

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

BY M. E. KAMWAYA,\* O. OSTER AND H. BRADACZEK

*Institut für Kristallographie der Freien Universität Berlin, Takustrasse 6, D-1000 Berlin 33,  
Federal Republic of Germany*

M. N. PONNUSWAMY AND S. PARTHASARATHY

*Department of Crystallography and Biophysics, University of Madras, Guindy Campus, Madras-600025, India*

AND R. NAGARAJ AND P. BALARAM

*Molecular Biophysics Unit, Indian Institute of Science, Bangalore-560012, India*

(Received 14 July 1980; accepted 27 May 1981)

### Abstract

$C_{13}H_{22}N_2O_5 \cdot H_2O$ ,  $M_r = 304.37$ , 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<sup>3</sup>,  $D_o = 1.20$ ,  $D_c = 1.231$  Mg m<sup>-3</sup>;  $Z = 4$ ;  $\lambda(\text{Cu } K\alpha) = 1.5418$  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.  $C^\alpha$  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.  $C^\beta$  is readily displaced from this best plane of the five-membered ring and deviates by 0.489 Å.  $N'$  and  $C^\gamma$  are on the same side of this plane in relation to the carboxyl  $C'$ . Thus *t*-Boc-Ala-Pro is  $C_s$ - $C^\gamma$ -*endo*( $C^\beta$ -*exo*). This derivative belongs to conformation  $B$ , since the dihedral angle  $\chi_1 = 28.55^\circ$  takes a positive value, and it has collagen-like characteristics, with  $N'-C^\alpha-C'-O$ , that is, the dihedral angle  $\psi_1$  Pro, equal to  $161^\circ$ . The  $C^\alpha$  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.

\* Present address: School of Physics, Universiti Sains Malaysia, Minden, Penang, Malaysia.

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  $C^\beta$  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  $C^\beta$ -CH<sub>2</sub> 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  $\theta$ - $2\theta$  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<sup>2</sup>) and scale factor were evaluated (Wilson, 1942) and used to

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  $4.0 \text{ \AA}^2$  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  $B_{\text{eq}} = 8\pi^2 U_{\text{eq}}$  for O, N and C (Willis & Pryor, 1975).

