

The Crystal and Molecular Structure of *allo*-4-Hydroxy-L-proline Dihydrate

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allo-4-Hydroxy-L-proline crystallizes from an aqueous solution as the dihydrate. The crystals are orthorhombic, space group $P2_12_12_1$, with $a=7.08$ (2), $b=22.13$ (3), $c=5.20$ (2) Å. The structure was solved by direct methods and refined by block-diagonal least squares. The final R for 733 observed reflexions is 0.054. The molecule exists as a zwitterion with hydroxyl and carboxyl groups *cis* to the pyrrolidine ring. The latter is puckered at the β -carbon atom, which deviates by -0.54 Å from the best plane formed by the four remaining atoms. The molecules are held together by a network of hydrogen bonds, the water molecules playing a dominant role in the stability of the structure.

Introduction

It has been found that if proline is replaced by hydroxyproline in one cyclic pentapeptide of the actinomycin-DNA complex, the biological activity of the antibiotic is diminished some twentyfold (Sobell, 1974). However, there is no appreciable change in activity if *allo*-hydroxyproline replaces proline in the pentapeptide chain. In this connexion it was considered worthwhile to determine the structure and conformation of *allo*-4-hydroxy-L-proline and the details are presented in this paper.

Experimental

Crystals of *allo*-4-hydroxy-L-proline dihydrate were grown from an aqueous solution as colourless needles elongated along c . The crystal data are recorded in Table 1. The cell dimensions and space group were obtained from Weissenberg and precession photographs.

Intensities were collected with Cu $K\alpha$ radiation for the layers $l=0$ to 4 by the multiple-film equi-inclination technique. The intensities were estimated visually and corrected for Lorentz, polarization and spot-

shape factors (Phillips, 1954). No absorption corrections were applied ($\mu t \approx 0.3$). As the crystals were hygroscopic they were enclosed in Lindemann capillaries.

Structure determination and refinement

With the overall temperature factor ($B=3.85$ Å²) and scale factor determined from a Wilson (1942) plot the normalized structure factors (E) were obtained. The structure was determined with *MULTAN* (Germain, Main & Woolfson, 1971) adapted by Ramakumar & Narasimha Murthy for an IBM 360/44 with 64K byte memory. 156 reflexions with $|E| \geq 1.25$ were used as the input. An E map computed with the phases obtained from the set with highest combined figure of merit revealed the structure. At the start it was not known definitely how many water molecules were present in the cell because of the inaccurate measured density; however, the E map indicated the presence of two molecules in the asymmetric unit. R on the basis of the positions obtained from the E map was 0.334. The positional and isotropic thermal parameters were refined by block-diagonal least squares with the program of Shiono (1968) modified for the IBM 360/44 by Swaminatha Reddy. R dropped to 0.136. At this stage a study of the $\sum |AF|^2$ versus $\langle |F_o| \rangle$ distribution suggested a weighting scheme $w = (0.95 + 0.012|F_o| + 0.0027|F_o|^2)^{-1}$. Further refinement with this scheme reduced R to 0.081. A difference map revealed the positions of all the H atoms which appeared with peak heights ranging from 0.3 to 0.6 e Å⁻³. Refinement of all non-hydrogen atoms with anisotropic temperature factors and an overall scale factor yielded an R of 0.054 for 733 observed reflexions. The hydrogen-atom positions were calculated with C-H = 1.0, and N-H and O-H = 0.9 Å. The shifts in the parameters at the end of the last cycle were $< 0.1\sigma$. The scattering factors

Table 1. *Crystal data*

<i>allo</i> -4-Hydroxy-L-proline dihydrate	
Molecular formula: C ₅ H ₉ NO ₃ · 2H ₂ O	
Molecular weight: 167.2	
Crystal system: orthorhombic	
$a =$	7.08 (2) Å
$b =$	22.13 (3)
$c =$	5.20 (2)
Space group: $P2_12_12_1$	
Absent reflexions: $h00$, h odd	
	$0k0$, k odd
	$00l$, l odd
$Z = 4$	
$D_c =$	1.363 g cm ⁻³
μ (Cu $K\alpha$) =	10.7 cm ⁻¹

