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Precision Neutron Diffraction Structure Determination of Protein and Nucleic Acid Components. IX. The Crystal and Molecular Structure of 4-Hydroxy-L-proline*

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A neutron diffraction study of 4-hydroxy-L-proline, $C_5H_9NO_3$, has been carried out. Space group $P2_12_12_1$; $Z=4$; $a=4{\cdot}995$ (3), $b=8{\cdot}307$ (3), $c=14{\cdot}193$ (5) Å. Full-matrix least-squares refinements, varying anisotropic thermal parameters for all atoms and including an anisotropic extinction correction, have led to a conventional R value of 0·032. The neutron diffraction results agree well with the results of new least-squares refinements based on data from an earlier X-ray diffraction study [Donohue & Trueblood (1952). *Acta Cryst.* **5**, 419–431]; the main new feature is the precise determination of position and thermal motion parameters for all hydrogen atoms. The structure consists of layers of hydroxyproline zwitterions approximately perpendicular to **a** and stabilized by a network of one O–H···O and two N–H···O hydrogen bonds. One of the N–H···O bonds is an unusual intramolecular hydrogen bond.

Introduction

A neutron diffraction study of 4-hydroxy-L-proline has been carried out in order to determine precise positions and thermal motion parameters for the hydrogen atoms. This work is part of a series of neutron diffraction studies, currently underway in this Laboratory, of the principal naturally occurring amino acids.

The structure of hydroxyproline has previously been studied by X-ray techniques (Zussman, 1951; Donohue & Trueblood, 1952*a*,*b*).

Crystal data

4-Hydroxy-L-proline;
$C_5H_9NO_3$, F.W. 131·13;
Orthorhombic; $a=4{\cdot}995$ (3), $b=8{\cdot}307$ (3), $c=14{\cdot}193$ (5) Å;
Space group $P2_12_12_1$; $Z=4$;
Density $\varrho_{calc}=1{\cdot}479$, $\varrho_{obs}=1{\cdot}474$ (3) g cm^{-3} (Donohue & Trueblood, 1952*b*);
Absorption coefficient $\mu=2{\cdot}43$ cm^{-1}.

Experimental

Large single crystals of 4-hydroxy-L-proline suitable for neutron diffraction were grown by slow evaporation from aqueous solution. The crystals are colorless prisms elongated in the **a** direction with bounding faces {001} and {011}. A well formed sample 13·2 mm^3 in volume and of dimensions 3·0 × 2·0 × 2·8 mm along **a**, **b**, and **c** respectively, was mounted on a four-circle neutron diffractometer at the Brookhaven High Flux Beam Reactor. Data were collected automatically at room temperature under the Multi-Spectrometer Control System (Beaucage, Kelley, Ophir, Rankowitz, Spinrad & van Norton, 1966), using a crystal-monochromated neutron beam of wavelength $\lambda=1{\cdot}0142$ Å. The orthorhombic cell constants were refined by least-squares techniques from the diffractometer setting angles observed for 27 reflections well distributed in reciprocal space, and they agree to within 0·1% with the cell constants found by Donohue & Trueblood.

Intensities were measured for 1325 hkl and $hk\bar{l}$ reflections with $d^*<1{\cdot}36$ Å^{-1} using the standard θ–2θ step scan technique. The observed systematic absences confirm that the space group is $P2_12_12_1$, as determined in the X-ray studies.

Background corrections were made using a method which divides the reflection profile into peak and background in such a way that $\sigma(I)/I$ is minimized. I is the integrated intensity, and $\sigma(I)$ its standard deviation based on counting statistics (Lehmann, Hamilton & Larsen, 1972). An absorption correction calculated by integration over a Gaussian grid was applied to the observed intensities, and calculated transmission coefficients ranged from 0·61 to 0·67. The neutron absorption coefficient $\mu=2{\cdot}43$ cm^{-1} was calculated assuming an incoherent scattering cross-section for hydrogen of 40 barns. Mass absorption coefficients for C, N and O were taken from *International Tables for X-ray Crystallography* (1962).

Squared observed structure factors were obtained as $F_o^2=I\sin 2\theta$, and were averaged for symmetry-related reflections. The agreement index is $R_c=\sum|F_o^2-\bar{F}_o^2|/\sum F_o^2$

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$=0{\cdot}031$, where $\bar{F}_o^2$ is the mean value for the symmetry-related reflections. Of the 874 unique reflections measured, 63 reflections having $F_o^2 < 3\sigma_{\text{count}}(F_o^2)$ were omitted from subsequent refinements.

