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A CONFORMATIONAL STUDY OF PROLINE DERIVATIVES

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ABSTRACT. From the study of the structures and molecular conformations of a number of proline derivatives, some conclusions were drawn. The widening effect of angle C^{α}-C'-N' is caused by steric repulsion between a hydrogen atom at C^{α} of the preceding prolyl residue with any other at either C^{α} or C^{δ} of the pyrrolidine ring *cis* to it. This effect is influenced by the distance between the said hydrogen atoms: the nearer this distance is, the greater is the steric repulsion and the wider is the angle of steric repulsion. The ratio of the angle of steric repulsion to the distance between C^{α} and the following C^{α} (or C^{δ}) *cis* to it is approximately 40 and 41 for peptides with *trans* and *cis* configurations, respectively. The torsion angle ranges for χ_1, χ_3, χ_4, θ and φ in these derivatives are widened more than usual. The highest vibration, which more often takes place at either the C^{β} or C^{γ} of the pyrrolidine ring, does so not necessarily at the one that is puckered. A $\Delta\Psi$-relationship is established, for the determination of α-helixity or collageneity, also in small peptides and amino acids that contain proline. The $\Delta\Psi$-relationship is versatile and gives about $+180^{\circ}$ and -180° for the two categories, respectively. The distance between the carbonyl and hydroxyl (or otherwise) terminal end atoms is minimal (2.2 Å) and constant, for all peptides. The ratios of the angles at the carbonyl carbons (O'-C'-N') or (O'-C'-O') to this distance is also constant: 56 and 57 for the *cis* and *trans* confirgurations, respectively; *i.e.* a proline O'-C'-N'- (or O'-C'-O')-test, hereinafter called the CT-test, has been established for the determination of *cis* and *trans* configurations. It is also established in these proline derivatives, that whereas puckering takes place at C^{β} for the C_S form, it does so at C^{γ} for the C_2 form.

KEY WORDS: Molecular conformations, Proline derivatives, Peptides, Amino acids

INTRODUCTION

When the cyclic amino acid proline appears along a polypeptide chain, it causes unfavourable steric interactions for the next sequence of the neighbouring amino acids, notably, that of its C^{β}-CH_2 group [1]. For this reason, proline has been regarded as a helix breaker. To study the cause of these neighbouring-group effects along a polypeptide chain after and before the position of a proline residue, it is essential to study peptides containing proline with different side residues at the amino and carboxyl terminal of proline.

The model systems, which were used in the conformational studies, were *t*-butyloxycarbonylalanylproline (*t*-BocAlaPro) [2], *t*-butyloxycarbonylglycylproline (*t*-BocGlyPro) [3], *t*-butyloxycarbonylprolyproline (*t*-BocProPro) [4], N-propionylproline [5] and the benzyl ester of proline hydrochloride [6].

EXPERIMENTAL

The crystals were grown from acetyl acetate [2, 3], ethyl acetate with a few drops of petroleum ether added [4, 5], and from methanol [6]. Approximate cell dimensions and space-group information were obtained from Weissenberg photographs. The θ-2θ mode (scan range 3^{o}-70^{o}) with the five-measurements technique was adopted [7, 8]. Independent reflexions were collected

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*Corresponding author. E-mail: kamwaya@chem.udsm.ac.tz from small crystals of diameter < 0.8 mm. Reflexions with $I < 2\sigma(I)$ were considered unobserved. Ni-filtered CuK_α radiation was used ($\lambda = 1.5418$ X). No absorption correction was applied.

