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Structure of *tert*-Butyloxycarbonyl-L-alanyl-L-proline Monohydrate (*t*-Boc-Ala-Pro)

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Abstract

$C_{13}H_{22}N_2O_5.H_2O$, M_r = 304·37, is orthorhombic, space group $P2_12_12_1$, with a = 20·751 (2), b = 13·457 (1), c = 5·875 (1) Å, V = 1640·49 Å^3, D_o = 1·20, D_c = 1·231 Mg m^{-3}; Z = 4; λ(Cu $K\alpha$) = 1·5418 Å; Z = 4; $F(000)$ = 656. Final R = 3·9% for 1664 observed reflexions. There is one molecule of water as well as an N—H···O hydrogen bond, rendering high stability to the crystal packing. The water-bridge bond is of the same type as that of a triple helix: $O_W\cdots O$ = 2·78, $O_W\cdots O'$ = 2·69 and $O_W\cdots OH$ = 2·50 Å. C^α is in the *trans* configuration. The absolute configuration of the non-centrosymmetric structure and, therefore, of the molecular conformation was determined by anomalous dispersion. The $N'C^\alpha C^\gamma C^\delta$ group in the pyrrolidine ring is fairly planar. C^β is readily displaced from this best plane of the five-membered ring and deviates by 0·489 Å. N′ and C^γ are on the same side of this plane in relation to the carboxyl C′. Thus *t*-Boc-Ala-Pro is C_s-C^γ-*endo*(C^β-*exo*). This derivative belongs to conformation *B*, since the dihedral angle χ_1 = 28·55° takes a positive value, and it has collagen-like characteristics, with $N'-C^\alpha-C'-O$, that is, the dihedral angle ψ_1 Pro, equal to 161°. The C^α atoms of the prolyl and alanyl residues are *trans* with respect to the peptide bond.

Introduction

Peptides containing proline residues have been the subject of intensive study because of the possibility of *cis–trans* isomerization about the *X*—Pro bond (Carver & Blout, 1976; Grathwohl & Wuthrich, 1976), variations in pyrrolidine-ring geometries (Detar & Luthra, 1977; Madison, 1977) and the widespread occurrence of Pro residues in proteins.

The cyclic amino acid proline is regarded as a helix breaker because its presence at the amino terminal end of a polypeptide causes steric interactions for the preceding amino acids, especially when there is a C^β atom in its side chain. In addition to steric interactions from neighbouring amino acids at the terminal end, there are steric interactions of the carboxyl group of the proline and on a polypeptide chain of the amide of the carboxyl group with the pyrrolidine, notably its C^β—CH_2 group (Schimmel & Flory, 1968).

The Ala-Pro sequence is of particular interest as it appears repetitively over a segment of the light chain of rabbit skeletal muscle myosin (Frank & Weeds, 1974). As part of a programme of investigation of *X*-Pro sequences, we describe the molecular structure of the dipeptide acid, *tert*-butyloxycarbonyl-L-Ala-L-Pro (*t*-Boc-Ala-Pro).

Experimental and results

The crystals were grown as colourless platelets from acetyl acetate. Approximate cell dimensions and space-group information were obtained from Weissenberg photographs. The θ–2θ mode (scan range 4–70°) with the five-measurements technique was adopted (Dreissig, 1969; Allen, Rogers & Troughton, 1971). 1775 independent reflexions were collected from a crystal 0·11 × 0·11 × 0·83 mm, of which 111 were considered unobserved with $I < 2\sigma(I)$. No absorption corrections were applied (diameter of crystal < 0·8 mm).

Structure determination and refinement

The phase problem was solved with *MULTAN* 77 (Main, Woolfson, Lessinger, Germain & Declercq, 1977). An overall temperature factor (B = 3·7 Å^2) and scale factor were evaluated (Wilson, 1942) and used to

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compute normalized structure factors (Karle & Hauptman, 1956). From three reflexions in the starting set and 200 E values > 1·50 all phases could be evaluated. The statistics of the E values confirmed a non-centrosymmetric structure. R was 21·87%. All non-hydrogen atoms including an O atom of one water molecule could be located. Least-squares refinement with an overall isotropic temperature factor of 4·0 Å^2 was carried out (Kruger, Ammon, Dickinson & Hall, 1976). Anisotropic temperature factors for all heavy atoms were used for further refinement and all H atoms were located from a difference synthesis. Further refinement reduced R to 0·042.