Structure refinement

All nine hydrogen atoms in the hydroxyproline molecule were located from a series of difference Fourier syntheses, starting with phases calculated from the positions of non-hydrogen atoms given by Donohue & Trueblood. The structure was refined by full-matrix least-squares techniques, using first isotropic and then anisotropic temperature factors for all atoms. Neutron scattering lengths used were $b_O=0{\cdot}577$, $b_N=0{\cdot}940$, $b_C=0{\cdot}665$ and $b_H=-0{\cdot}372$ (10^{-12} cm). The quantity minimized in the refinements was $\sum w|F_o^2-|F_c|^2|^2$; weights were chosen as $w=1/\sigma^2(F_o^2)$ with $\sigma^2(F_o^2)=\sigma^2_{\text{count}}(F_o^2)+(0{\cdot}01\,F_o^2)^2$, and with $\sigma_{\text{count}}(F_o^2)$ based on counting statistics.

The crystal used for data collection showed quite severe extinction, and an extinction correction (Zachariasen, 1967) was included in the refinements. A Type II anisotropic extinction correction (Coppens & Hamilton, 1970) was introduced in the final refinement cycles, which were based on the full, unaveraged data set of 1248 reflections having $F_o^2 < 3\sigma(F_o^2)$. 169 parameters were varied in the final cycles. The extinction correction factor E, which multiplies the calculated structure factor, is

$$E=\left[1+\frac{(2\bar{T}|F_c|^2 g\lambda^3)(10^4)}{V^2\sin 2\theta}\right]^{-1/4}$$

where $\bar{T}$ is the average beam path length through the crystal for a given reflection, V is the unit-cell volume and F_c is the calculated structure factor on an absolute scale. For Type II anisotropic extinction, $g=(\mathbf{N}'\mathbf{W}'\mathbf{N})^{-1/2}$ where $\mathbf{W}'$ is a symmetric tensor describing the anisotropy of domain size and $\mathbf{N}$ is a unit vector in the diffraction plane and perpendicular to the incident beam. The lowest value of E was 0·60 for the 020 reflection. The refined atomic parameters are given

Table 1. *Final atomic coordinates and thermal parameters* ($\times 10^4$)

The temperature factor is of the form

$$\exp\{-2\pi^2(u_{11}h^2a^{*2}+u_{22}k^2b^{*2}+u_{33}l^2c^{*2}+2u_{12}hka^*b^*+2u_{13}hla^*c^*+2u_{23}klb^*c^*)\}\,.$$

The atomic numbering scheme adopted by Donohue & Trueblood is given alongside the standard IUPAC designation for amino acids and peptides. The atomic coordinates given by Donohue & Trueblood, which describe the molecule in the D-configuration, are related to our coordinates by the transformation $1-x$, $1-y$, $1-z$.