	$x$	$y$	$z$	$B_{\text{eq}}$ or $U$ ( $\text{\AA}^2$ )
O <sub>1</sub>	0.8595 (1)	1.0332 (2)	0.3607 (4)	4.9 (1)
O <sub>2</sub>	0.9394 (1)	0.9320 (2)	0.2300 (5)	5.8 (1)
O <sub>3</sub>	0.7978 (1)	0.7196 (2)	0.3120 (4)	3.9 (1)
O <sub>4</sub>	0.7635 (1)	0.4380 (2)	0.2754 (4)	4.1 (1)
O <sub>5</sub>	0.8239 (1)	0.4970 (2)	0.5571 (4)	4.8 (1)
O <sub>w</sub>	0.7268 (1)	0.8753 (2)	0.1748 (4)	6.0 (1)
C <sub>1</sub>	0.8221 (2)	1.1922 (3)	0.2866 (10)	7.8 (3)
C <sub>2</sub>	0.8657 (2)	1.0906 (4)	-0.0352 (8)	7.1 (3)
C <sub>3</sub>	0.9406 (2)	1.1575 (3)	0.2684 (10)	6.9 (3)
C <sub>4</sub>	0.8740 (2)	1.1192 (3)	0.2135 (7)	4.6 (1)
C <sub>5</sub>	0.8924 (2)	0.9478 (2)	0.3481 (6)	3.9 (1)
C <sub>6</sub> <sup>α</sup>	0.8908 (1)	0.7819 (2)	0.5134 (5)	3.4 (1)
C <sub>7</sub> <sup>β</sup>	0.8833 (2)	0.7482 (3)	0.7607 (6)	5.0 (2)
C <sub>8</sub>	0.8549 (1)	0.7079 (2)	0.3619 (5)	3.0 (1)
C <sub>9</sub> <sup>α</sup>	0.8514 (1)	0.5456 (2)	0.1802 (5)	3.1 (1)
C <sub>11</sub> <sup>β</sup>	0.9052 (2)	0.4785 (2)	0.0882 (7)	4.6 (1)
C <sub>10</sub> <sup>γ</sup>	0.9597 (2)	0.4946 (3)	0.2527 (9)	6.4 (3)
C <sub>9</sub> <sup>β</sup>	0.9548 (1)	0.6016 (3)	0.3307 (7)	4.6 (2)
C <sub>13</sub> <sup>γ</sup>	0.8078 (1)	0.4889 (2)	0.3444 (5)	3.2 (1)
N <sub>1</sub>	0.8660 (1)	0.8813 (2)	0.4896 (5)	3.8 (1)
N <sub>2</sub>	0.8864 (1)	0.6259 (2)	0.2945 (4)	3.1 (1)
H <sub>1</sub> (O <sub>3</sub> )	0.795 (3)	0.436 (4)	0.490 (11)	0.19 (3)
H <sub>1w</sub> (O <sub>w</sub> )	0.757 (2)	0.823 (3)	0.194 (8)	0.12 (2)
H <sub>2w</sub> (O <sub>w</sub> )	0.744 (2)	0.894 (4)	0.075 (10)	0.16 (2)
H <sub>1</sub> (C <sub>2</sub> )	0.902 (2)	1.050 (3)	-0.080 (8)	0.11 (1)
H <sub>2</sub> (C <sub>2</sub> )	0.821 (3)	1.066 (4)	-0.060 (12)	0.19 (2)
H <sub>3</sub> (C <sub>2</sub> )	0.879 (2)	1.154 (3)	-0.115 (9)	0.13 (2)
H <sub>4</sub> (C <sub>3</sub> )	0.971 (2)	1.114 (2)	0.216 (6)	0.07 (1)
H <sub>5</sub> (C <sub>3</sub> )	0.938 (2)	1.185 (3)	0.438 (8)	0.12 (2)
H <sub>6</sub> (C <sub>3</sub> )	0.954 (2)	1.222 (3)	0.122 (9)	0.13 (2)
H <sub>7</sub> (C <sub>1</sub> )	0.828 (2)	1.240 (3)	0.210 (8)	0.10 (2)
H <sub>8</sub> (C <sub>1</sub> )	0.776 (2)	1.164 (3)	0.255 (8)	0.13 (2)
H <sub>9</sub> (C <sub>1</sub> )	0.836 (3)	1.223 (5)	0.515 (12)	0.20 (3)
H <sub>10</sub> (C <sub>6</sub> <sup>α</sup> )	0.936 (1)	0.782 (2)	0.481 (5)	0.04 (1)
H <sub>11</sub> (C <sub>7</sub> <sup>β</sup> )	0.838 (7)	0.746 (2)	0.822 (7)	0.07 (1)
H <sub>12</sub> (C <sub>7</sub> <sup>β</sup> )	0.900 (2)	0.685 (2)	0.775 (6)	0.08 (1)
H <sub>13</sub> (C <sub>7</sub> <sup>β</sup> )	0.907 (2)	0.791 (3)	0.872 (7)	0.09 (1)
H <sub>14</sub> (C <sub>9</sub> <sup>α</sup> )	0.823 (1)	0.374 (2)	0.049 (5)	0.05 (1)
H <sub>15</sub> (C <sub>11</sub> <sup>β</sup> )	0.891 (2)	0.404 (2)	0.035 (6)	0.07 (1)
H <sub>16</sub> (C <sub>11</sub> <sup>β</sup> )	0.921 (2)	0.507 (2)	-0.061 (6)	0.08 (1)
H <sub>17</sub> (C <sub>10</sub> <sup>γ</sup> )	1.004 (2)	0.482 (3)	0.187 (8)	0.12 (2)
H <sub>18</sub> (C <sub>10</sub> <sup>γ</sup> )	0.961 (3)	0.441 (5)	0.481 (13)	0.22 (3)
H <sub>19</sub> (C <sub>9</sub> <sup>β</sup> )	0.969 (1)	0.612 (2)	0.476 (6)	0.06 (1)
H <sub>20</sub> (C <sub>9</sub> <sup>β</sup> )	0.984 (2)	0.646 (3)	0.237 (8)	0.12 (2)
H <sub>1</sub> (N <sub>1</sub> )	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)<sub>*n*</sub>. 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<sup>α</sup> atoms of the prolyl and alanyl residues are *trans* with respect to the peptide bond C<sub>7</sub><sup>β</sup>-N<sub>2</sub><sup>γ</sup>. 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<sub>5</sub> of this group acts as a donor with respect to the strong hydrogen bonding (2.50 Å) with O<sub>w</sub>, O<sub>4</sub> accepts one of the H atoms from the water oxygen O<sub>w</sub> (2.78 Å). The water molecule donates the other H atom for a fairly strong hydrogen bond with symmetry-related (O<sub>3</sub>)<sup>1</sup> (2.69 Å). The water molecule is thus involved in three hydrogen bonds. The functional group N<sub>1</sub>-H(N<sub>1</sub>) of the alanyl residue is involved in hydrogen bonding with (O<sub>4</sub>)<sup>111</sup> 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.

