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X-ray Structure Analysis and Molecular Conformation of the Benzyl Ester of Proline Hydrochloride

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Abstract

$C_{12}H_{15}NO_2.HCl$, $M_r = 242{\cdot}734$, $a = 12{\cdot}379$ (1), $b = 11{\cdot}478$ (4), $c = 8{\cdot}711$ (1) Å, $V = 1237{\cdot}82$ Å^3, $D_x = 1{\cdot}30$, $D_c = 1{\cdot}302$ Mg m^{-3}, orthorhombic, space group $P2_12_12_1$, $Z = 4$, $F(000) = 512$. Final $R = 0{\cdot}032$ for 932 reflexions. The interionic distances are $Cl\cdots H_1N'$ 2·43, $Cl\cdots H_2N'$ 2·24 and $Cl\cdots N'$ 3·08 Å. The absolute configuration of the noncentrosymmetric structure and, therefore, of the molecular conformation was determined by anomalous dispersion. The $NC^{\beta}C^{\delta}$ group in the pyrrolidine ring is fairly planar. C^{γ} is furthest displaced from this plane and deviates by 0·584 Å. C^{β} and C^{γ} are on the same side of the plane in relation to the carboxyl C′. Therefore, the benzyl ester of proline hydrochloride can be denoted as C_2-C^{γ}-*endo* (C^{β}-*endo*). This derivative belongs to conformation *B*, since the dihedral angle $\chi_1 = 2{\cdot}2°$ at the ring takes a positive value.

Introduction

The cyclic amino acid proline is regarded as a helix breaker because its presence at the amino terminal 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 amino terminal, 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 of the proline, notably its C^{β}—CH_2 group (Schimmel & Flory, 1968).

To study the cause of these neighbouring-group effects along a polypeptide chain before and after the position of a proline residue, so as eventually to come to some conclusions concerning the protein conformation, it is essential to study peptides containing proline with different side residues at the amino and carboxyl terminal of proline or to study model substances so as to generalize certain aspects, especially to obtain the conformation of cyclic pyrrolidine systems in proline. The model system used here is the benzyl ester of proline hydrochloride.

Experimental and results

The crystals were grown from methanol. Approximate cell dimensions and space-group information were obtained from Weissenberg photographs. The θ–2θ mode (4–68°) with the three-measurements technique was adopted (Dreissig, 1969; Allen, Rogers & Troughton, 1971). 932 reflexions were collected of which 32 were considered unobserved with $I < 2\sigma(I)$. Only 75% of the expected reflexions were measured. Ni-filtered Cu $K\alpha$ radiation was used ($\lambda = 1{\cdot}5418$ Å). No absorption correction was applied (diameter of crystal < 0·8 mm).

Structure determination and refinement

The phase problem was solved with *MULTAN* 77 (Main, Lessinger, Woolfson, Germain & Declercq, 1977). An overall temperature factor ($B = 4{\cdot}1$ Å^2) and scale factor were evaluated (Wilson, 1942) and used to compute normalized structure factors (*E*'s) (Karle & Hauptman, 1956). From five reflexions in the starting set and 300 $E > 1{\cdot}09$ all phases could be evaluated. The statistics of the *E*'s confirmed a noncentrosymmetric structure. All non-H atoms could be located. *R* was 0·3262. Least-squares refinement with an overall isotropic temperature factor of 4·1 Å^2 was carried out (Stewart, 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 0·035.

Determination of the absolute configuration

Additional cycles including dispersion were required at the end of the refinement to differentiate the two

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enantiomorphic forms of the structure. The atomic coordinates of the solution structure as well as those with inverted coordinates were used, the latter having the same effect as taking f'' values negative. In this way both the real and imaginary part of the anomalous dispersion were taken into account. The values for Cu $K\alpha$ radiation for O, N and C were from Cromer & Liberman (1970). Through significance tests on both enantiomorphic forms, a choice between the two possibilities could be made (Hamilton, 1956). R showed deviations from the original value of R = 0·035. The anomalous-dispersion correction after inversion of the atomic positions gave a better fit: R^+ = 0·043, R^- = 0·032. Therefore, the latter enantiomorphic form was chosen to represent the true picture of the absolute configuration (Bijvoet, 1949).

