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Crystal Structure and Conformation of N-(t-Butoxycarbonyl)-L-Isoleucyl-L-Valine Methyl Ester (Boc-Ile-Val-OMe)

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Crystal Structure and Conformation of N-(t-Butoxycarbonyl)-L-Isoleucyl-L-Valine Methyl Ester (Boc-Ile-Val-OMe)[†]

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N-(t-Butoxycarbonyl)-L-Isoleucyl-L-Valine Methyl ester, $C_{17}H_{32}N_2O_5$, F.W. = 344.46, crystallizes in hexagonal space group $P6_5$ with parameters, $a = b = 20.285(5)$, $c = 27.160(5)$ Å, $V = 9679.1(6)$ Å^3, $Z = 18$, $Dcal = 1.064$ Mg cm^{-3}, $\lambda(CuK\alpha) = 1.5418$ Å, $\mu(CuK\alpha)$. The dipeptide Boc-Ile-Val-OMe has three crystallographically independent molecules (A, B, and C) in the asymmetric unit. The peptide units are in trans conformation and the three molecules adopt an extended conformation. The side chains of isoleucine and valine for the three molecules A, B, and C take up g^-t, g^-g^-, and g^-t; and g^-g^+, g^+t, and tg^- conformations, respectively. The molecules form an infinite ribbon of β-sheet structure, which in turn forms helical arrangement along the c-direction. They are stabilized by N–H ··· O and C–H ··· O types of intermolecular interactions.

Keywords: conformation; crystal; dipeptide; hydrogen bonds; isoleucine; valine

INTRODUCTION

Amino acids are building blocks of enzymes, antibodies, and nucleoproteins. They act as neurotransmitters and help vitamins and minerals to perform their tasks. Structural studies on peptide fragments are

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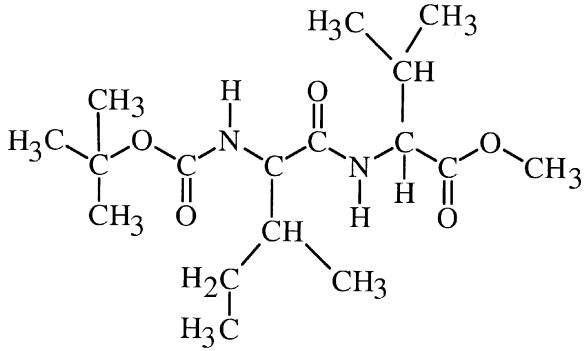

FIGURE 1 Chemical diagram of Boc-Ile-Val-OMe.

useful in theoretical-modeling studies designed to predict the three-dimensional structure of a protein purely from its amino-acid sequence. The study of their structure and conformation provides valuable information to understand the structure–activity relationship of bioactive peptides at the molecular level with the aim of producing and developing materials relevant to pharmacology and medicinal chemistry.

The dipeptide sequence Boc-Ile-Val-OMe occurs as C-terminal part of HIV-1 protease substrate (Ser-Gln-Asn-Tyr-Pro-Ile-Val, which is a gag fragment, 129–135), the minimum sequence to exhibit the activity [1]. On the basis of this importance, the crystal structure and conformation of the dipeptide (Fig. 1) has been determined.

EXPERIMENTAL

Crystals of Boc-Ile-Val-OMe were obtained by slow evaporation procedure using n-hexane as solvent. A thin, transparent, needle-shaped crystal of dimension $0.15 \times 0.15 \times 0.23$ mm was chosen for intensity data collection on an Enraf-Nonius CAD-4 diffractometer with graphite monochromated CuKα($\lambda = 1.5418$ Å) radiation. Accurate unit cell parameters were obtained from 20 reflections in the range $16 \leq \theta \leq 28^\circ$ from least-squares refinement. The intensities were measured to a maximum θ of 50° in the $\omega/2\theta$ scan mode. Three standard reflections, which monitored every hundred reflections for an intensity check, showed 3% decay for which decay correction was applied. Out of 3032 independent reflections collected, 2521 reflections with $I \geq 2\sigma(I)$ were used for structure analysis. The intensities were corrected for Lorentz and polarization effects.