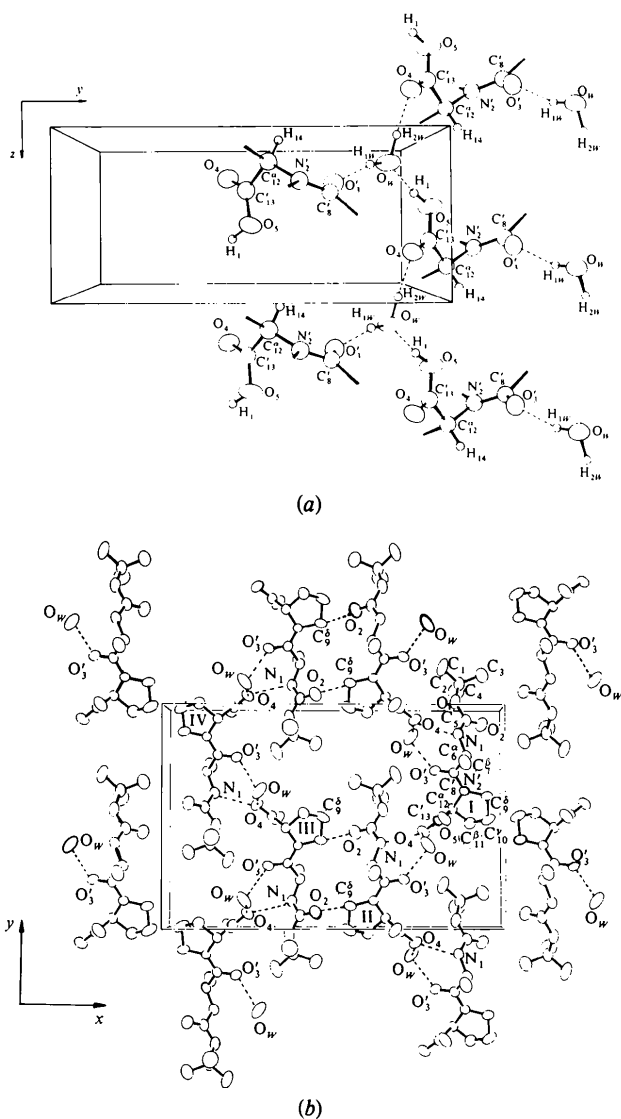


Fig. 1. (a) Hydration in *t*-Boc-Ala-Pro. Projection on the (100) plane showing three molecules joined to the same molecule of water and the packing scheme. (b) The cell of *t*-Boc-Ala-Pro with neighbouring molecules showing a network of hydrogen bonds and packing down *c*.

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

<i>D</i> — <i>H</i> ... <i>A</i>	<i>D</i> ... <i>A</i>	<i>H</i> ... <i>A</i>	<i>D</i> — <i>H</i> ... <i>A</i>
$O_w-H_{1w}\cdots(O_3)^I$	2.68 (7)	1.79 (7)	178.2
$O_w-H_{2w}\cdots(O_4)^{II}$	2.78 (3)	1.73 (1)	163.4
$O_3-H(O_3)\cdots O_w$	2.50 (4)	1.55 (6)*	161.7
$N_1-H(N_1)\cdots(O_4)^{III}$	3.12 (6)	2.34 (8)	170.3
$C_9^{\delta}-H_{19}(C_9^{\delta})\cdots(O_2)^{IV}$	3.24 (7)	2.48 (6)	134.3