RESULTS AND DISCUSSION

The crystals were found to be orthorhombic, space group $P2_12_12_1$ with Z = 4. Final R = 0.039 for *t*-BOC-Ala-Pro, R = 0.040 for *t*-BOC-Gly-Pro, R = 0.036 for *t*-BOC-Pro-Pro, R = 0.051 for N-propionylproline and R = 0.032 for the benzyl ester of proline hydrochloride. Molecules in the BOC-amino acids and in N-propionylproline are joined by hydrogen bonds. In addition there is a molecule of water in *t*-BOC-Ala-Pro, rendering high stability for the crystal packing. The absolute configuration of the non-centrosymmetric structures and molecular conformations were determined by anomalous dispersion. The groups $N^1C^\alpha C^\gamma C^\delta$ (C_S-form) of the five-membered ring for the BOC-amino acids and N-propionylproline on the one hand, and $N^1C^\beta C^\delta$ (C_2-form) for the benzyl ester of proline hydrochloride on the other, are fairly planar. Whereas it is C^β which is readily displaced from the mean plane in the first case, it is C^γ which is puckered in the benzyl ester of proline hydrochloride. The average deviation from the plane is about 0.523 Å which is in agreement with investigations in other proline derivatives [9-15]. The torsion angle χ_1 about the pyrrolidine ring takes positive values for the derivatives, indicating that they all belong to conformation B. Whereas there seem to exist two shiftal conformations for the BOC-amino acids of the form C_S-C^γ-endo (C^β-exo), C_S-C^γ-exo (C^β-exo) (preferably the former), and C_S-C^γ-exo(C^β-endo), C_S-C^γ-endo(C^β-exo) (preferably the former) for N-propionylproline, there is only one shiftal conformation found in the benzyl ester of proline hydrochloride, the C_2-C^γ-endo (C^β-endo). The C^α atom of the preceding prolyl residue in *t*-BOC-Ala-Pro and in *t*-BOC-Gly-Pro is in the *trans* configuration, while it is in the *cis* and *trans* configurations in *t*-BOC-Pro-Pro and only in the *cis* in N-propionylproline. The negative values of Ψ in *t*-BOC-Gly-Pro, *t*-BOC-Pro-Pro and N-propionylproline imply that they belong to the α-helix type of proline compounds, while *t*-BOC-Ala-Pro shows a collagenlike Ψ value of 161°. The range for the torsion angles in benzyl ester of proline hydrochloride and *t*-BOC-Pro-Pro in particular is by far wider than usual (Figure 1).

The angles O_1-C_5'-N_1' and C_6^α-C_{10}'-N_2' in the *t*-BOC-Pro-Pro are 109° and 119°, respectively, showing the angle-widening effect (steric repulsion) in the latter angle, caused by the hydrogen atom at C_6^α of the first ring with another at C_{14}^δ *cis* to it of the second ring. This effect seems to be influenced not only by the sum of the hydrogen atoms at the C^α's and C^δ's *cis* to each other, but more so by the distance between them: the nearer this distance is, the greater is the steric repulsion and the larger is the angle of steric repulsion (119°). The ratio of the angle of steric repulsion to the distance between C^α and the following C^αor C^δ *cis* to it is approximately 40 for peptides with a *trans* configuration and more than 41 for those with a *cis* configuration. The situation in the benzyl ester of proline hydrochloride can be likened to conformation A of DL-proline-hydrochloride [11] (Table 1).