Table 2. Final atomic coordinates ($\times 10^4$) and thermal parameters ($\times 10^4$) for the non-hydrogen atoms

$$T = \exp [-(\beta_{11}h^2 + \beta_{22}k^2 + \beta_{33}l^2 + 2\beta_{12}hk + 2\beta_{23}kl + 2\beta_{13}hl)].$$

	<i>x</i>	<i>y</i>	<i>z</i>	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
C $^\alpha$	2864 (8)	4013 (2)	9212 (13)	175 (11)	17 (1)	454 (31)	0 (3)	-64 (16)	-18 (5)
C $^\beta$	2728 (8)	3331 (2)	9716 (11)	182 (11)	16 (1)	257 (24)	0 (3)	-27 (15)	4 (4)
C $^\gamma$	616 (8)	3177 (2)	9416 (13)	223 (13)	16 (1)	366 (29)	-9 (3)	22 (17)	-5 (4)
C $^\delta$	-368 (8)	3738 (3)	10472 (14)	205 (13)	23 (1)	465 (34)	-7 (4)	59 (19)	-6 (5)
N	997 (6)	4249 (2)	10243 (9)	177 (11)	13 (1)	255 (24)	-6 (3)	-6 (14)	-2 (4)
C'	2957 (8)	4179 (2)	6327 (11)	209 (10)	15 (1)	281 (21)	1 (2)	2 (13)	2 (4)
O(1)	1742 (5)	4523 (2)	5397 (8)	226 (9)	17 (1)	379 (19)	6 (2)	-6 (12)	0 (3)
O(2)	4381 (6)	3966 (2)	5209 (9)	218 (9)	24 (1)	394 (21)	2 (2)	34 (13)	3 (4)
O $^\delta$	130 (6)	3107 (2)	6758 (8)	216 (8)	19 (1)	401 (20)	0 (2)	5 (13)	-9 (3)
W(1)	6480 (6)	2918 (2)	5307 (11)	209 (9)	26 (1)	618 (26)	-2 (3)	33 (15)	-22 (5)
W(2)	6929 (6)	4831 (2)	3572 (7)	249 (10)	27 (1)	346 (19)	9 (3)	-14 (12)	2 (4)

Table 3. Positional coordinates ($\times 10^3$) for hydrogen atoms with their isotropic temperature factors

	<i>x</i>	<i>y</i>	<i>z</i>	<i>B</i> (Å 2)
H $^\alpha$	405	416	1008	3.63
H $^\beta$ (1)	307	325	1153	3.04
H $^\beta$ (2)	341	312	831	3.04
H $^\gamma$	32	283	1058	3.99
H $^\delta$ (1)	97	277	616	4.18
H $^\delta$ (21)	-66	368	1225	4.49
H $^\delta$ (22)	-146	383	927	4.49
H(1)	48	455	896	2.96
H(2)	119	441	1202	2.96
W(1)H(1)	67	167	467	5.21
W(1)H(2)	267	200	500	5.21
W(2)H(1)	100	50	567	4.66
W(2)H(2)	267	17	467	4.66

were those of Cromer & Waber (1965) for non-hydrogen atoms and of Stewart, Davidson & Simpson (1965) for H.

Final positional and thermal parameters with their standard deviations are recorded in Tables 2 and 3.*

Discussion of the structure

The structure of *allo*-4-hydroxy-L-proline is illustrated in Fig. 1. The bond lengths, bond angles and torsion angles are listed in Table 4. The definition of the torsion angles and the atom labels are those adopted by the IUPAC-IUB Commission on Biochemical Nomenclature (1970). The average C-C length of 1.533 (8) is in agreement with the 1.525 (2) in 4-hydroxy-L-proline (Koetzle, Lehmann & Hamilton, 1973) and 1.525 (8) Å in 2,3-*cis*-3,4-*trans*-3,4-dihydroxy-L-proline (Karle, 1970). The C-O length of 1.435 (7) is consistent with the 1.427 (7) Å obtained by Karle (1970). However, the value obtained from the neutron-diffraction study of 4-hydroxy-L-proline (Koetzle *et al.*, 1973) is 1.403 (2) Å. C $^\alpha$ -N, 1.521 (7), is significantly longer than C $^\delta$ -N, 1.490 (7) Å.