The structure was solved by direct methods using the program SHELXS [2]. The structure was refined on F^2 by full-matrix least-squares procedures using the program SHELXL [3]. The nonhydrogen atoms were refined anisotropically and all the hydrogen atoms were identified from the difference Fourier map and were not refined. The final cycle of refinement converged to $R_1 = 0.097$ for the observed reflections. The maximum and minimum heights in the final difference Fourier map were found to be $\pm 0.32 e/Å^3$, respectively. The geometrical calculations and the figures were drawn by using the programs PARST [4] and PLATON [5] respectively.

The crystal data and relevant parameters pertaining to data collection, data reduction, structure solution, and least-squares refinement for Boc-Ile-Val-OMe are listed in Table 1. There are three crystallographically independent molecules (A, B, and C) in the asymmetric unit.

TABLE 1 Crystal Data

Parameter	Boc-Ile-Val-OMe
Empirical formula	$C_{17}H_{32}N_2O_5$
Molecular weight	344.46
Crystal system	Hexagonal
Space group	$P6_5$
Cell constants	
a	20.285(5) Å
b	20.285(5) Å
c	27.160(5) Å
Volume	9679.1(6) $Å^3$
Z	18 (three molecules in the asymmetric unit)
Dcal	1.064 g cm^{-3}
F(000)	3384
μ (CuKα)	6.4 cm^{-1}
Scan width	$1.2 + 0.14 \tan\theta$
Max. time spent on each reflection	60 sec
Standard reflections	(1 1 11), (2 5 3), (4 0 10)
Decay of standards	3% (decay correction applied)
Reflections measured	3032
$2\theta_{max}$	100
h	$0 \rightarrow 20$
k	$0 \rightarrow 20$
ℓ	$0 \rightarrow 27$
Reflections observed	2521 [Fo $\geq 2\sigma$ (Fo)]
No. of parameters	217 ($240 \times 3 = 720$)
Weighting scheme	Unit weights
$S[\Sigma w(\Delta F)^2/(N\text{-}P)]^{1/2}$	3.63
Final Residuals R	0.097
Max. and min. ($\Delta\rho$) e/$Å^3$	± 0.32

During the initial stages of isotropic refinement, the carboxy terminal oxygen atom O5′ of molecule B showed large thermal vibration and the bond distances involving the O5′ atom had abnormal values. The above features indicate that the O5′ atom might be disordered. A difference Fourier map was computed at this stage, leaving out the O5′ atom from the structure-factor calculations. The map showed two alternate positions, which suggest the possibility of disorder for the oxygen atom. The occupancy factors for the two alternate positions of oxygen atom (named as O5′ and O5″) were kept the same at a value of 0.5 and refined. The refinement of occupancy factors of O5′ and O5″ at different stages indicated that the assumed weights were correct for the two alternate positions. In the initial stages of refinement, the trial structure was refined in the space group $P6_1$, which inicated a D-configuration for both isoleucine and valine residues, which implies either a reversal for the sign of the z-coordinates or a change of space group to $P6_5$. The former option led to unacceptable intermolecular short contacts and hence the space group was changed to $P6_5$.

In addition, during the course of refinement, the two C^{γ} atoms of the valine side chain in molecules A and C showed large temperature factors, which suggest conformational disorder. However, difference Fourier maps computed by leaving out these two atoms did not show any alternative sites. Instead, the peaks corresponding to the deleted atoms showed up as slightly elongated and drawn out. The original positions of the C^{γ} atoms were therefore retained and further full-matrix least-squares refinement was carried out.

RESULTS AND DISCUSSION

The perspective views of the molecules are shown in Figure 2. The estimated standard deviations in bond lengths and bond angles are high because of the poor quality of the crystal, high mosaic spread, and a rather poor ratio of parameters to observations. Because it was difficult to regrow the crystals, repeating the data collection with better crystals has not been possible. However, the structure analysis is unambiguous and accurate enough to fulfill the important objective of deducing the conformational features of the molecule.

The peptide units are in trans conformation [$\omega = -176(1), -177(1)$ and $175(1)^{\circ}$] with only slight deviations from planarity [$\Delta\omega = 4(1)$, $3(1)$, and $5(1)^{\circ}$] for the molecules A, B, and C respectively. Table 2 shows the backbone torsion angles for the three molecules.