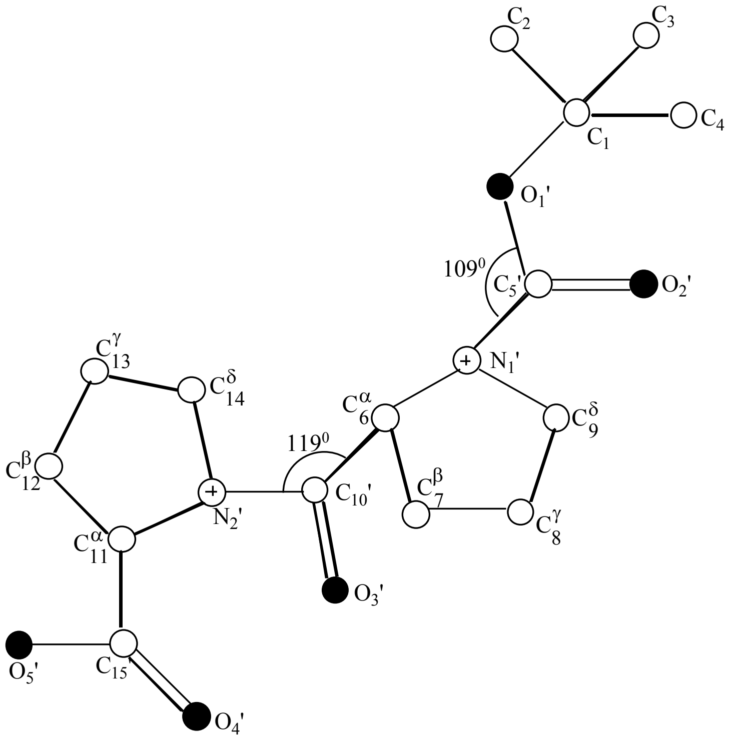

Figure 1. Schematic drawing of *t*-BOC-Pro-Pro showing the angle-widening effect (119°), caused by steric repulsion of a hydrogen atom at C_6^α of the first ring with another at C_{14}^δ of the second ring *cis* to it. The two rings are folded, in which C_7^β and C_{12}^β are displaced from their best plane by 0.4471 and -0.5682 Å, respectively.

Table 1. Steric repulsion is influenced by the distance between the hydrogen atom at C^α and any other at the following C^α (or C^δ) *cis* to it: the nearer this distance is, the larger is the angle of repulsion. The ratio of the angle of steric repulsion to the distance between C^α and the following C^α (or C^δ) *cis* to it is about 40 for peptides with a *trans* configuration and above 41 for those with a *cis* configuration.

	O_2'-C'-N' C° -C'-N'	*cis* C^α-C° *cis* C° -C^δ	Ratio	Configuration
t-BOC-Pro-Pro [4]	124.9° 118.7°	2.98 Å	41 40	*cis* *trans*
t-BOC-Ala-Pro [2]	118.04°	2.97 Å	40	*trans*
N-propionylproline [5]	117.4°	2.90 Å	41	*cis*
t-BOC-Gly-Pro [3]	116.9°	2.897 Å	40	*trans*

It may be generalized that the torsion angle ranges for χ_1, χ_2, χ_3, χ_4, θ and Ψ in these derivatives are widened more than usual, being 40 to -35°, -30 to -41°, 40 to -35°, 7 to -40°, -30 to 30°, and -45 to -95°, respectively. The ω-values for the two rings in *t*-BOC-Pro-Pro suggest *cis* and *trans* configurations, respectively, (Table 2a, Figure2). The ω-values given in the literature are ±0° and ±180° for the *cis* and *trans* configurations, respectively.

Table 2a. Conformational dihedral (torsion) angles.

	χ_1	χ_2	χ_3	χ_4	θ	φ	ω	ω-test
t-BOC-Ala-Pro [2]	29	-33	24	-6	-14	-72	170	*trans*
t-BOC-Gly-Pro [3]	29	-36	28	-10	-12	-71	178	*trans*
t-BOC-Pro-Pro [4]	27 38	-31 to -36	23 to 19	-5 to 6	-13 to -28	-67 -95	-7 -176	*cis* *trans*
N-propionylproline[5]	37	-39	26	-3	-21	-75	-8	*cis*
Benzyl ester of proline hydrochloride [6]	2	-25	39	-39	25	-57		
Oligo-peptides [11]	20 to -40	-30 to -40	20 to -35	-5 to -20	-15 to 15	-45		
DL-proline hydrochloride [11]	-35	23	-3.8	-184	33.7			

Further, a second Ψ_1-Ψ_2 relationship is established for the determination of collageneity and α-helixity also in small peptides and amino acids that contain proline. The Ψ_1-Ψ_2 relationship, where Ψ_1 is the torsion angle containing the double-bond part of the carboxyl group of the pyrrolidine ring (N_1'-$C_6\alpha$-C_{10}'-O_3' in *t*-BOC-Pro-Pro) and Ψ_2 being that of the hydroxyl group or any other (here N_1'-C_6^{α}-C_{10}'-N_2') can be useful as a quick check for collageneity or α-helixity. The Ψ_1-Ψ_2 relationship gives about +180° and -180° for the collagenlike and α-helix type of compounds, respectively. The other hitherto known test is that the torsion angle Ψ_1 is normally negative for the α-helix and very large (150° to 180°) for the collagenlike type of compounds (cf. *t*-BOC-Ala-Pro with Ψ_1 = 161°). The Ψ_1-Ψ_2 relationship is in complete agreement for the BOC-amino acids and N-propionylproline, where Ψ_1-Ψ_2 equals about +180° for *t*-BOC-Ala-Pro and about -180° for *t*-BOC-Gly-Pro, *t*-BOC-Pro-Pro and N-propionylproline, respectively. It must be admitted here that the rings of *t*-BOC-Pro-Pro behave differently since they are folded (C_7^{β} and C_{12}^{β} are displaced from their best plane by 0.4471 and -0.5682 Å, respectively). In the benzyl ester of proline hydrochloride, too, this generalization (of Ψ_1-Ψ_2) is not so obvious. The $\Delta\Psi$ relationship is thus Ψ_1-Ψ_2 for the preceding prolyl residue and Ψ_2-Ψ_1 for the following prolyl residue, indicating the versatile behaviour of proline along a polypeptide chain (Table 2b, Figure 2, Table 2c).