The molecule is a zwitterion with the hydroxyl and the carbonyl groups *cis* to the pyrrolidine ring. Ramachandran, Lakshminarayanan, Balasubramanian &

Table 4. Molecular dimensions

(a) Bond lengths (Å) and bond angles (°)			
C'-O(1)	1.240 (6)	C $^\alpha$ -C $^\beta$ -C $^\gamma$	105.2 (4)
C'-O(2)	1.256 (7)	C $^\beta$ -C $^\gamma$ -C $^\delta$	103.1 (5)
C'-C $^\alpha$	1.545 (9)	C $^\gamma$ -C $^\delta$ -N	107.3 (5)
C $^\alpha$ -C $^\beta$	1.535 (7)	C $^\delta$ -N-C $^\alpha$	109.1 (4)
C $^\alpha$ -N	1.521 (7)	N-C $^\alpha$ -C $^\beta$	102.5 (4)
C $^\beta$ -C $^\gamma$	1.534 (8)	C $^\delta$ -C $^\gamma$ -O $^\delta$	110.9 (5)
C $^\gamma$ -C $^\delta$	1.518 (8)	O $^\delta$ -C $^\gamma$ -C $^\delta$	108.6 (5)
C $^\gamma$ -O $^\delta$	1.435 (7)	N-C $^\alpha$ -C'	107.4 (4)
C $^\delta$ -N	1.490 (7)	C $^\beta$ -C $^\alpha$ -C'	113.9 (5)
		C $^\alpha$ -C'-O(1)	119.5 (5)
		C $^\alpha$ -C'-O(2)	113.2 (5)
		O(1)-C'-O(2)	127.2 (5)
(b) Torsion angles (°) (IUPAC-IUB designations)			
χ^1	O(1)-C'-C $^\alpha$ -N	9.2 (7)	
χ^2	O(2)-C'-C $^\alpha$ -N	-174.9 (9)	
χ^1	N-C $^\alpha$ -C $^\beta$ -C $^\gamma$	33.2 (5)	
$\chi^{2,1}$	C $^\alpha$ -C $^\beta$ -C $^\gamma$ -O $^\delta$	81.1 (5)	
$\chi^{2,2}$	C $^\alpha$ -C $^\beta$ -C $^\gamma$ -C $^\delta$	-35.1 (6)	
$\chi^{3,2,1}$	O $^\delta$ -C $^\gamma$ -C $^\delta$ -N	-94.0 (5)	
$\chi^{3,2,2}$	C $^\beta$ -C $^\gamma$ -C $^\delta$ -N	22.9 (6)	
χ^4	C $^\gamma$ -C $^\delta$ -N-C $^\alpha$	-3.4 (6)	
χ^5	C $^\delta$ -N-C $^\alpha$ -C $^\beta$	-18.3 (6)	
	C'-C $^\alpha$ -C $^\beta$ -C $^\gamma$	-82.9 (5)	
	C'-C $^\alpha$ -N-C $^\delta$	101.0 (5)	
	C $^\beta$ -C $^\alpha$ -C'-O(1)	122.3 (5)	
	C $^\beta$ -C $^\alpha$ -C'-O(2)	-61.8 (6)	

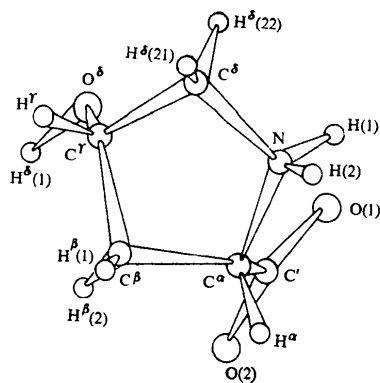


Fig. 1. A view of the molecule.

* A list of structure factors has been deposited with the British Library Lending Division as Supplementary Publication No. SUP 31951 (5 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 13 White Friars, Chester CH1 1NZ, England.

