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X-ray Structure Analysis and Molecular Conformation of *N*-Propionylproline

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Abstract

$C_8H_{13}NO_3$, $a = 14{\cdot}310\,(7)$, $b = 12{\cdot}520\,(6)$, $c = 5{\cdot}079\,(3)$ Å, $V = 909{\cdot}63$ Å^3, $M_r = 171{\cdot}18$, $D_m = 1{\cdot}250$, $D_c = 1{\cdot}249$ Mg m^{-3}, orthorhombic, space group $P2_12_12_1$, $Z = 4$, $F(000) = 368$; final $R = 5{\cdot}1\%$ for 963 observed reflections. The molecules are joined together in pairs by a hydrogen bond: $O' \cdots OH = 2{\cdot}58\,(1)$ Å. C^α is in the *cis* configuration. The absolute configuration of the noncentrosymmetric structure and, therefore, of the molecular conformation was determined by anomalous dispersion. The $NC^\alpha C^\gamma C^\delta$ group in the pyrrolidine ring is fairly planar. C^β deviates by $-0{\cdot}592\,(3)$ Å from the best plane of the five-membered ring; C^α, C^β and C^δ are on the same side whereas N and C^γ are on the opposite side of the plane in relation to the carboxyl C'. Therefore, *N*-propionylproline is C_s–C^γ-*exo* (C^β-*endo*). This derivative belongs to conformation *B*, since the dihedral angle $\chi_1 = 36{\cdot}8\,(4)^\circ$ at the ring takes a positive value.

Introduction

It is known that the presence of proline at the amino terminal of a polypeptide chain causes steric interactions for the preceding amino acids, especially when there is a C^β atom in its side chain. For this reason proline has been regarded as a helix breaker. 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, especially its C^β—CH_2 group (Schimmel & Flory, 1968).

To study the cause of these neighbouring-group effects along a polypeptide chain after and before 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 the 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 *N*-propionylproline.

Experimental and results

The crystals were grown from ethyl acetate. Approximate cell dimensions and space-group information were obtained from Weissenberg photographs. The θ–2θ mode (3–68°) with the three-measurements technique was adopted (Dreissig, 1969; Allen, Rogers & Troughton, 1971). 1041 reflexions were collected of which 963 were regarded as observed with $I > 2\sigma(I)$. Ni-filtered Cu $K\alpha$ radiation was used ($\lambda = 1{\cdot}5418$ Å). No absorption correction was applied (diameter of crystal $< 0{\cdot}8$ mm).

Structure determination and refinement

The phase problem was solved with *MULTAN* 77 (Main, Lessinger, Woolfson, Germain & Declercq,

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1977). An overall temperature factor ($B = 4\cdot6$ Å^2) 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 $>1\cdot28$ all phases could be evaluated. The statistics of the E's confirmed a noncentrosymmetric structure. R was 22·13%. All non-hydrogen atoms could be located. Least-squares refinement with an overall isotropic temperature factor of 5·0 Å^2 was carried out (Stewart, 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 0·053.

Determination of the absolute configuration

At the end of the refinement, additional cycles were carried out including dispersion to differentiate the two 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 parts of the anomalous dispersion were taken into account. The values for Cu $K\alpha$ radiation for O, N and C are from Cromer & Liberman (1970). Through significance tests of R of both enantiomorphs, a choice between the two possibilities could be made (Hamilton, 1956). The computed R values showed deviations from the original value of $R = 0\cdot053$. The anomalous-dispersion correction after inversion of the atomic positions gave a better fit ($R^+ = 0\cdot052$, $R^- = 0\cdot051$, $R^+/R^- = 1\cdot019$, significance $>99\cdot0\%$) for 1041 reflexions. In this way the latter enantiomorph was chosen to represent the true picture for the absolute configuration (Bijvoet, 1949).*

In the course of refinement, in the normal case as well as in the determination of the absolute configuration, it was noted that 15 reflexions, though apparently observed as strong, calculated weak. These reflexions were excluded in the final stage of refinement and are classified as unobserved in the listing.

Positional and thermal parameters are given in Table 1, bond distances and angles in Table 2, dihedral angles in Table 3. Fig. 1 is a view of the unit-cell contents and Fig. 2 a stereodrawing of the molecule.