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

\* 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  $O_1-C_4-C_1$  ( $101.4^\circ$ ) is greatly reduced from the regular tetrahedral value and the other C—C—C angles around  $C_4$  are widened by about  $2.6^\circ$  (on average). The angle  $C_4-O_1-C_5$  ( $122.4^\circ$ ) is about  $6^\circ$  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)	$C_{11}^{\beta}-C_{12}^{\alpha}-C_{13}^{\alpha}$	111.1 (2)
$O_1-C_5-N_1$	109.3 (3)		
$O_1-C_5-O_2$	126.3 (3)	$C_4-C_2$	1.521 (6)
$O_2-C_5-N_1$	124.4 (3)	$C_4-C_3$	1.510 (6)
$C_4-O_1-C_5$	122.4 (3)	$C_4-C_1$	1.520 (6)
$C_2-C_4-C_1$	110.8 (4)	$C_4-O_1$	1.476 (4)
$C_3-C_4-C_1$	111.5 (3)	$O_1-C_5$	1.338 (4)
$C_5-N_1-C_6^{\alpha}$	122.4 (3)	$C_5-O_2$	1.216 (4)
$N_1-C_6^{\alpha}-C_8^{\alpha}$	112.0 (2)	$C_5-N_1$	1.339 (4)
$N_1-C_6^{\alpha}-C_6^{\beta}$	109.4 (3)	$N_1-C_6^{\alpha}$	1.440 (4)
$C_6^{\alpha}-C_8^{\alpha}-N_2^{\prime}$	118.0 (3)	$C_6^{\alpha}-C_6^{\beta}$	1.530 (5)
$C_6^{\alpha}-C_8^{\alpha}-O_2^{\prime}$	121.6 (2)	$C_6^{\alpha}-C_8^{\alpha}$	1.530 (4)
$C_6^{\alpha}-C_8^{\alpha}-C_8^{\beta}$	108.1 (2)	$C_8^{\alpha}-O_1^{\prime}$	1.230 (3)
$O_2^{\prime}-C_8^{\alpha}-N_2^{\prime}$	120.2 (3)	$C_8^{\alpha}-N_2^{\prime}$	1.342 (4)
$C_8^{\alpha}-N_2^{\prime}-C_{12}^{\alpha}$	120.1 (2)	$N_2^{\prime}-C_{12}^{\alpha}$	1.464 (4)
$C_8^{\alpha}-N_2^{\prime}-C_9^{\beta}$	127.5 (2)	$C_{12}^{\alpha}-C_{11}^{\beta}$	1.533 (4)
$N_2^{\prime}-C_{12}^{\alpha}-C_{11}^{\beta}$	103.7 (2)	$C_{11}^{\beta}-C_{10}^{\gamma}$	1.504 (6)
$C_{12}^{\alpha}-C_{11}^{\beta}-C_{10}^{\gamma}$	103.6 (3)	$C_{10}^{\gamma}-C_9^{\delta}$	1.514 (6)
$C_{11}^{\beta}-C_{10}^{\gamma}-C_9^{\delta}$	106.4 (3)	$C_9^{\delta}-N_1^{\prime}$	1.474 (4)
$C_{10}^{\gamma}-C_9^{\delta}-N_1^{\prime}$	103.4 (3)	$C_{12}^{\alpha}-C_{13}^{\alpha}$	1.529 (4)
$C_9^{\delta}-N_1^{\prime}-C_{12}^{\alpha}$	112.3 (2)	$C_{13}^{\alpha}-O_4$	1.216 (4)
$N_1^{\prime}-C_{12}^{\alpha}-C_{13}^{\alpha}$	111.9 (2)	$C_{13}^{\alpha}-O_5$	1.298 (4)
$C_{12}^{\alpha}-C_{13}^{\alpha}-O_4$	121.3 (3)		
$C_{12}^{\alpha}-C_{13}^{\alpha}-O_5$	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)
		$O_1-C_5-N_1-C_6^{\alpha}$	179.9 (3)
		$C_5-N_1-C_6^{\alpha}-C_8^{\alpha}$	-95.4 (3)
		$N_1-C_6^{\alpha}-C_8^{\alpha}-N_2^{\prime}$	153.6 (3)
		$C_6^{\alpha}-C_8^{\alpha}-N_2^{\prime}-C_{12}^{\alpha}$	170.4 (3) $\omega$
		$C_8^{\alpha}-N_2^{\prime}-C_{12}^{\alpha}-C_{13}^{\alpha}$	-71.8 (3) $\phi$
		$N_2^{\prime}-C_{12}^{\alpha}-C_{13}^{\alpha}-O_4$	160.9 (3) $\psi_1$
		$N_2^{\prime}-C_{12}^{\alpha}-C_{13}^{\alpha}-O_5$	-21.8 (3) $\psi_2$
		$N_2^{\prime}-C_{12}^{\alpha}-C_{11}^{\beta}-C_{10}^{\gamma}$	28.5 (3) $\chi_1$
		$C_{12}^{\alpha}-C_{11}^{\beta}-C_{10}^{\gamma}-C_9^{\delta}$	-33.1 (4) $\chi_2$
		$C_{11}^{\beta}-C_{10}^{\gamma}-C_9^{\delta}-N_1^{\prime}$	24.2 (4) $\chi_3$
		$C_{10}^{\gamma}-C_9^{\delta}-N_1^{\prime}-C_{12}^{\alpha}$	-5.8 (4) $\chi_4$
		$C_9^{\delta}-N_1^{\prime}-C_{12}^{\alpha}-C_{11}^{\beta}$	-14.3 (3) $\chi_5 = \theta$
		$C_{13}^{\alpha}-C_{12}^{\alpha}-C_{11}^{\beta}-C_{10}^{\gamma}$	-91.8 (3) $\theta^{\prime}$
		$C_{13}^{\alpha}-C_{12}^{\alpha}-N_2^{\prime}-C_8^{\alpha}$	105.5 (3) $\theta^{\prime\prime}$
		$C_8^{\alpha}-N_2^{\prime}-C_9^{\delta}-C_{10}^{\gamma}$	171.2 (3) $\theta^{\prime\prime\prime}$
		$C_8^{\alpha}-N_2^{\prime}-C_{12}^{\alpha}-C_{11}^{\beta}$	168.4 (3) $\theta^{\prime\prime\prime\prime}$