		x	y	z	u_{11}	u_{22}	u_{33}	u_{12}	u_{13}	u_{23}
O^1	O(1)	0·9913 (4)	0·52266 (20)	0·80896 (10)	401 (8)	539 (9)	290 (7)	−190 (8)	−103 (6)	113 (7)
O^2	O(2)	0·7234 (4)	0·31631 (17)	0·83710 (10)	520 (9)	356 (7)	315 (6)	−142 (7)	−96 (7)	122 (5)
O^δ	O(3)	0·4045 (3)	0·47338 (19)	0·50219 (10)	364 (8)	510 (9)	226 (7)	−28 (7)	−10 (5)	−22 (6)
N	N	0·7255 (2)	0·63014 (9)	0·65941 (6)	330 (5)	236 (3)	261 (4)	−6 (4)	−47 (4)	19 (3)
C	C(1)	0·7927 (2)	0·43722 (12)	0·79175 (7)	283 (5)	258 (5)	193 (4)	−22 (4)	−20 (4)	16 (4)
C^α	C(2)	0·6227 (2)	0·48028 (13)	0·70571 (8)	295 (6)	226 (4)	257 (5)	−29 (4)	−66 (5)	19 (4)
C^β	C(3)	0·6396 (6)	0·35041 (16)	0·62986 (11)	1454 (21)	229 (6)	339 (7)	64 (11)	−385 (11)	−56 (5)
C^γ	C(4)	0·6617 (3)	0·43844 (16)	0·53629 (8)	484 (8)	427 (7)	236 (5)	191 (7)	−69 (5)	−99 (5)
C^δ	C(5)	0·8146 (3)	0·59075 (20)	0·56215 (9)	280 (7)	696 (10)	285 (6)	−60 (7)	41 (5)	−23 (7)
H^1		0·5713 (7)	0·71529 (31)	0·65975 (20)	612 (19)	309 (11)	490 (14)	69 (14)	−25 (14)	52 (11)
H^2		0·8838 (6)	0·67300 (33)	0·69789 (21)	568 (16)	417 (13)	478 (14)	−164 (13)	−157 (14)	14 (11)
H^α		0·4187 (6)	0·50294 (41)	0·72798 (24)	327 (14)	717 (19)	713 (20)	−38 (14)	−9 (12)	245 (17)
$H^{\beta1}$		0·8112 (17)	0·27701 (63)	0·64092 (29)	2859 (98)	871 (30)	661 (25)	1214 (52)	−694 (41)	−343 (22)
$H^{\beta2}$		0·4679 (18)	0·27349 (62)	0·63110 (31)	2881 (98)	849 (30)	599 (24)	−1104 (51)	−554 (39)	195 (21)
H^γ		0·7734 (9)	0·36872 (50)	0·48405 (23)	894 (27)	1086 (27)	536 (18)	589 (25)	−164 (19)	−424 (20)
$H^{\delta1}$		0·4202 (6)	0·49066 (35)	0·43475 (18)	486 (15)	636 (16)	299 (13)	−4 (14)	−56 (10)	41 (11)
$H^{\delta21}$		1·0276 (6)	0·56810 (68)	0·56472 (29)	298 (16)	1760 (46)	753 (22)	−13 (24)	81 (14)	−401 (31)
$H^{\delta22}$		0·7744 (10)	0·68872 (43)	0·51604 (24)	1021 (31)	842 (24)	438 (16)	317 (26)	3 (19)	188 (16)

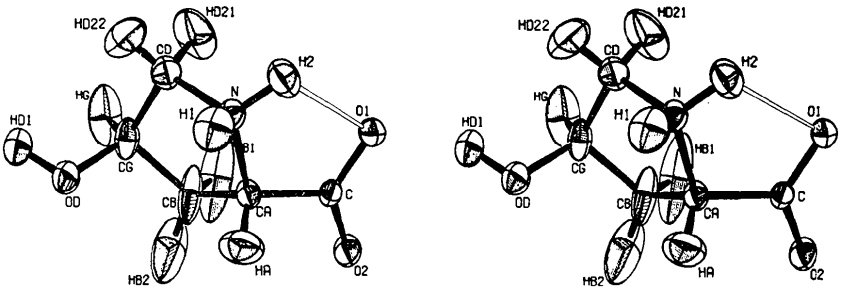


Fig. 1. Stereo drawing of the hydroxyproline molecule with thermal ellipsoids drawn to enclose 40% probability $CA\equiv C^\alpha$, $CB\equiv C^\beta$, $CG\equiv C^\gamma$, $OD\equiv O^\delta$, $CD\equiv C^\delta$.

in Table 1, and final values for the squared structure amplitudes are shown in Table 2 for those reflections included in the refinements. The anisotropic extinction tensor, defined above, is $W'_{11}=2{\cdot}3$ (3), $W'_{22}=0{\cdot}58$ (5), $W'_{33}=0{\cdot}51$ (5), $W'_{12}=0{\cdot}40$ (14), $W'_{13}=-0{\cdot}51$ (11), $W'_{23}=-0{\cdot}21$ (5). These values correspond to an average particle size described by an ellipsoid with principal axes as follows (direction cosines refer to the crystal axis system **a***, **b***, **c***).

R.m.s. (μ)	Direction cosines		
0·64	0·94	0·22	−0·26
1·39	0·32	−0·84	0·44
1·80	−0·12	−0·50	−0·86.

The extinction is markedly anisotropic and g is smaller along **a***, which corresponds to the long dimension of the crystal, than along **b*** or **c***. This anisotropy may be associated with the fact that the structure consists of layers of hydroxyproline molecules approximately perpendicular to **a**.