Table 1. *Atomic coordinates and equivalent isotropic temperature factors*

	x	y	z	B_{eq}* (Å^2)
Cl	0·2914 (1)	0·6518 (1)	−0·0953 (2)	4·1 (1)
O_1	0·2762 (3)	0·6457 (3)	0·4201 (5)	6·0 (2)
O_2	0·1711 (2)	0·8034 (2)	0·4351 (4)	4·2 (1)
N′	0·1649 (3)	0·5547 (3)	0·1803 (5)	3·2 (1)
C_1^{α}	0·1327 (3)	0·6717 (4)	0·2394 (6)	3·1 (1)
C_2^{β}	0·0101 (4)	0·6620 (5)	0·2747 (9)	4·8 (3)
C_3^{γ}	−0·0192 (4)	0·5371 (6)	0·2391 (8)	4·6 (3)
C_4^{δ}	0·0635 (4)	0·4978 (5)	0·1233 (8)	3·9 (2)
C_5'	0·2019 (3)	0·7028 (3)	0·3744 (6)	3·4 (1)
C_6	0·2367 (4)	0·8491 (5)	0·5644 (7)	4·5 (3)
C_7	0·1667 (3)	0·9347 (4)	0·6474 (6)	3·5 (2)
C_8	0·1709 (4)	1·0527 (4)	0·6150 (7)	4·3 (2)
C_9	0·1005 (5)	1·1286 (4)	0·6875 (8)	5·4 (1)
C_{10}	0·0258 (5)	1·0888 (5)	0·7886 (8)	5·6 (4)
C_{11}	0·0213 (5)	0·9715 (6)	0·8243 (8)	5·5 (3)
C_{12}	0·0924 (4)	0·8949 (4)	0·7548 (7)	4·4 (4)

* Willis & Pryor (1975).

Table 2. *Bond angles* (°) *and lengths* (Å)

$\mathrm{N'{-}C_4^{\delta}{-}C_3^{\gamma}}$	102·5 (5)	$\mathrm{O_1{-}C_5'{-}O_2}$	124·4 (4)
$\mathrm{N'{-}C_1^{\alpha}{-}C_2^{\beta}}$	105·4 (4)	$\mathrm{O_2{-}C_6{-}C_7}$	106·3 (4)
$\mathrm{N'{-}C_1^{\alpha}{-}C_5'}$	109·4 (3)	$\mathrm{C_6{-}C_7{-}C_8}$	121·5 (4)
$\mathrm{C_1^{\alpha}{-}C_2^{\beta}{-}C_3^{\gamma}}$	105·2 (4)	$\mathrm{C_7{-}C_8{-}C_9}$	120·0 (5)
$\mathrm{C_1^{\alpha}{-}C_5'{-}O_1}$	124·7 (4)	$\mathrm{C_8{-}C_9{-}C_{10}}$	120·9 (5)
$\mathrm{C_1^{\alpha}{-}C_5'{-}O_2}$	110·9 (3)	$\mathrm{C_9{-}C_{10}{-}C_{11}}$	120·1 (6)
$\mathrm{C_1^{\alpha}{-}N'{-}C_4^{\delta}}$	106·4 (3)	$\mathrm{C_{10}{-}C_{11}{-}C_{12}}$	119·6 (6)
$\mathrm{C_2^{\beta}{-}C_3^{\gamma}{-}C_4^{\delta}}$	105·0 (5)	$\mathrm{C_{11}{-}C_{12}{-}C_7}$	120·5 (5)
$\mathrm{C_2^{\beta}{-}C_1^{\alpha}{-}C_5'}$	114·9 (5)	$\mathrm{C_{12}{-}C_7{-}C_6}$	119·6 (4)
$\mathrm{C_5'{-}O_2{-}O_6}$	116·9 (4)	$\mathrm{C_{12}{-}C_7{-}C_8}$	118·9 (4)
$\mathrm{N'{-}C_4^{\delta}}$	1·500 (6)	$\mathrm{O_2{-}C_6}$	1·485 (7)
$\mathrm{N'{-}C_1^{\alpha}}$	1·493 (6)	$\mathrm{C_6{-}C_7}$	1·497 (7)
$\mathrm{C_1^{\alpha}{-}C_2^{\beta}}$	1·553 (7)	$\mathrm{C_7{-}C_8}$	1·385 (6)
$\mathrm{C_1^{\alpha}{-}C_5'}$	1·499 (7)	$\mathrm{C_8{-}C_9}$	1·384 (8)
$\mathrm{C_2^{\beta}{-}C_3^{\gamma}}$	1·510 (9)	$\mathrm{C_9{-}C_{10}}$	1·356 (9)
$\mathrm{C_3^{\gamma}{-}C_4^{\delta}}$	1·506 (8)	$\mathrm{C_{10}{-}C_{11}}$	1·383 (9)
$\mathrm{C_5'{-}O_1}$	1·198 (5)	$\mathrm{C_{11}{-}C_{12}}$	1·384 (8)
$\mathrm{C_5'{-}O_2}$	1·326 (5)	$\mathrm{C_{12}{-}C_7}$	1·389 (7)