Table 1. *Atomic coordinates and temperature factors*

$T = \exp(-8\pi^2 U \sin^2\theta/\lambda^2)$ for H atoms and $B_{eq} = 8\pi^2 U_{eq}$ for O, N and C (Willis & Pryor, 1975).

	x	y	z	B_{eq} or U (Å^2)
O_1	0·8595 (1)	1·0332 (2)	0·3607 (4)	4·9 (1)
O_2	0·9394 (1)	0·9320 (2)	0·2300 (5)	5·8 (1)
O_3'	0·7978 (1)	0·7196 (2)	0·3120 (4)	3·9 (1)
O_4	0·7635 (1)	0·4380 (2)	0·2754 (4)	4·1 (1)
O_5	0·8239 (1)	0·4970 (2)	0·5571 (4)	4·8 (1)
O_W	0·7268 (1)	0·8753 (2)	0·1748 (4)	6·0 (1)
C_1	0·8221 (2)	1·1922 (3)	0·2866 (10)	7·8 (3)
C_2	0·8657 (2)	1·0906 (4)	−0·0352 (8)	7·1 (3)
C_3	0·9406 (2)	1·1575 (3)	0·2684 (10)	6·9 (3)
C_4	0·8740 (2)	1·1192 (3)	0·2135 (7)	4·6 (1)
C_5	0·8924 (2)	0·9478 (2)	0·3481 (6)	3·9 (1)
C_6^{α}	0·8908 (1)	0·7819 (2)	0·5134 (5)	3·4 (1)
C_7^{β}	0·8833 (2)	0·7482 (3)	0·7607 (6)	5·0 (2)
C_8'	0·8549 (1)	0·7079 (2)	0·3619 (5)	3·0 (1)
C_{12}^{α}	0·8514 (1)	0·5456 (2)	0·1802 (5)	3·1 (1)
C_{11}^{β}	0·9052 (2)	0·4785 (2)	0·0882 (7)	4·6 (1)
C_{10}^{γ}	0·9597 (2)	0·4946 (3)	0·2527 (9)	6·4 (3)
C_9^{δ}	0·9548 (1)	0·6016 (3)	0·3307 (7)	4·6 (2)
C_{13}'	0·8078 (1)	0·4889 (2)	0·3444 (5)	3·2 (1)
N_1	0·8660 (1)	0·8813 (2)	0·4896 (5)	3·8 (1)
N_2'	0·8864 (1)	0·6259 (2)	0·2945 (4)	3·1 (1)
$H_1(O_5)$	0·795 (3)	0·436 (4)	0·490 (11)	0·19 (3)
$H_{1W}(O_W)$	0·757 (2)	0·823 (3)	0·194 (8)	0·12 (2)
$H_{2W}(O_W)$	0·744 (2)	0·894 (4)	0·075 (10)	0·16 (2)
$H_1(C_2)$	0·902 (2)	1·050 (3)	−0·080 (8)	0·11 (1)
$H_2(C_2)$	0·821 (3)	1·066 (4)	−0·060 (12)	0·19 (2)
$H_3(C_2)$	0·879 (2)	1·154 (3)	−0·115 (9)	0·13 (2)
$H_4(C_3)$	0·971 (2)	1·114 (2)	0·216 (6)	0·07 (1)
$H_5(C_3)$	0·938 (2)	1·185 (3)	0·438 (8)	0·12 (2)
$H_6(C_3)$	0·954 (2)	1·222 (3)	0·122 (9)	0·13 (2)
$H_7(C_1)$	0·828 (2)	1·240 (3)	0·210 (8)	0·10 (2)
$H_8(C_1)$	0·776 (2)	1·164 (3)	0·255 (8)	0·13 (2)
$H_9(C_1)$	0·836 (3)	1·223 (5)	0·515 (12)	0·20 (3)
$H_{10}(C_6^{\alpha})$	0·936 (1)	0·782 (2)	0·481 (5)	0·04 (1)
$H_{11}(C_7^{\beta})$	0·838 (7)	0·746 (2)	0·822 (7)	0·07 (1)
$H_{12}(C_7^{\beta})$	0·900 (2)	0·685 (2)	0·775 (6)	0·08 (1)
$H_{13}(C_7^{\beta})$	0·907 (2)	0·791 (3)	0·872 (7)	0·09 (1)
$H_{14}(C_{12}^{\alpha})$	0·823 (1)	0·374 (2)	0·049 (5)	0·05 (1)
$H_{15}(C_{11}^{\beta})$	0·891 (2)	0·404 (2)	0·035 (6)	0·07 (1)
$H_{16}(C_{11}^{\beta})$	0·921 (2)	0·507 (2)	−0·061 (6)	0·08 (1)
$H_{17}(C_{10}^{\gamma})$	1·004 (2)	0·482 (3)	0·187 (8)	0·12 (2)
$H_{18}(C_{10}^{\gamma})$	0·961 (3)	0·441 (5)	0·481 (13)	0·22 (3)
$H_{19}(C_9^{\delta})$	0·969 (1)	0·612 (2)	0·476 (6)	0·06 (1)
$H_{20}(C_9^{\delta})$	0·984 (2)	0·646 (3)	0·237 (8)	0·12 (2)
$H_1(N_1)$	0·833 (1)	0·894 (2)	0·540 (5)	0·03 (1)

