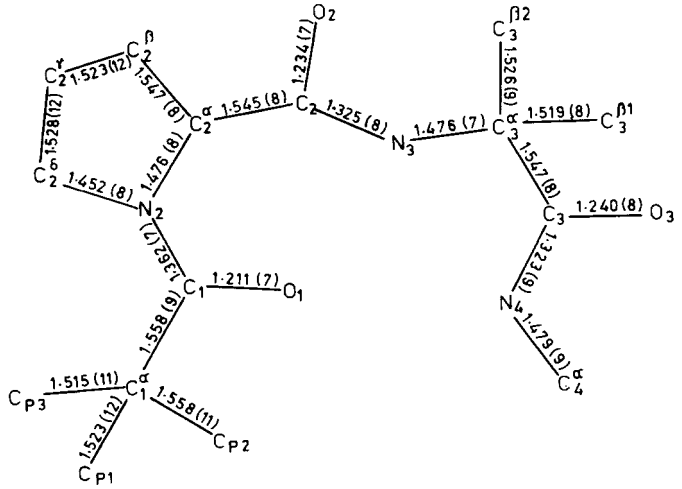

Fig. **3.** Bond lengths determined in Piv-Pro-Aib-NHMe. Values in parentheses are standard deviations.

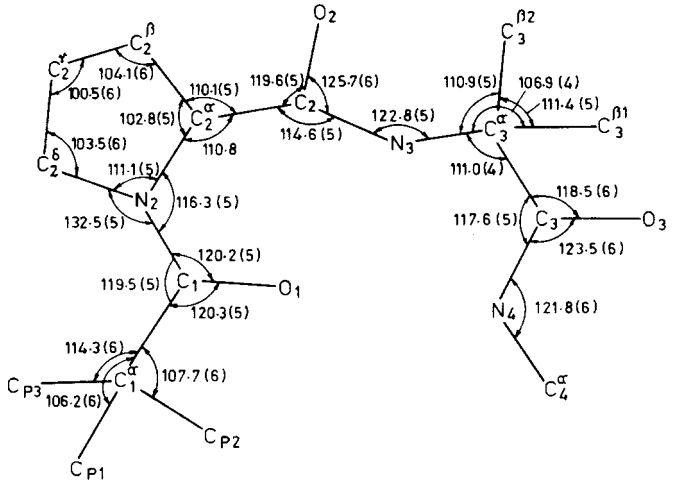

Fig. 4. Bond angles determined in Piv-Pro-Aib-NHMe. Values in parentheses are standard deviations.

The observation that the Pro-Aib segment adopts Type III β-turn conformations in the two tetrapeptides (Table V) is probably determined by the stereochemical requirements for repetitive β-turn or 3_{10} helical conformations. While Type III 0-turns can occur in succession, a Type II structure cannot be preceded by a β-turn and can be followed only by a Type III′ structure ($\phi_{i+1} = 60°, \psi_{i+1} = 30°, \phi_{i+2} = 60°, \psi_{i+2} = 30°$, where $i + 1$ and $i + 2$ are the corner residues). In Z-Aib-Pro-Aib-Ala-OMe,[7] the Aib-L-Pro segment is constrained to adopt only the Type III structure ($\phi_{\mathbf{Pro}}$

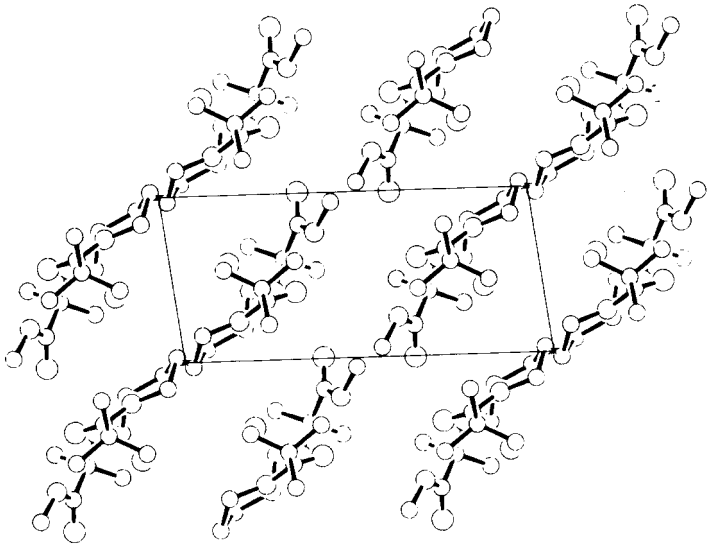

Fig. 5. Packing of Piv-Pro-Aib-NHMe in the crystal, viewed along the *b* axis.

TABLE V

Backbone and Proline Ring Conformational Angles (deg) for Pro-X β-Turns (X = Aih, L-Ala, or D-Ala)

Compound	Backbone				Proline Ring					Ref.
	ϕ_{Pro}	ψ_{Pro}	ϕ_X	ψ_X	δ	χ_1	χ_2	χ_3	χ_4	
Type II										
Piv-Pro-Aib-NHMe	−58	139	61	25	0.8	−26	40	−40	25	This study
Ac-Pro-Aib-NHMe[a]	−60	110	50	40	A 0	−20	33	−31	20	This study
(theoretical)					B 0	20	**−33**	31	**−20**	
Ibu-Pro-D-Ala-NIpr	**−62**	137	96	**3**	0	−17	−28	−29	18	*3*
Ibu-Pro-L-Ala-NIpr	**−59**	136	66	14	0	−4	6	−6	3	*37*
N-Ac-Pro-D-Ala-NHMe	−66	127	75	**12**	−3	22	**−33**	**31**	−18	*38*
Type III										
Boc-Pro-Aib-Ala-Aib-OMe	−53	−39	−48	−41	15	−30	35	−25	5	10
Z-Aib-Pro-Aib-Ala-OMe	−55	−36	−72	−11	7	−27	*37*	−31	14	7
Ac-Pro-Aib-NHMe[a]	−60	−30	−50	−40	**A 0**	−20	33	−31	20	This study
(theoretical)					B 0	20	−33	**31**	−20	

[a] The two sets of angles for the proline ring in the theoretically computed structures correspond to the lowest conformations for C^{γ}-*exo* (A) and C^{γ}-*endo* (B) puckering.

$\sim -60°$), necessarily forcing the Pro-Aib segment to also adopt a Type III conformation. In Boc-Aib-Ala-Aib-OBz[10] the presence of L-Ala renders Type III′ conformations less favored for the Aib-Ala β-turn when compared with a Type III structure. This, in turn, requires the Pro-Aib segment to adopt a Type III conformation. Thus, while Pro-Aib segments may intrinsically favor Type II β-turns, requirements for further optimal folding of the peptide chain in oligopeptides may result in the observation of Type III conformations.

These studies provide an illustrative example of the influence of long-range factors in determining peptide conformations. Peptides containing Pro and Aib residues can be used to specifically generate Type I, Type II (this study), and Type III 6-turn structures, which may prove valuable in further developing CD,[39] nmr,[40] and Raman[41] methods as diagnostic tools in studies of β-turn conformations.

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