Crystal Structure and Conformation of *N*-(*t*-Butoxycarbonyl)-L-valine *N*-Hydroxysuccinimide Ester

N. SUKUMAR, M. N. PONNUSWAMY,* and R. JAYAKUMAR†
Department of Crystallography and Biophysics, University of Madras, Guindy Campus, Madras-600025, India
† Chemical Laboratory, Central Leather Research Institute, Madras-600020, India

(Received November 19, 1992)

Synopsis. An X-ray crystal structure analysis of the title compound ($C_{14}H_{22}N_2O_6$) was carried out. There are two molecules (A & B) in the asymmetric unit. In both molecules valine side chains exist in a staggered conformation. The molecules in the crystalline state are stabilized by N–H···O and C–H···O hydrogen bonding.

The valine-containing peptides have the least tendency to form a β-turn, but have a high propensity to form a β-sheet structure.[1,2] Although the succinimide ring, due to its cyclic nature, forces its succeeding residue to adopt a β-turn conformation,[3—5] there is no evidence available regarding its effect on the preceding residue. The present study was aimed at gaining more information concerning the effect of the succinimide ring over the preceding residue of the polypeptide chain.

Experimental

Colorless needle crystals of dimension 0.05×0.13×0.18 mm were grown from methanol by slow evaporation at room temperature. A preliminary examination of the crystals and intensity data collection were performed on an Enraf–Nonius CAD4 diffractometer equipped with graphite monochromated Cu $K\alpha$ radiation (λ=1.5418 Å). Accurate cell constants were obtained from a least-squares refinement of the setting angles of 23 reflections in the range $19\leq 2\theta\leq 58°$. The crystal data are as follows: M_r=314.34, monoclinic, space group $P2_1$, a=8.365(4), b=17.466(3), c=11.582(2) Å, β=90.58(2)°, V=1692.1(9) Å^3, Z=4, D_x=1.234 g cm^{-3}, μ=7.1 cm^{-1}, $F(000)$=672.

A total of 2825 unique intensity data was measured at 298 K with the $w/2\theta$ scan technique to a maximum 2θ of 140°. Data were corrected for Lorentz and polarization effects as well as for empirical absorption based on a series of ψ scans. The transmission coefficients ranged from 0.840 to 0.998 with an average value of 0.944; 2174 reflections with intensities greater than $2.5\sigma(I)$ were used for the structure solution and refinement. Three standard reflections were monitored for every two hours, but didn't show any significant variation. The structure solution was carried out by direct methods using SHELXS86.[6] The structure was refined by a full-matrix least-squares technique.[7] All of the non-hydrogen atoms were refined anisotropically; the hydrogen atoms (obtained from difference Fourier) were refined isotropically. Convergence was reached at a final R=0.043 and wR=0.047, where $w=1.0/[\sigma(F)^2+0.00398F^2]$ and S=0.763. The final Fourier map was totally featureless, with maximum and minimum $\Delta\rho$ of 0.15 e/Å^3 and −0.19 e/Å^3, respectively. The atomic-scattering factors were taken from Cromer & Weber.[8] The computer programs used were PARST[9] and PLUTO.[10] All calculations were carried out using VAX730

Table 1. Positional and Equivalent Thermal Parameters of the Non-Hydrogen Atoms with Their esd's in Parentheses