Determination of the absolute configuration

At the end of the refinement additional cycles were required including dispersion to differentiate between the two enantiomorphic forms of the structure. The atomic coordinates of the solution structure were used.

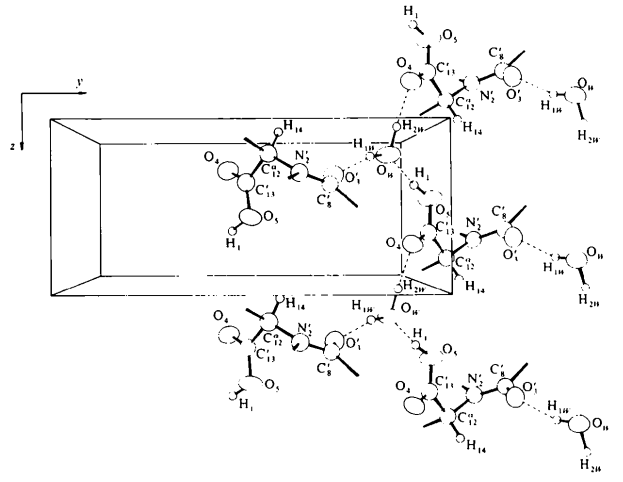
Corrections were taken into account by changing the sign of the imaginary part f''. R changed very little from the original 0·042 to R = 0·039. The values for Cu $K\alpha$ radiation for O, C and N were taken from Cromer & Liberman (1970). The slight deviation of R from the original indicated that the proposed structure from *MULTAN* was in the correct orientation (Hamilton, 1956; Bijvoet, 1949). Table 1 gives the atomic parameters.*

Packing and the water-bridge bond in *t*-Boc-Ala-Pro

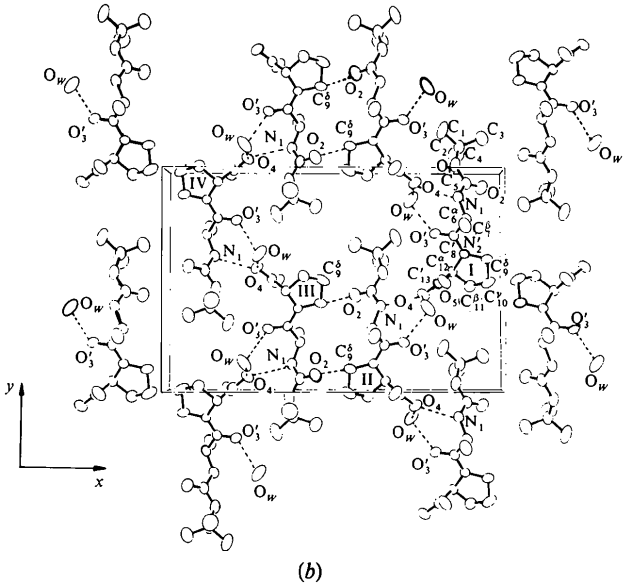
Different hypotheses have been proposed concerning the unusual presence and the role of hydroxyproline (Hyp) in collagen and collagen-like polymers (Ramachandran & Ramakrishnan, 1976; Ramachandran, Bansal & Bhatnagar, 1973; Inouye, Sakakibara & Prockop, 1976). In these studies it was found that a tripeptide unit is strongly retained by one mole of water. Traub (1974) has proposed a model with only *trans* peptide bonds including an interchain bridge for (Gly-L-Pro-L-4-Hyp)$_n$. Hospital, Courseille, Lorey & Roques (1979) found that the crystal packing of *N*-acetyl-L-4-hydroxyproline is stabilized by hydrogen bonds between three different molecules and the same molecule of water. The same situation prevails in *t*-Boc-Ala-Pro (Fig. 1*a*).