The torsion angles indicate that all the three molecules adopt an essentially extended conformation although there are significant differences among them. The mean plane of the carboxyl group makes

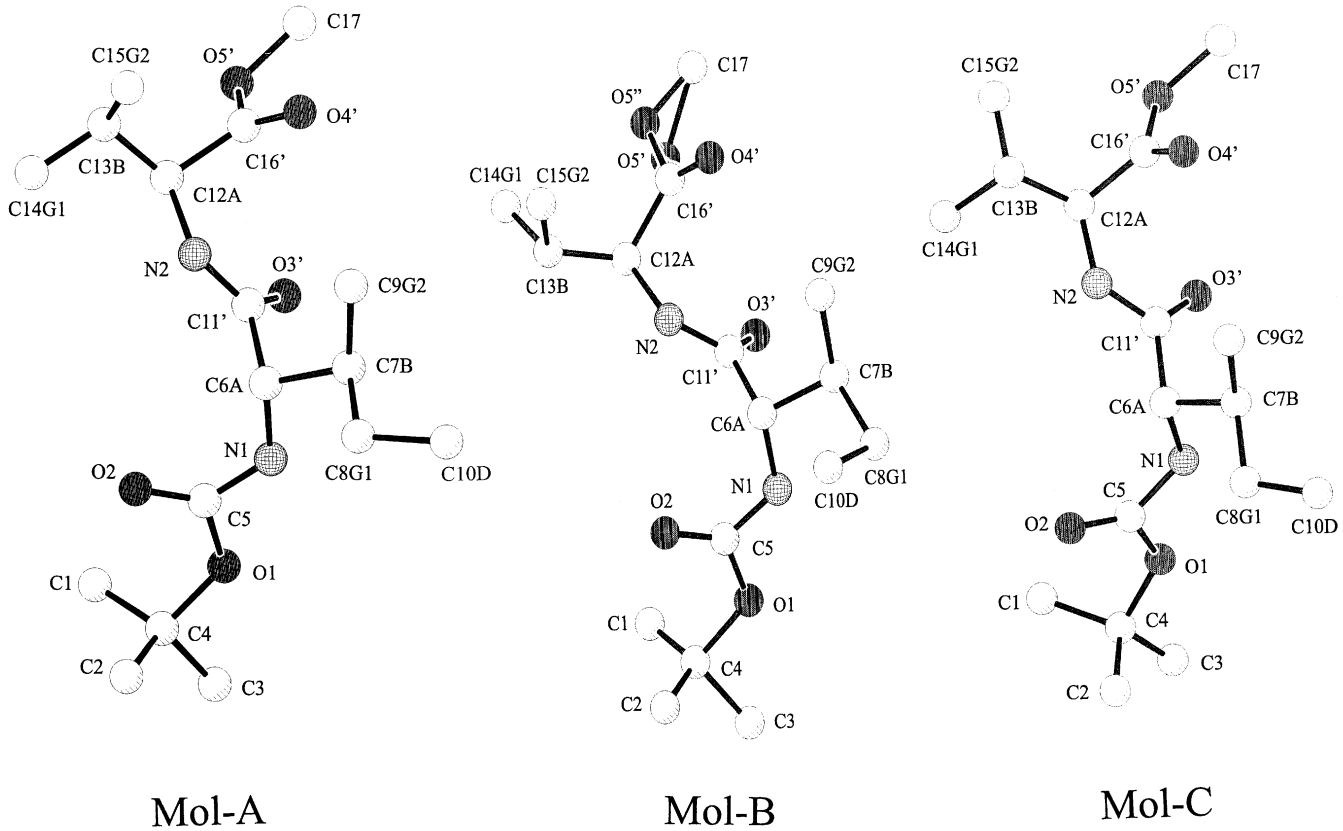

FIGURE 2 Perspective view of the molecules A, B, and C.

TABLE 2 Backbone Torsion Angles

Molecule	ϕ_1	ψ_1	ϕ_2	ψ_2
A	$-86(2)^\circ$	$138(1)^\circ$	$-59(2)^\circ$	$149(2)^\circ$
B	$-76(2)^\circ$	$128(1)^\circ$	$-74(2)^\circ$	$173(2)^\circ$
C	$-116(1)^\circ$	$137(1)^\circ$	$-57(2)^\circ$	$138(2)^\circ$

a dihedral angle of 106(1), 99(2), and 101(2)° with the adjacent peptide plane for molecules A, B, and C respectively.