Atom	x	y	z	U_{eq}[a]
Molecule A				
O1	−0.1642(3)	−0.1459(2)	0.2931(3)	0.059(2)
O2	0.0855(3)	−0.1023(2)	0.3356(3)	0.061(2)
O3′	0.3724(4)	−0.1879(3)	0.0255(3)	0.084(2)
O4	0.1368(3)	−0.2480(2)	−0.0011(2)	0.056(2)
O5	0.2622(4)	−0.3769(2)	−0.1128(3)	0.069(2)
O6	0.0621(5)	−0.1377(2)	−0.1635(3)	0.088(3)
N1	0.0419(4)	−0.1988(2)	0.2090(3)	0.048(2)
N2	0.1737(4)	−0.2525(2)	−0.1179(3)	0.052(2)
C1	−0.4196(7)	−0.1216(4)	0.3554(7)	0.102(5)
C2	−0.2202(8)	−0.0154(4)	0.3556(7)	0.098(4)
C3	−0.1911(8)	−0.1221(4)	0.5001(5)	0.094(4)
C4	−0.2481(5)	−0.0991(3)	0.3797(4)	0.059(3)
C5	−0.0054(5)	−0.1452(2)	0.2843(3)	0.044(2)
C6A	0.2100(5)	−0.2145(3)	0.1907(3)	0.052(2)
C7B	0.2698(7)	−0.2894(3)	0.2489(5)	0.074(3)
C8G1	0.2463(7)	−0.2847(4)	0.3776(5)	0.091(4)
C9G2	0.1998(9)	−0.3602(4)	0.1995(6)	0.099(5)
C10′	0.2532(5)	−0.2129(3)	0.0654(4)	0.053(2)
C11	0.1246(6)	−0.1962(3)	−0.1922(4)	0.060(3)
C12	0.1646(6)	−0.2251(3)	−0.3110(4)	0.066(3)
C13	0.2293(5)	−0.3061(3)	−0.2936(4)	0.061(3)
C14	0.2273(5)	−0.3205(3)	−0.1662(4)	0.053(2)
Molecule B				
O1	0.2363(4)	0.0410(2)	0.4997(2)	0.069(2)
O2	0.4219(4)	0.1206(2)	0.4226(3)	0.070(2)
O3′	0.1499(3)	0.1806(2)	0.2207(3)	0.065(2)
O4	0.3885(3)	0.1956(2)	0.1353(2)	0.048(2)
O5	0.2237(4)	0.2361(2)	−0.0634(3)	0.065(2)
O6	0.4572(5)	0.3268(2)	0.2649(3)	0.084(2)
N1	0.2718(4)	0.0415(2)	0.3122(3)	0.054(2)
N2	0.3391(4)	0.2691(2)	0.1096(3)	0.049(2)
C1	0.1451(9)	0.0155(4)	0.6838(5)	0.097(4)
C2	0.4375(8)	0.0310(5)	0.6530(5)	0.100(4)
C3	0.2509(9)	0.1449(4)	0.6416(5)	0.092(4)
C4	0.2713(6)	0.0603(3)	0.6207(4)	0.064(3)
C5	0.3209(5)	0.0722(3)	0.4145(3)	0.053(2)
C6A	0.3329(5)	0.0726(2)	0.2069(3)	0.047(2)
C7B	0.2852(6)	0.0213(3)	0.1027(4)	0.059(3)
C8G1	0.1062(7)	0.0218(4)	0.0777(4)	0.080(3)
C9G2	0.3783(7)	0.0411(3)	−0.0042(4)	0.077(3)
C10′	0.2738(5)	0.1536(2)	0.1924(3)	0.046(2)
C11	0.3821(5)	0.3310(3)	0.1750(4)	0.057(3)
C12	0.3131(7)	0.4002(3)	0.1159(5)	0.076(3)
C13	0.2485(6)	0.3709(3)	0.0008(4)	0.065(3)
C14	0.2638(5)	0.2852(3)	0.0044(4)	0.052(2)

a) $U_{eq}=1/3(U_{11}+U_{22}+U_{33})$.

Table 2. Bond Distances (Å) and Bond Angles (°) Involving Non-Hydrogen Atoms with Their esd's in Parentheses