Positional and thermal parameters are given in Table 1, bond angles and lengths in Table 2 and torsion angles in Table 3.[*] Fig. 1 is a view of the unit-cell contents and Fig. 2 shows an *ORTEP* (Johnson, 1965) drawing of the molecule with atom numbering.

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 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 conformation of a proline ring can best be expressed through three terms: the approximate symmetry of the ring (C_s or C_2), the atom C^{α}, C^{β} or C^{γ} outside the plane, and the direction of the shift of the atom from

Table 3. *Torsion angles* (°)

χ_1	$\mathrm{N'{-}C_1^{\alpha}{-}C_2^{\beta}{-}C_3^{\gamma}}$	2·2 (6)	θ''	$\mathrm{C_5'{-}C_1^{\alpha}{-}N'{-}C_4^{\delta}}$	145·8 (4)		$\mathrm{C_8{-}C_9{-}C_{10}{-}C_{11}}$	2·1 (10)
χ_2	$\mathrm{C_1^{\alpha}{-}C_2^{\beta}{-}C_3^{\gamma}{-}C_4^{\delta}}$	−25·2 (7)		$\mathrm{C_1^{\alpha}{-}C_5'{-}O_2{-}C_6}$	−176·4 (4)		$\mathrm{C_9{-}C_{10}{-}C_{11}{-}C_{12}}$	−0·9 (10)
χ_3	$\mathrm{C_2^{\beta}{-}C_3^{\gamma}{-}C_4^{\delta}{-}N'}$	38·5 (6)		$\mathrm{C_5'{-}O_2{-}C_6{-}C_7}$	−159·9 (4)		$\mathrm{C_{10}{-}C_{11}{-}C_{12}{-}C_7}$	−1·3 (9)
χ_4	$\mathrm{C_3^{\gamma}{-}C_4^{\delta}{-}N'{-}C_1^{\alpha}}$	−37·5 (5)		$\mathrm{O_2{-}C_6{-}C_7{-}C_8}$	−94·5 (5)		$\mathrm{C_{11}{-}C_{12}{-}C_7{-}C_8}$	2·2 (8)
θ	$\mathrm{C_4^{\delta}{-}N'{-}C_2^{\beta}{-}C_3^{\gamma}}$	24·5 (4)		$\mathrm{C_6{-}C_7{-}C_8{-}C_9}$	176·2 (5)		$\mathrm{C_{12}{-}C_7{-}C_8{-}C_9}$	−1·0 (8)
θ'	$\mathrm{C_5'{-}C_1^{\alpha}{-}C_2^{\beta}{-}C_3^{\gamma}}$	−118·3 (5)		$\mathrm{C_7{-}C_8{-}C_9{-}C_{10}}$	−1·2 (10)	ψ_1	$\mathrm{N'{-}C_1^{\alpha}{-}C_5'{-}O_1}$	4·7 (8)
						ψ_2	$\mathrm{N'{-}C_1^{\alpha}{-}C_5'{-}O_2}$	−176·3 (7)
						φ	$\mathrm{Cl{-}N'{-}C_1^{\alpha}{-}C_5'}$	−57·4 (3)