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

Plane 1: C <sub>4</sub> , O <sub>1</sub> , C <sub>5</sub> , O <sub>2</sub> , N <sub>1</sub> and C <sub>6</sub> <sup>α</sup>			
0.594x + 0.330y + 0.734z = 16.694			
C <sub>1</sub>	-0.045 (1)	O <sub>1</sub>	0.014 (6)
C <sub>5</sub>	0.041 (5)	O <sub>2</sub>	0.003 (6)
N <sub>1</sub>	0.014 (2)	C <sub>6</sub>	-0.028 (8)
Plane 2: C <sub>12</sub> <sup>α</sup> , C <sub>13</sub> , O <sub>4</sub> and O <sub>5</sub>			
0.605x - 0.793y - 0.078z = 4.775			
C <sub>12</sub> <sup>α</sup>	0.003 (8)	C <sub>13</sub>	-0.013 (6)
O <sub>4</sub>	0.005 (2)	O <sub>5</sub>	0.004 (6)
N <sub>2</sub> <sup>β</sup>	-0.466 (1)	C <sub>11</sub> <sup>β</sup>	1.436 (7)
Plane 3: C <sub>9</sub> <sup>δ</sup> , C <sub>10</sub> <sup>γ</sup> , C <sub>12</sub> <sup>α</sup> and N <sub>2</sub> <sup>γ</sup>			
0.259x + 0.341y - 0.904z = 6.099			
C <sub>9</sub> <sup>δ</sup>	0.029 (7)	C <sub>10</sub> <sup>γ</sup>	-0.018 (1)
C <sub>12</sub> <sup>α</sup>	0.019 (4)	N <sub>2</sub> <sup>γ</sup>	-0.032 (1)
C <sub>11</sub> <sup>β</sup>	0.488 (9)	C <sub>13</sub>	-1.346 (7)
C <sub>8</sub> <sup>γ</sup>	-0.181 (9)		
Plane 4: C <sub>6</sub> <sup>α</sup> , C <sub>8</sub> <sup>α</sup> , O <sub>3</sub> , N <sub>2</sub> <sup>γ</sup> and C <sub>12</sub> <sup>α</sup>			
0.294x + 0.447y - 0.845z = 7.639			
C <sub>6</sub> <sup>α</sup>	-0.051 (4)	C <sub>8</sub> <sup>α</sup>	0.036 (3)
O <sub>3</sub>	0.006 (4)	N <sub>2</sub> <sup>γ</sup>	0.069 (1)
C <sub>12</sub> <sup>α</sup>	-0.060 (4)	C <sub>9</sub> <sup>δ</sup>	0.159 (9)
N <sub>1</sub>	0.464 (4)		