Table 1. *Atomic positional parameters and isotropic thermal parameters* (Å^2)

	x	y	z	B_{eq}*/B†
O_1'	0·7219 (2)	0·6648 (2)	−0·0061 (7)	5·6 (1)
O_2	0·9938 (2)	0·7373 (2)	−0·1162 (6)	6·3 (1)
O_3	1·0949 (2)	0·7145 (2)	0·2117 (6)	6·1 (1)
N'	0·8626 (2)	0·6098 (2)	0·1191 (6)	3·5 (0)
C_1	0·7408 (5)	0·8664 (5)	0·2688 (20)	10·0 (7)
C_2^{α}	0·8058 (4)	0·7769 (4)	0·3098 (13)	7·3 (4)
C_3	0·7936 (2)	0·6810 (3)	0·1268 (8)	4·4 (3)
C_4^{α}	0·9541 (2)	0·6209 (3)	0·2441 (8)	3·7 (1)
C_5^{β}	0·9917 (3)	0·5069 (3)	0·2289 (10)	4·8 (1)
C_6^{γ}	0·9567 (3)	0·4676 (3)	−0·0318 (9)	5·1 (2)
C_7^{δ}	0·8575 (2)	0·5140 (3)	−0·0487 (8)	4·2 (1)
C_8'	1·0147 (2)	0·6978 (3)	0·0910 (8)	4·0 (1)
$H_1(C_1)$	0·769 (3)	0·935 (4)	0·380 (11)	0·12 (2)
$H_2(C_2)$	0·697 (5)	0·860 (6)	0·038 (17)	0·21 (2)
$H_3(C_1)$	0·786 (2)	0·873 (2)	0·094 (6)	0·03 (1)
$H_4(C_2^{\alpha})$	0·880 (4)	0·804 (5)	0·283 (14)	0·17 (2)
$H_5(C_2^{\alpha})$	0·755 (5)	0·784 (6)	0·438 (15)	0·17 (3)
$H_6(C_4^{\alpha})$	0·804 (3)	0·461 (3)	0·017 (8)	0·07 (1)
$H_7(C_5^{\beta})$	0·843 (3)	0·543 (3)	−0·247 (9)	0·07 (1)
$H_8(C_5^{\gamma})$	0·958 (3)	0·380 (4)	−0·043 (12)	0·14 (2)
$H_9(C_6^{\gamma})$	1·003 (3)	0·513 (3)	−0·186 (10)	0·09 (1)
$H_{10}(C_6^{\gamma})$	0·954 (3)	0·464 (4)	0·037 (11)	0·11 (2)
$H_{11}(C_7^{\delta})$	1·055 (3)	0·512 (3)	0·238 (10)	0·08 (1)
$H_{12}(C_7^{\delta})$	0·946 (2)	0·642 (3)	0·391 (7)	0·05 (1)
$H_{13}(O_3)$	1·130 (3)	0·767 (4)	0·106 (12)	0·13 (2)

* Willis & Pryor (1975).

† For H atoms $T = \exp(-8\pi^2 U \sin^2 \theta/\lambda^2)$.

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

$C_1-C_2^{\alpha}-C_3'$	116·1 (5)	$C_1-C_2^{\alpha}$	1·471 (8)
$C_2^{\alpha}-C_3'-O_1'$	123·4 (3)	$C_2^{\alpha}-C_3'$	1·529 (7)
$C_2^{\alpha}-C_3'-N'$	117·4 (3)	$C_3'-O_1'$	1·245 (4)
$O_1'-C_3'-N'$	119·1 (3)	$C_3'-N'$	1·329 (4)
$C_3'-N'-C_4^{\alpha}$	126·0 (3)	$N'-C_4^{\alpha}$	1·462 (4)
$N'-C_4-C_5$	101·8 (3)	C_4-C_5	1·527 (5)
$C_4^{\alpha}-C_5^{\beta}-C_6^{\gamma}$	103·6 (3)	$C_5^{\beta}-C_6^{\gamma}$	1·499 (7)
$C_5^{\beta}-C_6^{\gamma}-C_7^{\delta}$	103·5 (3)	$C_6^{\gamma}-C_7^{\delta}$	1·537 (5)
$C_6^{\gamma}-C_7^{\delta}-N'$	103·3 (3)	$C_7^{\delta}-N'$	1·474 (4)
$C_7^{\delta}-N'-C_4^{\alpha}$	111·8 (2)	$C_4^{\alpha}-C_8'$	1·511 (5)
$N'-C_4^{\alpha}-C_8{}'$	110·5 (3)	$C_8'-O_2$	1·201 (5)
$C_4^{\alpha}-C_8'-O_3$	111·2 (3)	$C_8'-O_3$	1·317 (4)
$O_2-C_8'-O_2$	124·0 (3)		
$C_3'-N'-C_7^{\delta}$	121·7 (3)		
$C_5^{\beta}-C_4^{\alpha}-C_8'$	111·5 (3)		
$C_4^{\alpha}-C_8'-O_2$	124·8 (3)		