The final unweighted and weighted R values based on F^2 are

$$R_F^2=[\sum|F_o^2-|F_c|^2|]/\sum F_o^2=0{\cdot}044$$

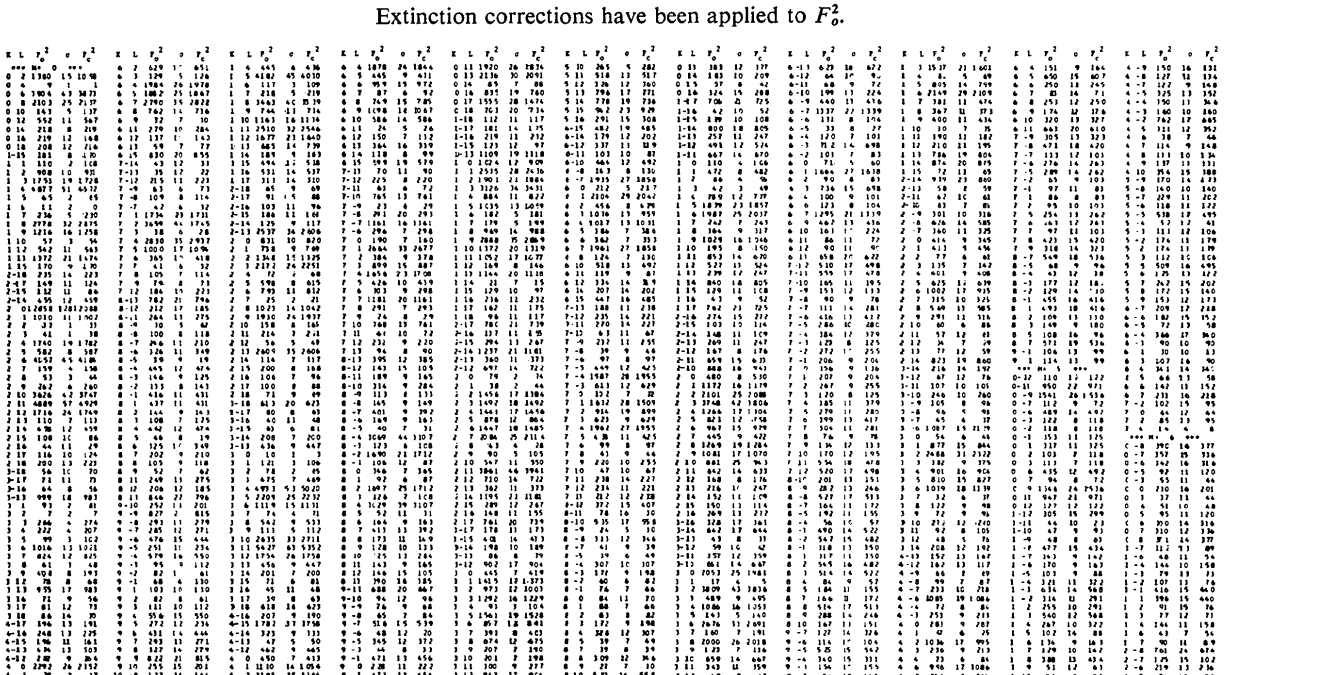
$$R_{wF}^2=\{[\sum w|F_o^2-|F_c|^2|^2]/\sum wF_o^4\}^{1/2}=0{\cdot}056$$

and the conventional R value is $R_F=0{\cdot}032$. In the last cycle, no parameter shifted by more than 2% of its e.s.d. Using an isotropic extinction correction, $R_{wF}^2=0{\cdot}068$, so significantly improved agreement was obtained using the anisotropic extinction correction.