Atoms	Lengths		Atoms	Lengths	
	Mol-A	Mol-B		Mol-A	Mol-B
O1–C4	1.477(6)	1.468(5)	O1–C5	1.333(5)	1.336(5)
O2–C5	1.218(5)	1.198(6)	O3′–C10′	1.187(6)	1.188(5)
O4–N2	1.393(4)	1.380(5)	O4–C10′	1.379(5)	1.382(5)
O5–C14	1.198(6)	1.208(6)	O6–C11	1.196(6)	1.213(6)
N1–C5	1.342(5)	1.360(5)	N1–C6A	1.450(5)	1.434(5)
N2–C11	1.367(6)	1.366(6)	N2–C14	1.389(6)	1.395(6)
C1–C4	1.511(7)	1.509(9)	C2–C4	1.507(9)	1.525(9)
C3–C4	1.523(8)	1.507(9)	C6A–C7B	1.552(7)	1.552(6)
C6A–C10′	1.499(6)	1.508(5)	C7B–C8G1	1.508(8)	1.522(8)
C7B–C9G2	1.481(9)	1.510(7)	C11–C12	1.506(7)	1.502(7)
C12–C13	1.527(7)	1.522(7)	C13–C14	1.497(7)	1.503(7)

Atoms	Angles		Atoms	Angles	
	Mol-A	Mol-B		Mol-A	Mol-B
C4–O1–C5	121.8(3)	120.6(3)	N2–O4–C10′	113.9(3)	112.9(3)
C5–N1–C6A	121.3(4)	119.0(3)	O4–N2–C14	121.0(4)	120.5(3)
O4–N2–C11	120.2(4)	122.7(3)	C11–N2–C14	117.2(4)	116.0(4)
C2–C4–C3	112.5(5)	113.2(5)	C1–C4–C3	112.9(5)	110.5(5)
C1–C4–C2	111.5(5)	110.4(5)	O1–C4–C3	109.2(4)	110.9(4)
O1–C4–C2	109.6(4)	109.3(4)	O1–C4–C1	100.7(4)	102.1(4)
O2–C5–N1	124.0(4)	123.6(4)	O1–C5–N1	110.1(4)	109.0(4)
O1–C5–O2	125.9(4)	127.5(4)	N1–C6A–C10′	112.4(4)	109.3(3)
N1–C6A–C7B	113.9(4)	110.6(3)	C7B–C6A–C10′	110.9(4)	112.0(3)
C6A–C7B–C9G2	114.3(5)	112.0(4)	C6A–C7B–C8G1	109.8(4)	112.9(4)
C8G1–C7B–C9G2	111.9(5)	110.9(4)	O4–C10′–C6A	110.9(3)	108.9(3)
O3′–C10′–C6A	126.6(4)	128.9(4)	O3′–C10′–O4	122.5(4)	122.2(4)
O6–C11–N2	124.7(4)	124.0(4)	N2–C11–C12	105.4(4)	106.6(4)
O6–C11–C12	129.9(5)	129.3(5)	C11–C12–C13	105.8(4)	105.1(4)
C12–C13–C14	106.2(4)	106.3(4)	N2–C14–C13	105.1(4)	105.2(4)
O5–C14–C13	129.9(5)	131.8(4)	O5–C14–N2	125.0(4)	123.0(5)

and MicroVAX II computers available in the department.

Results and Discussion

The final positional parameters of the atoms are listed in Table 1.[11] A schematic diagram of the molecules is shown in Fig. 1. The bond lengths, bond angles, and selected torsion angles are given in Tables 2 and 3. The differences in the bond lengths and bond angles between the two molecules are within the estimated errors, and

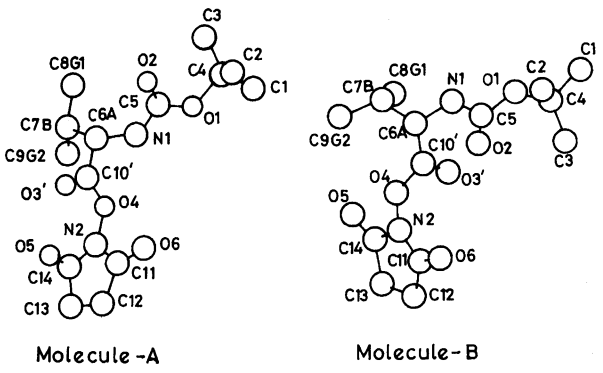

Fig. 1. Schematic representation of the molecules.