Table 3. *Dihedral angles* (°)

	$C_1-C_2^{\alpha}-C_3'-N'$	165·7 (5)	θ'	$C_8'-C_4^{\alpha}-C_5^{\beta}-C_6^{\gamma}$	−81·1 (4)	χ_2	$C_4^{\alpha}-C_5^{\beta}-C_6^{\gamma}-C_7^{\delta}$	−39·4 (4)
ω	$C_2^{\alpha}-C_3'-N'-C_4^{\alpha}$	−8·0 (6)	θ''	$C_8'-C_4^{\alpha}-N'-C_7^{\delta}$	97·9 (3)	χ_3	$C_5^{\beta}-C_6^{\gamma}-C_7^{\delta}-N'$	26·3 (4)
	$C_3'-N'-C_4^{\alpha}-C_8'$	−75·3 (4)	θ'''	$C_3'-N'-C_7^{\delta}-C_6^{\gamma}$	170·5 (3)	χ_4	$C_6^{\gamma}-C_7^{\delta}-N'-C_4^{\alpha}$	−3·1 (4)
ψ_1	$N'-C_4^{\alpha}-C_8'-O_2$	−5·9 (2)	θ^{IV}	$C_3'-N'-C_4^{\alpha}-C_5^{\beta}$	166·1 (4)	θ	$C_7^{\delta}-N'-C_4^{\alpha}-C_5^{\beta}$	−20·7 (4)
ψ_2	$N'-C_4^{\alpha}-C_8'-O_3$	175·5 (3)	χ_1	$N'-C_4^{\alpha}-C_5^{\beta}-C_6^{\gamma}$	36·8 (4)			

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* Lists of structure factors and anisotropic thermal parameters have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 35663 (10 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

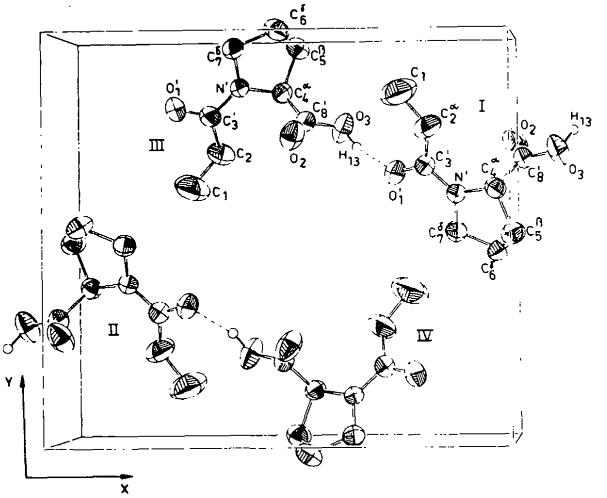

Fig. 1. The unit cell of *N*-propionylproline. Molecules are joined together in pairs by a hydrogen bond [$O'_1\cdots O_3$ 2·584 (9), $O'_1\cdots H_{13}(O_3)$ 1·65 (2) Å]. Symmetry code: (I) x, y, z; (II) $1-x, 1-y, -z+\frac{1}{2}$; (III) $\frac{3}{2}-x, -\frac{1}{2}+y, \frac{1}{2}-z$; (IV) $-\frac{1}{2}+x, \frac{3}{2}-y, -z$.

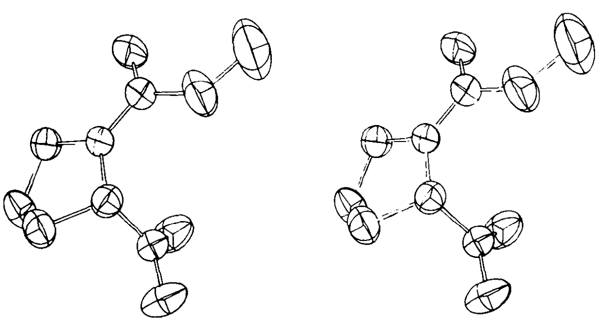

Fig. 2. Stereodrawing (Johnson, 1965) of the molecule perpendicular to the *xy* plane.