Tegoni (1970) have found two local minima in conformational space for the pyrrolidine ring, one with C^γ displaced on the same side as C' , which is energetically more stable than the other with C^γ on the opposite side to C' (conformations *A* and *B* respectively). A positive value of the conformational angle χ^5 ($C^\delta-N-C^\alpha-C^\beta$) corresponds to *A* and a negative value to *B*. The small value for the conformational angle χ^4 ($C^\gamma-C^\delta-N-C^\alpha$) indicates that the pyrrolidine ring has the usual envelope conformation but with C^β away from the plane through the remaining four atoms (Table 5). The observed conformation is the same as in *N*-(*tert*-butyloxycarbonyl)-L-proline (Benedetti, Ciajolo & Maisto, 1974). The deviation of C^β in our structure is *trans* to the carboxyl group with an intramolecular distance of 3.11 Å between C' and O^δ . From the PMR study of *allo*-4-hydroxy-L-proline (Abraham & McLauchlan, 1962) it was surmised that C^δ should be out of plane, favouring the formation of an intramolecular hydrogen bond between the hydroxyl and carboxyl groups. In the structure of 4-hydroxy-L-proline (Donohue & Trueblood, 1952; Koetzle *et al.*, 1973) C^γ is displaced from the plane through the remaining four atoms of the pyrrolidine ring. The conformation of the ring in the present structure may be described as C_s - C^β -*exo* (Ashida & Kakudo, 1974). The angle between the two planes N, C^α , C^γ , C^δ and C^α , C^β , C^γ is 35° 36'. Atom N deviates from the plane through C^α , C' , O(1) and O(2) by 0.16 Å.

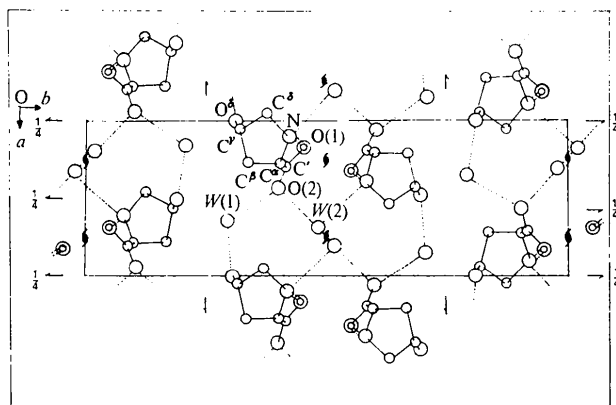


Fig. 2. Packing of the molecules viewed down *c*.

Table 5. *Least-squares planes through various sets of atoms*

Equation to the plane $Ax + By + Cz = D$ with respect to crystallographic axes where x, y, z are in Å.

Atoms defining planes and equations to planes	Deviation (Å)
(1) $C^\alpha, C^\gamma, C^\delta$ and N $-0.32304x + 0.20793y$ $-0.92326z = -3.21472$	$C^\alpha = -0.014$ $C^\gamma = 0.014$ $C^\delta = -0.023$ N = 0.023 $C^{\beta*} = -0.541$
(2) $C', C^\alpha, O(1)$ and O(2) $-0.54453x - 0.80400y$ $-0.23800z = -9.38187$	$C' = 0.023$ $C^\alpha = -0.006$ O(1) = -0.009 O(2) = -0.008 N* = 0.165
(3) $C', C^\alpha, N, O(1)$ and H(1) $-0.53461x - 0.79938y$ $-0.27419z = -9.43924$	$C' = 0.028$ $C^\alpha = -0.057$ N = 0.084 O(1) = -0.008 H(1) = -0.064

* Atoms not included in the calculation of the least-squares planes.

Crystal packing and hydrogen bonding

A view of the molecular packing and hydrogen-bonding scheme is shown in Fig. 2. The hydrogen-bond distances and angles are recorded in Table 6. All the protons available for hydrogen bonding, except that belonging to the hydroxyl group, are involved in intramolecular hydrogen bonds. The only direct contact between the *allo*-hydroxyproline molecules is through the hydrogen bridge $N-H(2) \cdots O(1)$ formed between the molecules translated along *c*. Except for this contact the molecules are bridged by waters of crystallization. The hydroxyproline molecules which are generated by translation along *a* are linked by water molecule *W*(1) whereas those related by the screw axis parallel to *c* are bridged by *W*(2). One of the protons associated with *W*(2) forms a hydrogen bond with its symmetry-related counterpart, thus forming 'staircase' hydrogen bonding about the screw axis parallel to *c*. Thus the water molecules play an important role in the stability of the crystal structure. As in the structure of 4-hydroxy-L-proline (Koetzle *et al.*, 1973), an intramolecular hydrogen