Table 3. Some Selected Torsion Angles (°) Involving Non-Hydrogens with Their esd's in Parentheses

	Atoms	Angles	
		Mol-A	Mol-B
θ	C4–O1–C5–N1	172.8(4)	176.2(4)
ω	O1–C5–N1–C6A	−174.7(3)	172.9(3)
ϕ	C5–N1–C6A–C10′	−128.7(4)	−65.9(5)
ψ	N1–C6A–C10′–O4	−41.1(5)	147.9(3)
ω	O4–N2–C14–C13	171.5(3)	−172.5(3)
χ_{11}	N1–C6A–C7B–C8G1	−60.2(6)	67.4(5)
χ_{12}	N1–C6A–C7B–C9G2	66.5(6)	−166.5(4)
γ_1	C2–C4–O1–C5	63.6(5)	−65.4(6)
γ_2	C1–C4–O1–C5	−178.8(4)	177.8(4)
γ_3	C3–C4–O1–C5	−59.7(5)	60.1(6)
γ_4	C4–O1–C5–N1	172.8(4)	176.2(4)

are in good agreement with the average values of the peptides reported in the literature.[12,13] The peptide unit is nearly planar. As can be seen from the values of ϕ and ψ (Table 3), molecules A and B adopt a structure that is very similar to an extended and a folded conformation, respectively. The peptide linkage exists in the *trans* conformation. The value of ω (O1–C5–N1–C6A)

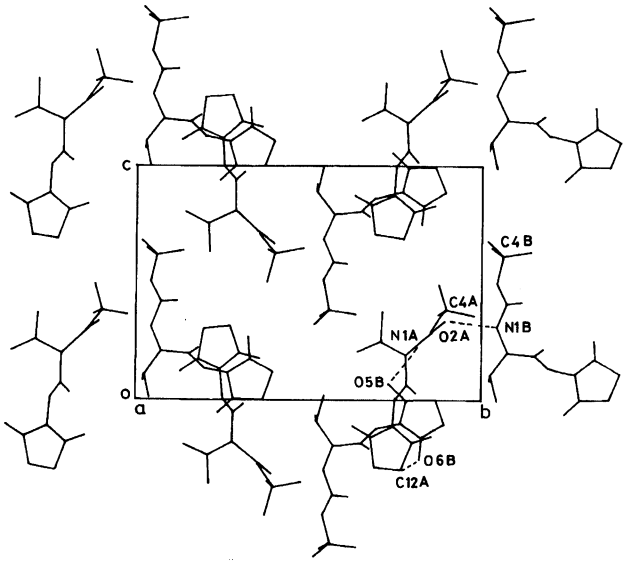

Fig. 2. Packing of the molecules viewed down the a-axis. The hydrogen bonding [N1(B)···O2(A) (x, y, z)=2.970(5) Å, N1(A)···O5(B) ($-x$, $y-1/2$, $-z$)=2.999(5) Å, C12(A)···O6(B) ($-x+1$, $y-1/2$, $-z$)=3.329(6) Å.] A and B in the parentheses represent molecules A and B, respectively.

for molecules A and B are −174.7(3)° and 172.9(3)°, respectively.

In both molecules, the t-boc group adopts a trans–trans conformation about the θ (O1–C5) and ω (C5–N1) bonds (172.8(4) : −174.7(3)° for molecule-A and 176.2(4) : 172.9(3)° for molecule-B, respectively).[14] The succinimide ring shows a considerable flexibility, as observed in other related structures.[3—5] The conformation of the succinimide ring can be described by a puckering amplitude of 0.053(4) Å and a phase (ϕ) of 10.89° for molecule A and 0.079(5) Å and −120.29° for molecule B, calculated for the sequence N2, C11, C12, C13, and C14.[15] The N2 atom is puckered in molecule-A, whereas in molecule-B the C12 atom is puckered. The value of ϕ is intermediate between an envelope and a twist conformation for both molecules.