The atoms C^β, $H^{\beta1}$, and $H^{\beta2}$ all have very large thermal motion parameters u_{11}. In order to provide a check of the thermal parameters an independent full-matrix least-squares refinement of the structure was carried out using the X-ray data of Donohue & Trueblood (these authors have reported the results of

Table 2. *Squared structure amplitudes* (× 100)

Extinction corrections have been applied to F_o^2.



diagonal least-squares refinements using a single isotropic temperature factor for the entire molecule). Our refinements were based on F^2 and weights were assigned as $w=1/\sigma^2(F_o^2)$ with $\sigma^2(F_o^2)=2500+(0{\cdot}06\,F_o^2)^2$. Anisotropic thermal parameters were varied for non-hydrogen atoms, and positional and isotropic thermal parameters were refined for the hydrogen atoms. Scattering factors for hydrogen were those of Stewart, Davidson & Simpson (1965), and an isotropic extinction correction was included in the refinement, assuming a constant beam path length through the crystal for all reflections. Final agreement factors for the X-ray data are $R_{F2}=0{\cdot}132$, $R_{wF2}=0{\cdot}173$, and $R_F=0{\cdot}065$. This latter value may be compared with the final $R_F=0{\cdot}148$ reported by Donohue & Trueblood.

The final X-ray values of the anisotropic thermal parameters u_{ij} for C^β are $u_{11}=0{\cdot}139$ (7), $u_{22}=0{\cdot}026$ (2), $u_{33}=0{\cdot}034$ (2), $u_{12}=0{\cdot}004$ (3), $u_{13}=-0{\cdot}034$ (4), $u_{23}=0{\cdot}002$ (2Å)^2, so these results confirm the high value of u_{11} for C^β found by neutron diffraction. Comparisons of the X-ray and neutron structure parameters for non-hydrogen atoms using the method of half-normal probability plots (Abrahams & Keve, 1971) and employing χ^2 tests as we have previously discussed (Hamilton, 1969) show that there is excellent agreement between the positional parameters from the two refinements. The X-ray thermal parameters, particularly u_{22}, are systematically slightly larger than the neutron values, possibly due to the fact that the X-ray data were not corrected for absorption.

Our refinements based on the X-ray data have resulted in small shifts in the positional parameters of the non-hydrogen atoms given by Donohue & Trueblood (1952*b*). The maximum changes in atomic positions are 0·030 Å for C^β and 0·047 Å for C^γ, both of which have quite large and anisotropic thermal parameters. The hydrogen atom positions given by Donohue & Trueblood are qualitatively correct when compared with the neutron diffraction results.

The calculations described in this paper were performed on CDC 6600 computers using programs from the Brookhaven Crystallographic Computing Library which have been described briefly by Schlemper, Hamilton & La Placa (1971).

The molecular structure

The molecular structure of hydroxyproline is illustrated in Fig. 1. The molecule is a zwitterion with the hydroxyl and carboxyl groups in the *trans* configuration relative to the ring, as had been found earlier (Donohue & Trueblood, 1952*b*). Molecular bond distances and bond angles are given in Tables 3 and 4. Torsion angles, labeled according to the IUPAC conventions (IUPAC-IUB Commission on Biochemical Nomenclature, 1970), are given in Table 5.

The puckered pyrrolidine ring is in the 'envelope' form (Eliel, Allinger, Angyal & Morrison, 1965), with C^γ lying 0·516 Å from the plane through N, C^α, C^β and C^δ, and with the latter four atoms coplanar to within