The carboxyl group is un-ionized with C<sub>13</sub>-O<sub>5</sub> = 1.298 and C<sub>13</sub>-O<sub>4</sub> = 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<sub>5</sub>-C<sup>β</sup>-*exo* conformation (Ashida & Kakudo, 1974). The β-carbon (C<sub>11</sub><sup>β</sup>) 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<sub>6</sub><sup>α</sup>, C<sub>8</sub><sup>α</sup>, O<sub>3</sub> and N<sub>2</sub><sup>γ</sup> and large for C<sub>12</sub><sup>α</sup> and this is consistent with the observation of Ramachandran, Lakshminarayanan & Kolaskar (1973) on non-planarity of the peptide unit. The dihedral angles Δω and θ<sub>N</sub> (Ramachandran, Lakshminarayanan & Kolaskar, 1973) are -8.8 and 4.9° respectively. Various torsion angles are listed in Table 5. The torsion angles φ, C<sub>8</sub><sup>γ</sup>-N<sub>2</sub><sup>γ</sup>-C<sub>12</sub><sup>α</sup>-C<sub>13</sub><sup>γ</sup> (-71.8°), and ψ<sub>2</sub>, N<sub>2</sub><sup>γ</sup>-

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

Backbone conformational angles		
O <sub>1</sub> -C <sub>5</sub> -N <sub>1</sub> -C <sub>6</sub> <sup>α</sup>	ωAla	179.9 (3)
C <sub>5</sub> -N <sub>1</sub> -C <sub>6</sub> <sup>α</sup> -C <sub>8</sub> <sup>α</sup>	φAla	-95.4 (3)
N <sub>1</sub> -C <sub>6</sub> <sup>α</sup> -C <sub>7</sub> <sup>γ</sup> -N <sub>2</sub> <sup>γ</sup>	ψAla	153.6 (3)
C <sub>6</sub> <sup>α</sup> -C <sub>8</sub> <sup>α</sup> -N <sub>2</sub> <sup>γ</sup> -C <sub>12</sub> <sup>α</sup>	ωPro	170.4 (3)
C <sub>7</sub> <sup>γ</sup> -N <sub>2</sub> <sup>γ</sup> -C <sub>12</sub> <sup>α</sup> -C <sub>13</sub> <sup>γ</sup>	φPro	-71.8 (3)
N <sub>2</sub> <sup>γ</sup> -C <sub>12</sub> <sup>α</sup> -C <sub>13</sub> <sup>γ</sup> -O <sub>4</sub>	ψ <sub>1</sub> Pro	160.9 (3)
N <sub>2</sub> <sup>γ</sup> -C <sub>12</sub> <sup>α</sup> -C <sub>13</sub> <sup>γ</sup> -O <sub>5</sub>	ψ <sub>2</sub> Pro	-21.8 (3)
Pyrrolidine-ring dihedral angles		
C <sub>9</sub> <sup>δ</sup> -N <sub>2</sub> <sup>γ</sup> -C <sub>12</sub> <sup>α</sup> -C <sub>11</sub> <sup>β</sup>	θ	-14.3 (3)
N <sub>2</sub> <sup>γ</sup> -C <sub>12</sub> <sup>α</sup> -C <sub>11</sub> <sup>β</sup> -C <sub>10</sub> <sup>γ</sup>	χ <sub>1</sub>	28.5 (3)
C <sub>12</sub> <sup>α</sup> -C <sub>11</sub> <sup>β</sup> -C <sub>10</sub> <sup>γ</sup> -C <sub>9</sub> <sup>δ</sup>	χ <sub>2</sub>	-33.1 (4)
C <sub>11</sub> <sup>β</sup> -C <sub>10</sub> <sup>γ</sup> -C <sub>9</sub> <sup>δ</sup> -N <sub>2</sub> <sup>γ</sup>	χ <sub>3</sub>	24.2 (4)
C <sub>10</sub> <sup>γ</sup> -C <sub>9</sub> <sup>δ</sup> -N <sub>2</sub> <sup>γ</sup> -C <sub>12</sub> <sup>α</sup>	χ <sub>4</sub>	-5.8 (4)

C<sub>12</sub><sup>α</sup>-C<sub>13</sub><sup>γ</sup>-O<sub>5</sub> (-21.8°), compare well with the values obtained for other prolyl peptides (Ashida & Kakudo, 1974).