The side-chain conformations for valine in the two molecules show significant differences, as observed in most of the valine containing peptides which have two crystallographically independent molecules in the asymmetric unit.[16] The valine γ-atoms are found to occur in a possible staggered conformation.[17] In the present study, they also occurred in a staggered conformation with the following values: χ_{11}(N1–C6A–C7B–C8G1)= −60.2(6)° and χ_{12}(N1–C6A–C7B–C9G2)=67.4(5)° for molecule A and 66.5(6)°, −166.5(4)° for molecule B.

Energy calculations of the molecules were performed using the DTMM program.[18] The energies of molecules A and B were found to be 10.6 kcal mol^{-1} and 9.3 kcal mol^{-1}, respectively, thus indicating that both the conformations are energetically preferable.

The molecular packing viewed down the a-axis is shown in Fig. 2. The molecules in the crystalline state are stabilized by N–H···O and C–H···O hydrogen bonding. The molecules are aggregated into separate layers in helical fashion along the b-axis.

One of the authors (NS) is indebted to the Council of Scientific and Industrial Research, India, for the award of Senior Research Fellowship.

References

1) P. Y. Chou and G. D. Fasmen, *Adv. Enzymol. Relat. Area Mol. Biol.*, **47**, 45 (1978).
2) G. E. Schulz and R. H. Schirmer, "Principle of Protein Structure," Springer-Verlag, New York (1978).
3) S. Capasso, C. A. Mattia, L. Mazzarella, and A. Zagari, *Int. J. Pept. Protein Res.*, **23**, 248 (1984).
4) S. Capasso, C. A. Mattia, L. Mazzarella, and A. Zagari, *Int. J. Pept. Protein Res.*, **24**, 85 (1984).
5) S. Capasso, L. Mazzarella, F. Sica, and A. Zagari, *Int. J. Pept. Protein Res.*, **24**, 588 (1984).
6) G. M. Sheldrick, "SHELXS86; Program for the Solution of Crystal Structures," University of Göttingen, Germany (1986).
7) G. M. Sheldrick, "SHELX76; A Program for Crystal Structure Determination," University of Cambridge, England (1976).
8) D. T. Cromer and J. T. Weber, "International Tables for X-Ray Crystallography," The Kynoch Press, Birmingham (1974), Vol. IV, Table 2.2B.
9) M. Nardelli, "PARST A System of Computer Routines for Calculating Molecular Parameters from the Results of Crystal Structure Analysis," *Comput. Chem.*, **7**, 95 (1983).
10) W. D. S. Motherwell and W. Clegg, "PLUTO; Program for Plotting Molecular and Crystal Structure," University of Cambridge, England (1978).
11) A list of the structure factors, anisotropic thermal parameters, H-atom coordinates, bond lengths and bond angles involving H-atoms, torsion angles, conformational parameters of the succinimide ring, and least-squares planes data are deposited as Document No. 66018 at the Office of the Editor of Bull. Chem. Soc. Jpn.
12) M. Ramanadham and R. Chidambaram, "Advances in Crystallography," ed by R. Srinivasan, Oxford & IBH Publishing Co., New Delhi (1978).
13) F. A. Momany, R. F. McGuire, A. W. Burgess, and H. A. Scheraga, *J. Phys. Chem.*, **79**, 2361 (1975).
14) E. Benedetti, C. Pedone, C. Toniolo, G. Nemathy, M. S. Pottle, and H. A. Scheraga, *Int. J. Pept. Protein Res.*, **16**, 156 (1980).
15) D. Cremer and J. A. Pople, *J. Am. Chem. Soc.*, **97**, 1345 (1975).
16) H. Ayato, I. Tanaka, and T. Ashida, *J. Am. Chem. Soc.*, **103**, 5902 (1981).
17) J. S. Richardson, "Adv. Protein Chem.," ed by C. B. Anfinson, J. T. Edsall, and F. M. Richards, Academic Press, New York (1981), Vol. 34, p. 167.
18) M. J. C. Crabbe and J. R. Appleyard, "DTMM; Desktop Molecular Modeller," Version 1.2, Oxford University Press, Oxford (1990).