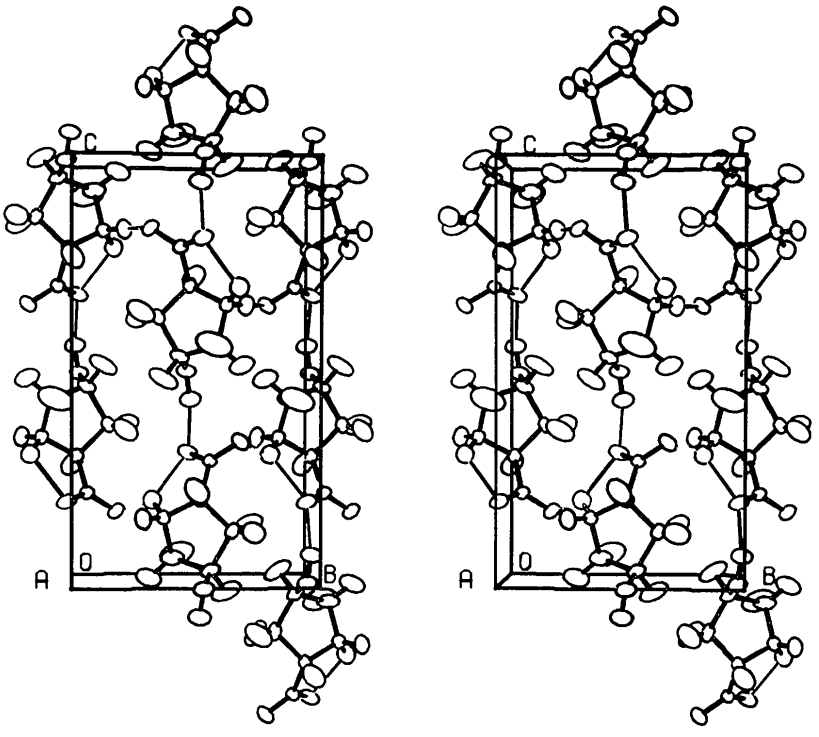


Fig. 2. Stereo drawing of one unit cell of the crystal structure. Molecular bonds are drawn thick, hydrogen bonds thin.

Table 3. *Molecular bond distances* (Å)

	Neutron		X-ray†
	Uncorrected	Corrected for thermal motion*	
$C—O^1$	1·244 (2)	1·247	1·245 (6)
$C—O^2$	1·242 (2)	1·245	1·244 (5)
$C—C^\alpha$	1·530 (2)	1·530	1·538 (5)
$C^\alpha—N$	1·498 (1)	1·498	1·495 (5)
$C^\alpha—C^\beta$	1·526 (2)	1·539	1·515 (6)
$C^\alpha—H^\alpha$	1·084 (3)	1·095	0·87 (6)
$N—H^1$	1·046 (3)	1·049	0·95 (9)
$N—H^2$	1·025 (3)	1·030	0·77 (10)
$C^\beta—C^\gamma$	1·520 (2)	1·525	1·523 (6)
$C^\beta—H^{\beta 1}$	1·064 (6)	1·093	0·96 (9)
$C^\beta—H^{\beta 2}$	1·070 (7)	1·102	1·10 (7)
$C^\gamma—O^\delta$	1·403 (2)	1·403	1·414 (6)
$C^\gamma—C^\delta$	1·523 (2)	1·523	1·501 (8)
$C^\gamma—H^\gamma$	1·094 (3)	1·107	0·96 (8)
$C^\delta—N$	1·487 (2)	1·489	1·496 (5)
$C^\delta—H^{\delta 21}$	1·081 (4)	1·097	0·86 (9)
$C^\delta—H^{\delta 22}$	1·063 (4)	1·074	1·01 (10)
$O^\delta—H^{\delta 1}$	0·971 (3)	0·972	0·81 (6)

* The minimum correction to be added to the uncorrected bond length R_{12} is defined as $(|u_2^{1/2}-u_1^{1/2}|)^2/2R_{12}$, where u_2 and u_1 are total mean square displacements normal to the bond for the two atoms.

† Taken from our refinements of Donohue and Trueblood's data.

Table 4. *Molecular bond angles* (°)

$O^1—C—O^2$	125·6 (1)	$C^\beta—C^\gamma—C^\delta$	103·0 (1)
$O^1—C—C^\alpha$	117·8 (1)	$O^\delta—C^\gamma—C^\beta$	109·5 (2)
$O^2—C—C^\alpha$	116·6 (1)	$O^\delta—C^\gamma—C^\delta$	111·7 (1)
$N—C^\alpha—C^\beta$	105·0 (1)	$H^\gamma—C^\gamma—C^\beta$	112·0 (3)
$C—C^\alpha—N$	110·8 (1)	$H^\gamma—C^\gamma—O^\delta$	110·1 (2)
$C—C^\alpha—C^\beta$	111·5 (1)	$H^\gamma—C^\gamma—C^\delta$	110·3 (3)
$H^\alpha—C^\alpha—C$	109·3 (2)		
$H^\alpha—C^\alpha—N$	107·8 (2)		
$H^\alpha—C^\alpha—C^\beta$	112·4 (2)		
$C^\alpha—N—C^\delta$	109·1 (1)	$N—C^\delta—C^\gamma$	104·9 (1)
$H^1—N—C^\alpha$	107·9 (2)	$H^{\delta 21}—C^\delta—N$	107·5 (2)
$H^1—N—C^\delta$	111·9 (2)	$H^{\delta 21}—C^\delta—C^\gamma$	110·9 (3)
$H^2—N—C^\alpha$	108·6 (2)	$H^{\delta 22}—C^\delta—N$	110·3 (2)
$H^2—N—C^\delta$	109·9 (2)	$H^{\delta 22}—C^\delta—C^\gamma$	113·1 (2)
$H^1—N—H^2$	109·3 (2)	$H^{\delta 21}—C^\delta—H^{\delta 22}$	109·9 (4)
$C^\alpha—C^\beta—C^\gamma$	106·3 (1)	$H^{\delta 1}—O^\delta—C^\gamma$	107·3 (2)
$H^{\beta 1}—C^\beta—C^\alpha$	110·2 (3)		
$H^{\beta 2}—C^\beta—C^\alpha$	111·5 (4)		
$H^{\beta 1}—C^\beta—C^\alpha$	110·3 (4)		
$H^{\beta 2}—C^\beta—C^\gamma$	111·1 (3)		
$H^{\beta 1}—C^\beta—H^{\beta 2}$	107·5 (5)		