A view of the packing down **c** is shown in Fig. 1(*b*). The C$^{\alpha}$ atoms of the prolyl and alanyl residues are *trans* with respect to the peptide bond C_8'—N_2'. The observed hydrogen-bond distances and angles are given in Table 2. Both the O atoms of the carboxyl group are involved in hydrogen bonding. While O_5 of this group acts as a donor with respect to the strong hydrogen bonding (2·50 Å) with O_W, O_4 accepts one of the H atoms from the water oxygen O_W (2·78 Å). The water molecule donates the other H atom for a fairly strong hydrogen bond with symmetry-related $(O_3')^{I}$ (2·69 Å). The water molecule is thus involved in three hydrogen bonds. The functional group N_1—H(N_1) of the alanyl residue is involved in hydrogen bonding with $(O_4)^{III}$ of the symmetry-related carboxyl group (3·12 Å). The structure is thus highly stabilized by a network of O—H···O and N—H···O hydrogen bonds (Table 2).

* Lists of structure factors and anisotropic thermal parameters have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 36270 (7 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.



(*a*)



(*b*)

Fig. 1. (*a*) Hydration in *t*-Boc-Ala-Pro. Projection on the ($\bar{1}$00) plane showing three molecules joined to the same molecule of water and the packing scheme. (*b*) The cell of *t*-Boc-Ala-Pro with neighbouring molecules showing a network of hydrogen bonds and packing down **c**.

Table 2. *Hydrogen-bond distances* (Å) *and angles* (°)

D—H···*A*	*D*···*A*	H···*A*	*D*—H···*A*
O_W—H_{1W}···$(O_3')^{I}$	2·68 (7)	1·79 (7)	178·2
O_W—H_{2W}···$(O_4)^{II}$	2·78 (3)	1·73 (1)	163·4
O_5—H(O_5)···O_W	2·50 (4)	1·55 (6)*	161·7
N_1—H(N_1)···$(O_4)^{III}$	3·12 (6)	2·34 (8)	170·3
C_9^{δ}—$H_{19}(C_9^{\delta})$···$(O_2)^{IV}$	3·24 (7)	2·48 (6)	134·3

Symmetry code: (I) x,y,z; (II) $\frac{3}{2}-x, -\frac{1}{2}+y, z+\frac{1}{2}$; (III) $-\frac{1}{2}+x, 1-y, -z+\frac{1}{2}$; (IV) $1-x, \frac{3}{2}-y, -z+\frac{1}{2}$.

* Theoretical value.

Intramolecular features

The bond lengths and angles are given in Table 3. The least-squares plane through the *t*-Boc group and the deviations of the atoms from this plane are given in Table 4 (plane 1). The C_4—O_1 bond is *cis* to C_5—O_2 and the methyl groups are staggered with respect to O_2. The angle O_1—C_4—C_1 (101·4°) is greatly reduced from the regular tetrahedral value and the other C—C—C angles around C_4 are widened by about 2·6° (on average). The angle C_4—O_1—C_5 (122·4°) is about 6° larger than that usually found in ester groupings. These features are in agreement with those observed in prolyl peptides containing the *t*-Boc group (Marsh, Narasimha Murthy & Venkatesan, 1977; Gadret, Leger & Carpy, 1977; Ashida & Kakudo, 1974; Benedetti, Ciajolo & Maisto, 1974).

Table 3. *Bond angles* (°), *lengths* (Å) *and torsion angles* (°)