The conformation of the Boc group is characterized mainly by two dihedral angles, ω_0 and θ_0 [7]. In ester groupings, CO−O−C, two stable conformations are found with respect to rotation around each of these two bonds, that is, *trans* and *cis* with dihedral angles near 180° and 0° [8]. In the present study, the observed conformational angles ω_0 and θ_0 [$\omega_0 = 175(1)$, 176(1), and $180(1)^\circ$; $\theta_0 = 175(1)$, $-173(1)$ and $-180(1)^\circ$] indicate that the Boc group adopts the *trans-trans* conformation in all the three molecules A, B, and C. The three methyl groups are staggered with respect to the O1-C4 bond in all the three molecules.

A statistical analysis of the side chain conformations of isoleucine in proteins reveals (i) the prefernce of χ_1 and χ_2 for angles near +60° and 180°, with the ethyl group in the g^+ position and the methyl group in the t position; (ii) that the C^δ atom is generally trans to C^α atom; (iii) that the g^+t conformation is most frequent (47%), g^+g^+ comes next (16%), and g^-t and tt together comprise most of the remaining cases (24%) [9]. In the present study, the conformational angles $\chi_1(\chi_{11})$ and χ_2 are $-69(2)$ and $165(2)^\circ$ for molecule A, $-55(1)$ and $-63(2)^\circ$ for molecule B, and $-64(2)$ and $168(2)^\circ$ for molecule C, representing g^-t, g^-g^-, and g^-t conformations respectively for the three molecules A, B, and C. The molecules A and C assume one of the preferred g^-t conformations whereas the molecule B exists in the less favored g^-g^- conformation as observed in Ile-Val fragment of apolar decapeptide [10] and in Boc-Ile-Ala-Obzl [11].

The relevant torsion angles required to describe the conformation of the side chain of valine are χ_{11} ($N-C^\alpha-C^\beta-C^{\gamma 1}$) and χ_{12} ($N-C^\alpha-C^\beta-C^{\gamma 2}$) [12]. Because $C^{\gamma 1}$ and $C^{\gamma 2}$ are linked to C^β in a tetrahedral configuration, the value of $\chi_{12} = \chi_{11} + 120^\circ$. A survey of the literature reveals that the valine side chain can assume any one of the three configurations, namely, g^+t, g^- g^+, or tg^-, with tg^- being the most frequent, g^- g^+ occurs with moderate propensity, and g^+t has low probability of occurrence [13]. The conformational angles χ'_{11} [N2-C12A-C13B-C14G1] and χ''_{12} [N2-C12A-C13B-C15G2] are $-64(2)$

and 61(2)° for molecule A, 71(2) and –168(1)° for molecule B, and −179(2) and −68(2)° for molecule C, representing g^-g^+, g^+t, and tg^- conformations respectively. It is interesting to observe that the molecule B adopts the less favored g^+t conformation as observed in Boc-Pro-Val-Gly-NH_2 [13]. The side chain conformations of the valine residues are usually different if the structure contains more than one crystallographically independent molecule in the asymmetric unit [14,15]. As exhibited in many earlier studies, in the present study the side chain conformation of valine residues showed significant differences among the molecules A, B, and C.

It has been found that the bond angle τ ($NC^{\alpha}C'$) of valine residue is smaller than the regular tetrahedral value because of the presence of the bulky hydrophobic side chain [13]. In the present study, the τ-angle of molecules A and B are compressed by about 2° and 5° respectively whereas in molecule C, it is broadened by 2°. This elongation in molecule C can be attributed to the steric interaction between the propyl and carboxyl groups.

Figure 3 shows the packing of molecules Boc-lle-Val-OMe viewed down the c-axis. Table 3 lists the hydrogen bonding parameters observed in the structure. The molecules are stabilized by $N-H\cdots O$ and $C-H\cdots O$ types of interactions. Molecules related by the six-fold