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[*] Lists of structure factors, anisotropic thermal parameters and H atom parameters have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 36006 (10 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

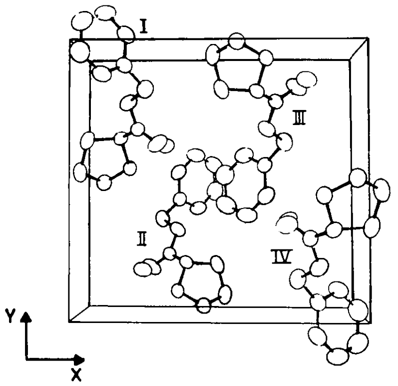

Fig. 1. The unit cell of the benzyl ester of proline hydrochloride: projection on the (001) plane. Symmetry code: (I) x, y, z; (II) $\frac{1}{2} - x, -\frac{1}{2} + y, -z$; (III) $\frac{1}{2} + x, \frac{3}{2} - y, -z + 1$; (IV) $1 - x, 1 - y, -z + \frac{3}{2}$.

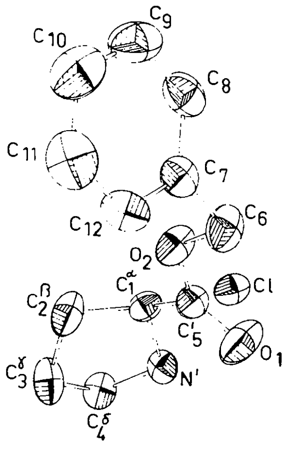

Fig. 2. Molecule of the benzyl ester of proline hydrochloride [Cl···N′ 3·08 (4), Cl···H_1N′ 2·43 (2), Cl···H_2N′ 2·23 (5) Å].

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 dihedral angle χ_1 takes negative values, while in class *B* the values are positive (Balasubramanian, Lakshminarayanan, Sabesan, Tegoni, Venkatesan & Ramachandran, 1971).

In this structure N—C_1^α—C_5' is 109·4° which is comparable to the normal 110°. The N′$C_2^\beta C_4^\delta$ group is planar, and the displacements from this plane are C_1^α = −0·348, C_2^β = 0·000, C_3^γ = 0·584 and C_5' = 0·386 Å (mean estimated standard deviation of 0·002 Å). Thus C_3^γ is most readily displaced from this plane, and is on the same side as the carboxyl C_5'. This structure may, therefore, be denoted as C_2-C^γ-*endo* (C^β-*endo*). The positive dihedral angle $\chi_1 = 2{\cdot}2°$ indicates that the structure belongs to conformation *B*. The other dihedral angles are $\chi_2 = -25{\cdot}2$, $\chi_3 = 38{\cdot}5$, $\chi_4 = -37{\cdot}5$ and $\theta = 24{\cdot}5°$. The interionic distances are Cl···H_1N′ 2·43 (2), Cl···H_2N′ 2·23 (5) and Cl···N′ 3·08 (4) Å. The ranges of the dihedral angles at the peptide ring are larger for conformation *B* in comparison to those given by Balasubramanian *et al.* (1971). This situation is comparable to that for conformation *A* of DL-proline hydrochloride (Mitsui *et al.*, 1969).

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