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 above or below the plane. In the half-chair form three atoms lie in the plane and the remaining two may be situated above or below this plane (Kilpatrick, Pitzer & Spitzer, 1947; Pitzer & Donath, 1960). Therefore, the conformations can be denoted according to their symmetry elements 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 is about 0·5 Å. This picture has also 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). 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 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). Earlier spectroscopic studies by Oster (1973) showed that C^α of *N*-propionylproline takes the rare *cis* configuration, which is confirmed in the present paper.

It is known that the energy difference between the *cis* and *trans* isomers is about 8·374 kJ mol^{-1}, the *trans* having lower energy (Ramachandran, Ramakrishnan & Sasisekharan, 1963). In proline this difference is smaller, since during transition from *cis* to *trans* the contact between C^α_i and C^δ_{i+1} (Fig. 3) is reduced and this is the reason for the occurrence of *cis* bonds in small cyclic peptides at the *N*-terminal end of proline (Karle, Karle, Wieland, Burgemeister, Faulstich & Witkop, 1973; Wieland & Birr, 1976) as well as in isolated cases in globular protein structures (Wright, Alden & Kraut, 1969; Wyckoff, Tsernoglou, Hanson, Knox, Lee & Richards, 1970; Quiocho & Lipscomb, 1971; Epp, Lattman, Schiffer, Huber & Palm, 1975). In this structure the angle C^α_2—C'_3—N′ is 117·4 (3)°, almost equal to the normal value of 118° found mostly in peptide bonds with a *cis* configuration (Pauling, 1960).

The angle O'_1—C'_3—N′ is 119·1 (3)°, comparable to the mean value reported by Ashida & Kakudo (1974) for the oligopeptides L-Len-L-Pro-Gly, tosyl-L-Pro-L-Hyp, *p*-bromo-Z-Gly-L-Pro-Len-Gly, *o*-bromo-Z-Gly-L-Pro, Z-Gly-L-Pro and Aoc-L-Pro, where it is 120·3° and differing from the values cited by Corey & Pauling (1963) and Marsh & Donohue (1967), where it is 125 and 123·5° respectively. The angle N′—C^α_4—C'_8

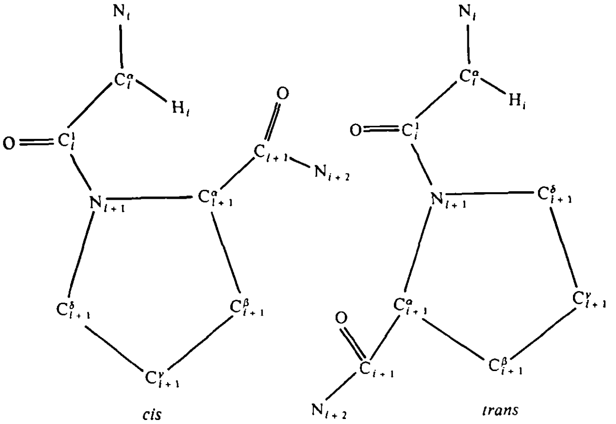

Fig. 3. *cis* and *trans* forms. During the transition to the *trans* form the distance between C^α_i and C^δ_{i+1} for proline is reduced.

is 110·5 (3)°, which is comparable to the normal value of 110°. The N$'$C$_4^\alpha$C$_6^\beta$C$_7^\delta$ group is fairly planar. C$_5^\beta$ is most displaced from this plane and deviates by −0·592 (3) Å. C$_4^\alpha$, C$_5^\beta$ and C$_7^\delta$ are on the same side, while N$'$ and C$_6^\gamma$ are on the opposite side of the plane in relation to the carboxyl C$_8'$. The displacements from the plane are $\mathrm{N}' = 0{\cdot}0176$, $\mathrm{C}_4^\alpha = -0{\cdot}0111$, $\mathrm{C}_5^\beta = -0{\cdot}592$, $\mathrm{C}_6^\gamma = 0{\cdot}010$, $\mathrm{C}_7^\delta = -0{\cdot}0166$ and $\mathrm{C}_8' = -2{\cdot}828$ Å. This prolyl residue can thus be denoted as C_s–C$^\nu$-*exo* (C$^\beta$-*endo*). The dihedral angle $\chi_1 = 36{\cdot}8\ (4)^\circ$ indicates that *N*-propionylproline belongs to conformation *B*. The other dihedral angles are $\chi_2 = -39{\cdot}4$, $\chi_3 = 26{\cdot}3$, $\chi_4 = -3{\cdot}1$, $\theta = -20{\cdot}7$, $\omega = -8{\cdot}0$, $\varphi = -75{\cdot}3$, $\psi_1 = -5{\cdot}9$ and $\psi_2 = 175{\cdot}5^\circ$ (OH group).

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