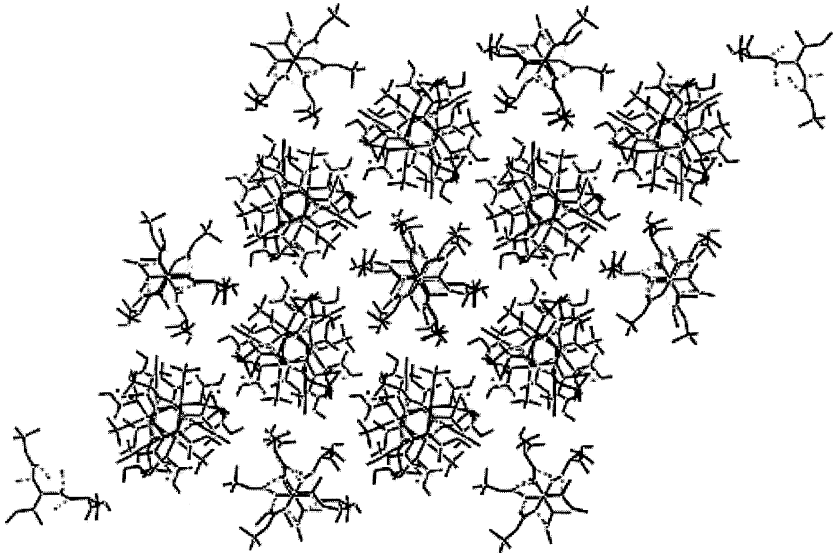

FIGURE 3 Packing diagram of the molecules viewed down c-axis.

TABLE 3 Hydrogen Bonding Parameters Observed in the Molecule of Boc-lle-val-OMe

D-H···Ac*	D···Ac	H···Ac	<D-H···Ac	Position of acceptor
N1(A)-H···O2 (A)	2.93(1)	1.92(1)	162.9(6)	y, −x+y+1, z+1/6
N2(A)-H···O3′ (A)	2.85(2)	2.26(1)	119.1(8)	x−y+ 1, x, z−1/6
N2(B)-H···O3′ (C)	2.89(2)	2.06(1)	135.2(7)	−x+y, −x+1, z−1/3
N1(C)-H···O2′ (B)	2.91(1)	2.34(1)	119.7(7)	−y+1, x−y+1, z−1/3
N2(C)-H···O3′ (B)	2.88(2)	2.00(1)	147.8(8)	x, y, z
C6A(A)-H···O3′ (A)	3.43(2)	2.47(1)	144.6(8)	x−y+1, x, z−1/6
C17(A)-H···O4′ (B)	3.39(2)	2.48(1)	152(1)	y−1, −x+y, z+1/6
C1(B)-H···O4′ (C)	3.44(3)	2.52(2)	144(1)	y−1, −x+y, z+1/6
C6A(B)-H···O3′ (C)	3.31(2)	2.47(1)	142.8(7)	−x+y, −x+1, z+1/3

*D = Donor; H = Hydrogen; Ac = Acceptor. A, B, and C in parentheses represent molecules A, B, and C respectively.

screw axis interact through backbone N−H···O hydrogen bonds to form an infinite ribbon of β-sheet structure, which in turn forms the helical arrangement along c-direction (Fig. 4).

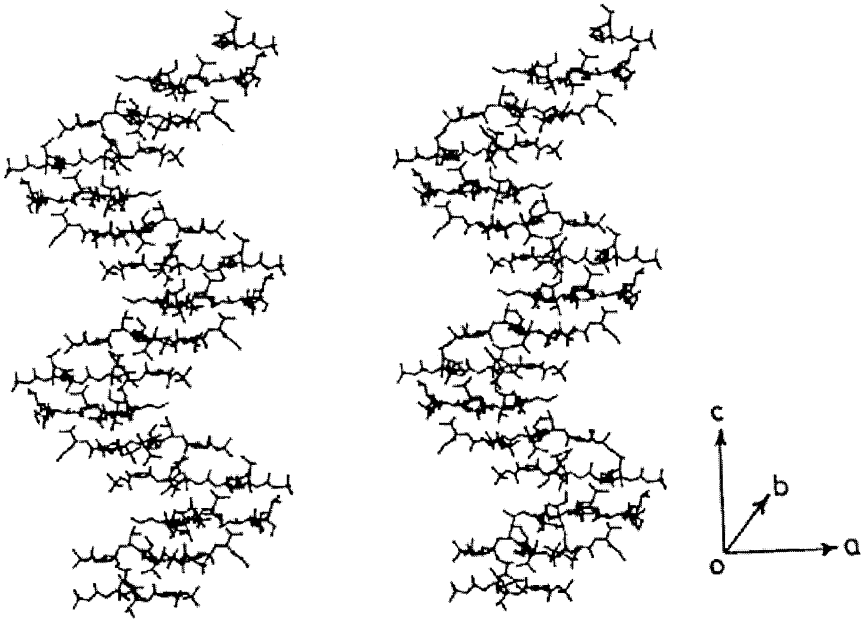

FIGURE 4 Stereoview of the dipeptide forming helical arrangement along c-direction.

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