Table 5. *Torsion angles* (°)

IUPAC Designation	Atoms Involved	Angle
φ^1	$H^1—N—C^\alpha—C$	−120·7 (2)
φ^2	$H^2—N—C^\alpha—C$	−2·3 (2)
ψ^1	$O^1—C—C^\alpha—N$	−3·2 (2)
ψ^2	$O^2—C—C^\alpha—N$	178·7 (1)
χ^1	$N—C^\alpha—C^\beta—C^\gamma$	−18·3 (2)
$\chi^{2,1}$	$C^\alpha—C^\beta—C^\gamma—O^\delta$	−87·0 (2)
$\chi^{2,2}$	$C^\alpha—C^\beta—C^\gamma—C^\delta$	32·0 (2)
$\chi^{3,1,1}$	$H^{\delta 1}—O^\delta—C^\gamma—C^\beta$	−158·8 (2)
$\chi^{3,1,2}$	$H^{\delta 1}—O^\delta—C^\gamma—C^\delta$	87·7 (2)
$\chi^{3,2,1}$	$O^\delta—C^\gamma—C^\delta—N$	84·0 (1)
$\chi^{3,2,2}$	$C^\beta—C^\gamma—C^\delta—N$	−33·5 (2)
χ^4	$C^\gamma—C^\delta—N—C^\alpha$	23·1 (1)
χ^5	$C^\delta—N—C^\alpha—C^\beta$	−3·1 (1)

0·037 (3) Å. The dihedral angle between the planes $N—C^\alpha—C^\beta—C^\delta$ and $C^\beta—C^\gamma—C^\delta$ is 33·5°.

An apparent alternation in the C–C X-ray bond lengths, described as possibly significant by Donohue & Trueblood, is not confirmed by the more precise neutron diffraction results, which show all four C–C bond lengths in the molecule to be between 1·52 and 1·53 Å, with a mean C–C distance of 1·525 (2) Å. In our neutron diffraction study, the $C^\gamma—O^\delta$ bond distance involving the hydroxyl group is found to be 1·403 (2) Å. This value is only slightly shorter than the corresponding C–O distances of 1·414 (1) and 1·413 (4) Å determined by neutron diffraction in DL-serine and in L-serine monohydrate (Frey, Lehmann, Koetzle & Hamilton, 1972) respectively. Although Donohue & Trueblood had reported an anomalously long $C^\gamma—O^\delta$ bond length of 1·460 Å, this value shifted to 1·414 (6) Å after our refinement of the X-ray data.

Hydrogen bonding

As mentioned above, the crystal structure is made up of layers of hydroxyproline zwitterions approximately perpendicular to **a** and is stabilized by a network of three unique hydrogen bonds, one for each hydrogen covalently bonded to nitrogen or oxygen. A stereoview of the molecular packing and hydrogen bonding scheme is shown in Fig. 2, and hydrogen bond distances and angles are summarized in Table 6.