### 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<sub>s</sub> (envelope) form or C<sub>2</sub> (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<sub>s</sub> symmetry, in which C<sup>α</sup>, C<sup>β</sup>, and very often C<sup>γ</sup>, 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<sub>s</sub> or C<sub>2</sub>); C<sup>α</sup>, C<sup>β</sup> or C<sup>γ</sup> 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<sub>1</sub> 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<sup>α</sup> of proline peptides of Boc-amino acids that possess a C<sup>β</sup> 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<sup>α</sup> 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  $C_6^{\alpha}-C_8^{\beta}-N_2'$  is widened to  $118^\circ$ , differing from the normal  $114^\circ$  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  $C_6^{\alpha}$  and the H atoms bonded to  $C_8^{\beta}$  of the pyrrolidine ring.  $C_{10}^{\gamma}$  is deformed.  $O_3'-C_8^{\beta}-N_2'$  is  $120^\circ$  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  $123.5^\circ$ , respectively.  $N_2'-C_{12}^{\alpha}-C_{13}^{\beta}$  is  $112^\circ$ , which is comparable to the usual value of  $110^\circ$ . The  $N_2'C_{12}^{\alpha}C_{10}^{\gamma}C_9^{\delta}$  group is fairly planar.  $C_{11}^{\beta}$  is most readily displaced from this plane and deviates by  $0.489 \text{ \AA}$ .  $N_2'$  and  $C_{10}^{\gamma}$  are on the same side of the plane in relation to the carboxyl  $C_{13}^{\beta}$ . This prolyl residue can, therefore, be regarded as  $C_5-C^{\gamma}$ -endo ( $C^{\beta}$ -exo). The positive torsion angle  $\chi_1$  implies that *t*-Boc-Ala-Pro belongs to conformation *B*. This proline derivative shows collagen-like characteristics where  $N_2'-C_{12}^{\alpha}-C_{13}^{\beta}-O_4$ , that is, the dihedral angle  $\psi_1$  Pro, is  $161^\circ$ .