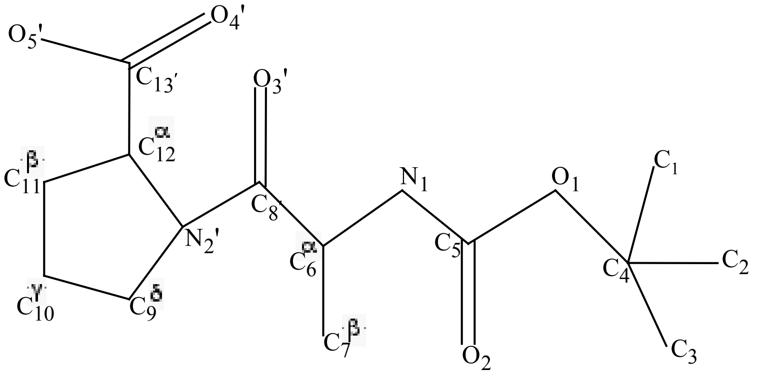

Figure 2. The molecule of *t*-BOC-Ala-Pro [2].

Table 2b. Conformational dihedral (torsion) and bond angles. The $\Delta\Psi$ relation gives about +180° for the collagenlike type of peptides and -180° for the α-helix type.

	Ψ (°)	Angle at C_i' (°)	angle at C_i^{α} (°)
t-BOC-Ala-Pro [2]	161 -21 (OH group)	118	112
t-BOC-Gly-Pro [3]	-25 156 (OH group)	117.4	111
t-BOC-Pro-Pro [4]	-39 141 (ON group) 12 -169 (OH group)	109 119	110 111
N-propionylproline [5]	-6 176 (OH group)	116.9	111
Benzylester ofproline hydrochloride [6]	5 -176 (OC group)		
Oligopeptides [15]		114 to 118	110
DL-prolineHydrochloride [11]	11 -168		

Table 2c. Conformational torsion angles (°) of particular interest in *t*-BOC-Ala-Pro [2]. Values in parantheses refer to standard deviations.

Backbone conformational angles

O_1-C_5-N_1-C_6^{α}	179.9(3)	ωAla
C_5-N_1-C_6^{α}-C_8'	-95.4(3)	φAla
N_1-C_6^{α}-C_8'-N_2'	153.6(3)	ψAla
C_6^{α}-C_8'-N_2'-C_{12}^{α}	170.4(3)	ωPro
C_8'-N_2'-C_{12}^{α}-C_{13}'	-71.8(3)	φPro
N_2'-C_{12}^{α}-C_{13}'-O_4'	160.9(3)	ψ_1Pro
N_2'-C_{12}^{α}-C_{13}'-O_5'	-21.8(3)	ψ_2Pro

Pyrrolidine ring dihedral angles

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_{12}^{α}-C_{11}^{β}-C_{10}^{γ}- 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^{I}
C_{13}'-C_{12}^{α}- N_2'-C_9^{δ}	105.5(3)	θ^{II}
C_8'-N_2'- C_9^{δ}- C_{10}^{γ}	171.2(3)	θ^{III}
C_8'-N_2'-C_{12}^{α}-C_{11}^{β}	168.4(3)	θ^{IV}

It is observed in *t*-BOC-Ala-Pro that atom C_{11}^{β} is readily displaced from the best plane ($N_2'C_{12}^{\alpha}C_{10}^{\gamma}C_9^{\delta}$) of the five-membered ring and deviates by 0.489 Å (Table 2d, plane 3).

Table 2d. Least-squares planes and deviations (Å) of atoms from best plane in *t*-BOC-Ala-Pro [2] calculated according to Schomaker, Waser, Marsh and Bergman [16]. Values in paranthes refer to standard deviations.