Table 6. *Hydrogen-bond distances and angles*

A-H...B	A...B (Å)	H...B (Å)	A-H...B (°)
N—H(2).....O(1) (I)	2.798	1.816	165.6
N—H(1).....O(1)*	2.646	2.059	114.9
N—H(1).....W(2) (II)	3.032	2.201	138.7
W(1)—W(1)H(2).....O ^δ (III)	2.767	2.003	147.5
W(1)—W(1)H(1).....O(2)	2.762	1.678	169.6
W(2)—W(2)H(1).....O(2)	2.760	1.704	173.2
W(2)—W(2)H(2).....W(2) (IV)	2.824	1.863	149.7

Symmetry code

(I)	x	y	$1+z$	(III)	$1+x$	y	z
(II)	$\frac{1}{2}-x$	$1-y$	$\frac{1}{2}+z$	(IV)	$\frac{1}{2}-x$	$1-y$	$\frac{1}{2}+z$

* Intramolecular hydrogen bond.

bond N–H(1)···O(1) (Table 6) also seems to exist in the present molecule. N, H(1), O(1), C' and C^α are nearly coplanar with deviations less than 0.08 Å. From the hydrogen-bond distances and angles involving N, H(1), O(1) and W(2) (Table 6) it may be concluded that there is a bifurcated hydrogen bond. All pertinent intermolecular distances were examined. Apart from the hydrogen bonds, no contact appears to be incompatible with accepted van der Waals radii.

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Anorthite Quenched from 1530 °C. I. Structure Refinement

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Anorthite (CaAl₂Si₂O₈) quenched from 1530°C shows only 'a' and 'b' type reflexions ($h+k+l$ = even). Two alternative refinements have been carried out, both in the space group $I\bar{1}$: in the first only the Ca atoms are split and the framework atoms are anisotropically refined ($R=0.058$); in the second the T and O atoms of the framework are also split and isotropically refined ($R=0.062$). On the basis of crystallochemical considerations the split model is assumed to be a better approximation to reality. Among all the possible configurations compatible with the split model, one was chosen on the basis of both crystallochemical considerations and a postulated analogy with low-temperature primitive anorthite. The resulting space group is $P\bar{1}$ which is not supported by the diffractometric evidence (absence of 'c' and 'd' reflexions with $h+k+l$ = odd). This absence can be ascribed to the existence of 'c' antiphase domains small enough to render undetectable the 'c' and 'd' reflexions. The quenchability of such 'c' microdomains may be due to (or favoured by) an imperfect Al–Si alternation induced in the framework by the severe heating. The values of Al occupancies of the T sites indicate a partial Al–Si disorder. Average Al occupancies are $t_1(0)=0.56$, $t_1(m)=0.46$, $t_2(0)=0.51$, $t_2(m)=0.47$.

Introduction

The structure of primitive anorthite at room temperature has been solved (Kempster, Megaw & Radoslovich, 1962; Megaw, Kempster & Radoslovich, 1962) and its structural features are now well established. However, there is no general agreement on the significance of the structural variations that take place with thermal treatment, although much research has been done in recent years. All the papers published on this subject report the following diffractometric data: (a)

the 'a' reflexions ($h+k$ = even, l = even) remain sharp to the melting point; (b) the 'b' reflexions ($h+k$ = odd, l = odd) remain sharp almost to the melting point; (c) the intensities of 'c' ($h+k$ = even, l = odd) and 'd' reflexions ($h+k$ = odd, l = even) decrease abruptly with temperature increase and are nearly zero above 230°C (Laves, Czank & Schulz, 1970). This fact can be interpreted either as a systematic extinction or as a decrease in intensity below the detection threshold.

The structures of anorthite refined at high temperature by Czank (1973) [Smith (1974) reports the main