#### References

- ABRAHAM, R. J. & MCLAUCHLAN, K. A. (1962). *Mol. Phys.* **5**, 195–203.
- ALLEN, F. H., ROGERS, D. & TROUGHTON, P. G. H. (1971). *Acta Cryst.* **B27**, 1325–1337.
- ASHIDA, T. & KAKUDO, M. (1974). *Bull. Chem. Soc. Jpn.* **47**, 1129–1133.
- BALASUBRAMANIAN, R., LAKSHMINARAYANAN, A. V., SABESAN, M. N., TEGONI, G., VENKATESAN, K. & RAMACHANDRAN, G. N. (1971). *Int. J. Protein Res.* **3**, 25–33.
- BENEDETTI, E., CIAJOLO, M. R. & MAISTO, A. (1974). *Acta Cryst.* **B30**, 1783–1788.
- BUVOET, J. M. (1949). *Proc. K. Ned. Akad. Wet. Ser. B*, **52**, 313–314.
- CARVER, J. P. & BLOUT, E. R. (1976). *Treatise on Collagen*, edited by G. N. RAMACHANDRAN, Vol. 1, 441–526. New York: Academic Press.
- COREY, R. B. & PAULING, L. (1953). *Proc. R. Soc. London Ser. B*, **141**, 10–33.
- CROMER, D. T. & LIBERMAN, D. (1970). Report LA 4403, UC-34. Los Alamos Scientific Laboratory, Univ. of California.
- DESLAURIERS, R. & SMITH, I. C. P. (1974). *J. Biol. Chem.* **249**, 7006–7010.
- DETAR, D. F. & LUTHRA, N. P. (1977). *J. Am. Chem. Soc.* **99**, 1232–1244.
- DREISSIG, W. (1969). Dissertation, Freie Univ. Berlin.
- FRANK, G. & WEEDS, A. G. (1974). *Eur. J. Biochem.* **44**, 317–334.
- GADRET, P. M., LEGER, J. M. & CARPY, A. (1977). *Acta Cryst.* **B33**, 1067–1071.
- GRATHWOHL, C. & WUTHRICH, K. (1976). *Biopolymers*, **15**, 2025–2041.
- HAMILTON, W. C. (1956). *Acta Cryst.* **18**, 502–510.
- HOSPITAL, M., COURSEILLE, C., LOREY, F. & ROQUES, B. P. (1979). *Biopolymers*, **18**, 1141–1148.
- INOUE, K., SAKAKIBARA, S. & PROCKOP, D. J. (1976). *Biochim. Biophys. Acta*, **420**, 133–139.
- KARLE, J. & HAUPTMAN, H. (1956). *Acta Cryst.* **9**, 635–651.
- KILPATRICK, J. E., PITZER, K. S. & SPITZER, R. (1947). *J. Am. Chem. Soc.* **69**, 2483–2488.
- KRUGER, G. T., AMMON, H. L., DICKINSON, C. & HALL, S. R. (1976). XRAY 76. Tech. Rep. TR-446. Computer Science Center, Univ. of Maryland, College Park, Maryland.
- LEUNG, Y. C. & MARSH, R. E. (1958). *Acta Cryst.* **11**, 17–31.
- MADISON, V. (1977). *Biopolymers*, **16**, 2671–2692.
- MAIN, P., WOOLFSON, M. M., LESSINGER, L., GERMAIN, G. & DECLERCQ, J. P. (1977). *MULTAN 77. A System of Computer Programs for the Automatic Solution of Crystal Structures from X-ray Diffraction Data*. Univ. of York, England, and Louvain-la-Neuve, Belgium.
- MARSH, R. E. & DONOHUE, J. (1967). *Adv. Protein Chem.* **22**, 235–256.
- MARSH, R. E., NARASIMHA MURTHY, M. R. & VENKATESAN, K. (1977). *J. Am. Chem. Soc.* **99**, 1251–1256.
- MATHIESON, A. McL. & WELSH, H. K. (1952). *Acta Cryst.* **5**, 599–604.
- MATSUZAKI, T. & IITAKA, Y. (1971). *Acta Cryst.* **B27**, 507–516.
- MITSUI, Y., TSUBOI, M. & IITAKA, Y. (1969). *Acta Cryst.* **B25**, 2182–2192.
- OSTER, O. (1973). Dissertation, Univ. Tübingen.
- PAULING, L. (1960). *The Nature of the Chemical Bond*, 3rd ed., p. 281. Ithaca: Cornell Univ. Press.
- PAULING, L., COREY, R. E. & BRANSON, H. R. (1951). *Proc. Natl Acad. Sci. USA*, **37**, 205–211.
- PITZER, K. S. & DONATH, W. E. (1960). *J. Am. Chem. Soc.* **81**, 3213–3219.
- RAMACHANDRAN, G. N., BANSAL, M. & BHATNAGAR, R. S. (1973). *Biochim. Biophys. Acta*, **332**, 166–171.
- RAMACHANDRAN, G. N., LAKSHMINARAYANAN, A. V. & KOLASKAR, A. S. (1973). *Biochim. Biophys. Acta*, **303**, 8–13.
- RAMACHANDRAN, G. N. & RAMAKRISHNAN, C. (1976). *Biochemistry of Collagen*, edited by G. N. RAMACHANDRAN & A. H. REDDI, pp. 45–48. New York: Plenum.
- SABESAN, M. N. & VENKATESAN, K. (1971). *Z. Kristallogr.* **134**, 230–242.
- SCHIMMEL, P. R. & FLORY, P. J. (1968). *J. Mol. Biol.* **34**, 105–120.
- SCHOMAKER, V., WASER, J., MARSH, R. E. & BERGMAN, G. (1959). *Acta Cryst.* **12**, 600–604.
- SHAMALA, N. & VENKATESAN, K. (1973). *Cryst. Struct. Commun.* **21**, 5–8.
- SUNDARALINGAM, M. & PUTKEY, E. P. (1970). *Acta Cryst.* **B26**, 790–800.
- TRAUB, W. (1974). *Isr. J. Chem.* **12**, 435–439.
- VENKATRAM PRASAD, B. V., SHAMALA, N., NAGARAJ, R., CHANDRASEKARAN, R. & BALARAM, P. (1979). *Biopolymers*, **18**, 1635–1646.
- WILLIS, B. T. M. & PRYOR, A. W. (1975). *Thermal Vibrations in Crystallography*, pp. 101–102. Cambridge Univ. Press.
- WILSON, A. J. C. (1942). *Nature, London*, **150**, 151–152.