Plane 1: C_4, O_1, C_5, N_1 and C_6^{α} $(0.594x + 0.330y + 0.734z = 16.694)$

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^{α}	-0.028(8)

Plane 2: C_{12}', C_{13}', O_4' and O_5' $(0.605x - 0.793y - 0.078z = 4.775)$

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' $(0.259x + 0.341y - 0.904z = 6.099)$

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)

Plane 4: C_6^{α}, C_8', O_3', N_2' and C_{12}^{α} $(0.294x + 0.447y - 0.845z = 7.639)$

C_6^{α}	-0.051(4)	C_8'	0.036(3)
O_3'	0.060(4)	N_2'	0.069(1)
C_{12}^{α}	-0.060(4)	C_9^{δ}	0.159(9)
N_1	0.464(4)		

The highest vibration which more often takes place at either C^{β}or C^{α} of the pyrrolidine ring, occurs, as a rule, at one of these atoms that lies in the best plane of the ring (this can be recognized through its large equivalent thermal parameter) and not necessarily at one that is puckered (Table 3).

Table 3. Puckering seems to occur, as a rule, at atom C^{β} for the C_S-form, and at atom C^{γ} for the C_2-form.

	N	C^{α}	C^{β}	C^{γ}	C^{δ}	Form
	$B_{eq}(Å)^2$					
t-BOC-Ala-Pro [2]	3.1	3.1	4.6 (puckering)	6.4 (highest vibration)	4.6	C_S
t-BOC-Pro-Pro [4]	5.0	4.9	7.9 (puckering)	9.4 (highest vibration)	7.3	C_S
N-propionyl proline [5]	3.5	3.7	4.8 (puckering)	5.1 (highest vibrations)	4.2	C_S
Benzyl ester of proline hydrochloride [6]	3.2	3.1	4.8 (highest vibration)	4.6 (puckering)	3.9	C_2
t-BOC-Gly-Pro [3]	4.8	5.1	7.3 (puckering) (highest vibration)	7.1	5.9	C_S

It appears from Table 3 that there is a correlation between puckering and vibration of highest magnitude on the one hand, and the conformational C_S and C_2 forms on the other. It can be concluded in these derivatives, that puckering takes place at C^{β} for the C_S form and at C^{γ} for the C_2 form. This effect may be referred to as the conformational correlation for the C_S and C_2

forms. And besides, the non-contact distance between the carboxyl oxygens (or otherwise terminal end atoms) is minimal (2.2 Å) and constant for all peptides, maintaining this as a universal stable property of the carboxyl group, while the angle between them is reduced for the *cis* configuration. The ratio of the angle at the carbonyl carbon to this distance is also a constant (it is 56 and 57 for the *cis* and *trans* configurations, respectively). This is another method for the determination of *cis* and *trans* configurations without resorting to the rather tedious torsion angle calculations. It may be referred to as the proline CT-test (Table 4).

Table 4. Conformational data at the carbonyl carbon of proline and the proline CT-test and are in complete agreement with the conformational data in Table 1.

	O'-C'-N' O'-C'-O'	O'.....N' O'.....O'	Ratio	CT-test
t-BOC-Ala-Pro [2]	124.3°	2.2 Å	57	*trans*
t-BOC-Gly-Pro [3]	124.7°	2.2 Å	57	*trans*
t-BOC-Pro-Pro [4]	122.6° 124.7°	2.2 Å 2.2 Å	56 57	*cis* *trans*
N-propionylproline [5]	124.0°	2.2 Å	56	*cis*
Benzylester of proline hydrochloride [6]	124.4°	2.2 Å	57	*trans*

CONCLUSION

The observations made about the angle of steric repulsion (Table 1), the ω-test (Table 2a) and the proline CT-test (Table 4) can be used to determine the *cis* and *trans* configurations of small peptides that contain the amino acid proline, on the one hand, while the $\Delta\psi$ relation decides the collagenlike or α-helix type of polypeptides, on the other (Table 2b). Further, it is also established in these proline derivatives, that whereas puckering takes place at C^{β} for the C_S form, it does so at C^{γ} for the C_2 form (Table 3).

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