The structure contains the unusual intramolecular hydrogen bond $N—H^2\cdots O^1$. The $H^2\cdots O^1$ distance is 2·082 (3) Å, or more than 0·3 Å smaller than 2·4 Å, the appropriate sum of van der Waals radii, assuming a radius of 1·0 Å for hydrogen as proposed by Baur (1972) from structural data for crystalline hydrates. H^2 also possesses a short intermolecular contact with the carboxyl oxygen O^2 of another molecule. The $H^2\cdots O^2$ distance is 2·348 (4) Å while the $N—H^2\cdots O^2$ angle is 135·1 (2)°, so that this contact is only 0·05 Å shorter than the expected van der Waals distance and should definitely not be called a hydrogen bond, although a weak interaction may contribute to the stability of the crystal structure. By contrast, the intramolecular hydrogen bond appears to be much stronger, in spite of being severely bent [the $N—H^2\cdots O^1$ angle is 113·2 (2)°]. The atoms N, H^2, O^1, C, C^α are nearly coplanar, with the least-squares plane passing within 0·030 Å of all five atoms. The torsion angle ψ^1 ($O^1—C—C^\alpha—N$) is −3·2 (2)°. Donohue & Trueblood, who have preferred not to call the short intramolecular contact a hydrogen bond, have noted that if N and the carboxyl group are coplanar, the close approach of H^2 and O^1 is dictated by the steric constraints of the cyclic hydroxyproline molecule. Although the α-nitrogen generally lies fairly close to the plane of the carboxyl group in amino acids, substantial deviations from this planar configuration have been found in β-L-glutamic acid, where the torsion angle ψ^1 is −42·3 (2)° (Lehmann, Koetzle & Hamilton, 1972*b*) and in L-

Table 6. *Hydrogen bond distances and angles*

A–H···B–C	A···B	H···B	∠A–H···B	∠H···B–C
$N-H^1\cdots O^2-C$	2·724 (2) Å	1·695 (4) Å	167·0 (3)°	131·0 (2)°
$N-H^2\cdots O^1-C$*	2·658 (2)	2·082 (3)	113·2 (2)	89·3 (1)
$O^\delta-H^{\delta 1}\cdots O^1-C$	2·792 (2)	1·843 (3)	164·9 (3)	110·3 (1)

* Intramolecular hydrogen bond.

histidine, with a ψ^1 angle of $-25{\cdot}9$ (6)° (Lehmann, Koetzle & Hamilton, 1972*c*). For a particular amino acid, the observed configuration of the carboxyl group is very likely that which gives the most efficient hydrogen bonding in the crystal. Thus, in hydroxyproline the carboxyl group is oriented to allow the closest possible approach of H^2 and the hydrogen bond acceptor O^1.

Adjacent layers of hydroxyproline molecules in the structure are connected by extremely strong hydrogen bonds $N-H^1\cdots O^2$ inclined 29·7° to **a** and with an $H^1\cdots O^2$ distance of 1·695 (4) Å, while the $O^\delta-H^{\delta 1}\cdots O^1$ hydrogen bonds join chains of molecules along **c**. These two intermolecular hydrogen bonds are both slightly bent.

In previous neutron diffraction studies of amino acids, we have observed a systematic inverse correlation of N–H and H···O bond lengths in N–H···O hydrogen bonds. The present results for hydroxyproline agree with this observation: the N–H bond involving the strongly hydrogen-bonded H^1 is found to be about 0·02 Å longer than that involving H^2.

Thermal motion

A glance at Fig. 1 indicates that the thermal motion parameters from our neutron diffraction study are not interpretable in terms of rigid-body motions. Intramolecular bond lengths were corrected for the effects of thermal motion using the minimum correction of Busing & Levy (1964) and the corrected values are included in Table 3. The average corrected C–H bond length is 1·095 (4) Å, almost identical to the expected value of 1·096 Å (*Tables of Interatomic Distances and Configuration in Molecules and Ions*, 1965). The minimum correction previously has been found to give reasonable corrected bond lengths in the case of L-lysine.HCl.$2H_2O$ (Koetzle, Lehmann, Verbist & Hamilton, 1972) and in other amino acids as well.

We have referred earlier to the large thermal parameters found for C^β, $H^{\beta 1}$ and $H^{\beta 2}$. It is impossible on the basis of the present neutron diffraction results to decide if these thermal parameters result from genuine thermal motion, which might be described in terms of restricted pseudo-rotation of the five-membered ring (Kilpatrick, Pitzer & Spitzer, 1947) or if the crystal is statically disordered, containing molecules with two or more distinct ring conformations of nearly equal energy. In any event, the fact that very large thermal parameters are associated only with the β-methylene group indicates that the pyrrolidine ring in the solid state may be quite flexible.

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