O_1—C_4—C_2	110·0 (3)	C_2—C_4—C_3	113·3 (3)
O_1—C_4—C_3	109·2 (3)	O_4—C_{13}'—O_5	124·3 (3)
O_1—C_4—C_1	101·4 (3)	C_{11}^{β}—C_{12}^{α}—C_{13}'	111·1 (2)
O_1—C_5—N_1	109·3 (3)		
O_1—C_5—O_2	126·3 (3)		
O_2—C_5—N_1	124·4 (3)	C_4—C_2	1·521 (6)
C_4—O_1—C_5	122·4 (3)	C_4—C_3	1·510 (6)
C_2—C_4—C_1	110·8 (4)	C_4—C_1	1·520 (6)
C_3—C_4—C_1	111·5 (3)	C_4—O_1	1·476 (4)
C_5—N_1—C_6^{α}	122·4 (3)	O_1—C_5	1·338 (4)
N_1—C_6^{α}—C_8'	112·0 (2)	C_5—O_2	1·216 (4)
N_1—C_6^{α}—C_7^{β}	109·4 (3)	C_5—N_1	1·339 (4)
C_6^{α}—C_8'—N_2'	118·0 (3)	N_1—C_6^{α}	1·440 (4)
C_6^{α}—C_8'—O_3'	121·6 (2)	C_6^{α}—C_7^{β}	1·530 (5)
C_7^{β}—C_6^{α}—C_8'	108·1 (2)	C_6^{α}—C_8'	1·530 (4)
O_3'—C_8'—N_2'	120·2 (3)	C_8'—O_3'	1·230 (3)
C_8'—N_2'—C_{12}^{α}	120·1 (2)	C_8'—N_2'	1·342 (4)
C_8'—N_2'—C_9^{δ}	127·5 (2)	N_2'—C_{12}^{α}	1·464 (4)
N_2'—C_{12}^{α}—C_{11}^{β}	103·7 (2)	C_{12}^{α}—C_{11}^{β}	1·533 (4)
C_{12}^{α}—C_{11}^{β}—C_{10}^{γ}	103·6 (3)	C_{11}^{β}—C_{10}^{γ}	1·504 (6)
C_{11}^{β}—C_{10}^{γ}—C_9^{δ}	106·4 (3)	C_{10}^{γ}—C_9^{δ}	1·514 (6)
C_{10}^{γ}—C_9^{δ}—N_2'	103·4 (3)	C_9^{δ}—N_2'	1·474 (4)
C_9^{δ}—N_2'—C_{12}^{α}	112·3 (2)	C_{12}^{α}—C_{13}'	1·529 (4)
N_2'—C_{12}^{α}—C_{13}'	111·9 (2)	C_{13}'—O_4	1·216 (4)
C_{12}^{α}—C_{13}'—O_4	121·3 (3)	C_{13}'—O_5	1·298 (4)
C_{12}^{α}—C_{13}'—O_5	114·3 (3)		

C_4—O_1—C_5—N_1	−175·7 (3)	
C_4—O_1—C_5—O_2	5·0 (5)	
O_1—C_5—N_1—C_6	179·9 (3)	
C_5—N_1—C_6^{α}—C_8'	−95·4 (3)	
N_1—C_6^{α}—C_8'—N_2'	153·6 (3)	
C_6^{α}—C_8'—N_2'—C_{12}^{α}	170·4 (3)	ω
C_8'—N_2'—C_{12}^{α}—C_{13}'	−71·8 (3)	φ
N_2'—C_{12}^{α}—C_{13}'—O_4	160·9 (3)	ψ_1
N_2'—C_{12}^{α}—C_{13}'—O_5	−21·8 (3)	ψ_2
N_2'—C_{12}^{α}—C_{11}^{β}—C_{10}^{γ}	28·5 (3)	χ_1
C_{12}^{α}—C_{11}^{β}—C_{10}^{γ}—C_9^{δ}	−33·1 (4)	χ_2
C_{11}^{β}—C_{10}^{γ}—C_9^{δ}—N_2'	24·2 (4)	χ_3
C_{10}^{γ}—C_9^{δ}—N_2'—C_{12}^{α}	−5·8 (4)	χ_4
C_9^{δ}—N_2'—C_{12}^{α}—C_{11}^{β}	−14·3 (3)	$\chi_5 = \theta$
C_{13}'—C_{12}^{α}—C_{11}^{β}—C_{10}^{γ}	−91·8 (3)	θ'
C_{13}'—C_{12}^{α}—N_2'—C_9^{δ}	105·5 (3)	θ''
C_8'—N_2'—C_9^{δ}—C_{10}^{γ}	171·2 (3)	θ'''
C_8'—N_2'—C_{12}^{α}—C_{11}^{β}	168·4 (3)	θ^{IV}

Table 4. *Least-squares planes and deviations* (Å) *of atoms* (*Schomaker, Waser, Marsh & Bergman*, 1959)

Plane 1: C_4, O_1, C_5, O_2, N_1 and C_6^{α}

$0{\cdot}594x + 0{\cdot}330y + 0{\cdot}734z = 16{\cdot}694$

C_1	−0·045 (1)	O_1	0·014 (6)
C_5	0·041 (5)	O_2	0·003 (6)
N_1	0·014 (2)	C_6	−0·028 (8)

Plane 2: C_{12}^{α}, C'_{13}, O_4 and O_5

$0{\cdot}605x - 0{\cdot}793y - 0{\cdot}078z = 4{\cdot}775$

C_{12}^{α}	0·003 (8)	C'_{13}	−0·013 (6)
O_4	0·005 (2)	O_5	0·004 (6)
N'_2	−0·466 (1)	C_{11}^{β}	1·436 (7)

Plane 3: C_9^{δ}, C_{10}^{γ}, C_{12}^{α} and N'_2

$0{\cdot}259x + 0{\cdot}341y - 0{\cdot}904z = 6{\cdot}099$

C_9^{δ}	0·029 (7)	C_{10}^{γ}	−0·018 (1)
C_{12}^{α}	0·019 (4)	N'_2	−0·032 (1)
C_{11}^{β}	0·488 (9)	C'_{13}	−1·346 (7)
C'_8	−0·181 (9)		

Plane 4: C_6^{α}, C'_8, O'_3, N'_2 and C_{12}^{α}

$0{\cdot}294x + 0{\cdot}447y - 0{\cdot}845z = 7{\cdot}639$

C_6^{α}	−0·051 (4)	C'_8	0·036 (3)
O'_3	0·006 (4)	N'_2	0·069 (1)
C_{12}^{α}	−0·060 (4)	C_9^{δ}	0·159 (9)
N_1	0·464 (4)		

The carboxyl group is un-ionized with C'_{13}–O_5 = 1·298 and C'_{13}–O_4 = 1·216 Å and these agree with the average values 1·306 (11) and 1·203 (9) Å for the un-ionized carboxyl group observed for amino acids (Sundaralingam & Putkey, 1970). The carboxyl group is practically planar (Table 4, plane 2).

The pyrrolidine ring exists in the C_s-C^{β}-*exo* conformation (Ashida & Kakudo, 1974). The β-carbon (C_{11}^{β}) is significantly out of the plane of the remaining four atoms by 0·489 Å (Table 4, plane 3). The same tendency is observed in *tert*-butyloxycarbonylglycyl-L-proline and its benzyl ester in which the deviations of the β-carbon are 0·516 and 0·529 Å respectively (Marsh, Narasimha Murthy & Venkatesan, 1977). However, in the other structures containing a pyrrolidine ring the γ-carbon deviates from the plane formed by the remaining four atoms (Mathieson & Welsh, 1952; Leung & Marsh, 1958; Shamala & Venkatesan, 1973; Venkatram Prasad, Shamala, Nagaraj, Chandrasekaran & Balaram, 1979) while in DL-proline hydrochloride it is the α-carbon that is displaced (Matsuzaki & Iitaka, 1971).

The deviation of the atoms in the peptide group from the least-squares plane (Table 4, plane 4) is small for C_6^{α}, C'_8, O'_3 and N'_2 and large for C_{12}^{α} and this is consistent with the observation of Ramachandran, Lakshminarayanan & Kolaskar (1973) on non-planarity of the peptide unit. The dihedral angles $\Delta\omega$ and θ_N (Ramachandran, Lakshminarayanan & Kolaskar, 1973) are −8·8 and 4·9° respectively. Various torsion angles are listed in Table 5. The torsion angles φ, C'_8–N'_2–C_{12}^{α}–C'_{13} (−71·8°), and ψ_2, N'_2–C_{12}^{α}–C'_{13}–O_5 (−21·8°), compare well with the values obtained for other prolyl peptides (Ashida & Kakudo, 1974).

Table 5. *Torsion angles* (°) *of particular interest in* *t*-Boc-Ala-Pro

Backbone conformational angles

O_1–C_5–N_1–C_6^{α}	ωAla	179·9 (3)
C_5–N_1–C_6^{α}–C'_8	φAla	−95·4 (3)
N_1–C_6^{α}–C'_8–N'_2	ψAla	153·6 (3)
C_6^{α}–C'_8–N'_2–C_{12}^{α}	ωPro	170·4 (3)
C'_8–N'_2–C_{12}^{α}–C'_{13}	φPro	−71·8 (3)
N'_2–C_{12}^{α}–C'_{13}–O_4	ψ_1Pro	160·9 (3)
N'_2–C_{12}^{α}–C'_{13}–O_5	ψ_2Pro	−21·8 (3)

Pyrrolidine-ring dihedral angles

C_9^{δ}–N'_2–C_{12}^{α}–C_{11}^{β}	θ	−14·3 (3)
N'_2–C_{12}^{α}–C_{11}^{β}–C_{10}^{γ}	χ_1	28·5 (3)
C_{12}^{α}–C_{11}^{β}–C_{10}^{γ}–C_9^{δ}	χ_2	−33·1 (4)
C_{11}^{β}–C_{10}^{γ}–C_9^{δ}–N'_2	χ_3	24·2 (4)
C_{10}^{γ}–C_9^{δ}–N'_2–C_{12}^{α}	χ_4	−5·8 (4)

Discussion and molecular conformation

Several five-membered ring systems, including pyrrolidine, are not planar. In the envelope form four atoms lie in a plane, while the fifth is found above or below the plane. In the half-chair form three atoms lie in the same plane and each of the remaining two may be situated above or below this plane (Kilpatrick, Pitzer & Spitzer, 1947; Pitzer & Donath, 1960). Further, the conformations can also be denoted according to their symmetry element such as C_s (envelope) form or C_2 (half-chair) form. The difference in energy of the two forms is small. The deviation from the ring plane is about 0·5 Å. This picture has been confirmed through X-ray crystal analysis (Mitsui, Tsuboi & Iitaka, 1969; Sabesan & Venkatesan, 1971; Benedetti, Ciajolo & Maisto, 1974) and spectroscopic studies (Abraham & McLauchlan, 1962; Deslauriers & Smith, 1974). An approximate C_s symmetry, in which C^{α}, C^{β}, and very often C^{γ}, lie outside the ring plane is normally encountered. According to Ashida & Kakudo (1974) the conformations of a proline ring can best be expressed through three terms: the approximate symmetry of the ring (C_s or C_2); C^{α}, C^{β} or C^{γ} outside the plane in relation to the carboxyl C′ (*-endo* or *-exo*). In addition, proline rings can be divided into two classes: in class *A* the torsion angle χ_1 takes negative values, while in class *B* the values are positive (Balasubramanian, Lakshminarayanan, Sabesan, Tegoni, Venkatesan & Ramachandran, 1971). In his spectroscopic studies, Oster (1973) stated that C^{α} of proline peptides of Boc-amino acids that possess a C^{β} atom at their side chains (*t*-Boc-Ala-Pro inclusive) do not show *cis–trans* isomers. It is confirmed in the present paper that C^{α} in *t*-Boc-Ala-Pro takes the *trans* configuration, the dihedral angle ωPro = 170° being comparable to the normal value of 180° for peptides with a *trans* configuration.

In this structure C_6^{α}–C_8'–N_2' is widened to 118°, differing from the normal 114° found mostly in peptide bonds with a *trans* configuration (Pauling, Corey & Branson, 1951), but equal to that found in bonds with a *cis* configuration (Pauling, 1960). This effect can be attributed to steric repulsion between C_6^{α} and the H atoms bonded to C_9^{δ} of the pyrrolidine ring. C_{10}^{γ} is deformed. O_3'–C_8'–N_2' is 120° almost equal to that reported for several oligopeptides by Ashida & Kakudo (1974), but less than as cited by Corey & Pauling (1953) and Marsh & Donohue (1967), where it is 120·5, 125 and 123·5°, respectively. N_2'–C_{12}^{α}–C_{13}' is 112°, which is comparable to the usual value of 110°. The $N_2'C_{12}^{\alpha}C_{10}^{\gamma}C_9^{\delta}$ group is fairly planar. C_{11}^{β} is most readily displaced from this plane and deviates by 0·489 Å. N_2' and C_{10}^{γ} are on the same side of the plane in relation to the carboxyl C_{13}'. This prolyl residue can, therefore, be regarded as C_s-C^{γ}-*endo* (C^{β}-*exo*). The positive torsion angle χ_1 implies that *t*-Boc-Ala-Pro belongs to conformation *B*. This proline derivative shows collagen-like characteristics where N_2'–C_{12}^{α}–C_{13}'–O_4, that is, the dihedral angle ψ_1 Pro, is 161°.

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