The Structure of Hydroxyproline

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The crystal structure of hydroxyproline is orthorhombic ($P2_12_12_1$) with four molecules in a cell of dimensions $a = 5.01$, $b = 8.35$, $c = 14.1$ A. In a previous paper, general features of the structure were discussed. Further refinement has led to the electron-density maps of two projections which are presented here. Details of the structure are compared with those observed in other compounds.

Introduction and experimental

In a recent short communication (Zussman, 1951) the general features of the hydroxyproline structure were described. The structure analysis was undertaken in order to establish the general configuration of the molecule, and this was achieved by examination of the a- and b-axis projections. Further refinement of each projection has now been carried out, and Figs. 1 and 2 show electron-density maps thus obtained.
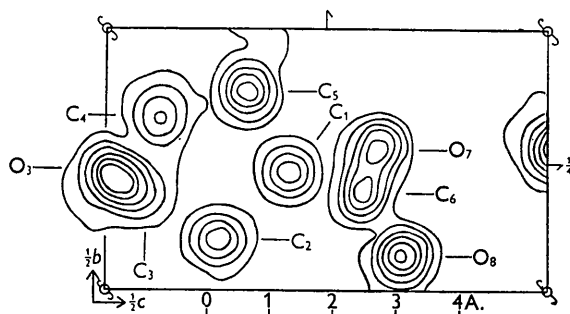

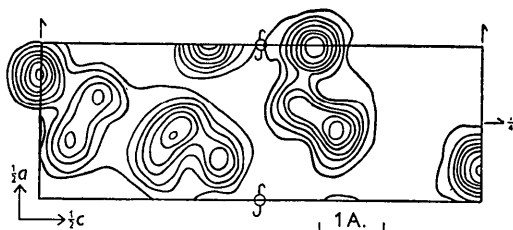Fig. 1. Electron density projected on (100). Contours at intervals of 2 e.A.$^{-2}$.

Fig. 2. Electron density projected on (010). Contours at intervals of 2 e.A.$^{-2}$.

For the a-axis projection the reliability index R for the structure originally published was 22%; this was reduced to 20%, and finally, by postulating positions for the hydrogen atoms, to 16%. For the b-axis projection the final value of R is 24%. For the sake of brevity, the usual table of F_o and F_c has been omitted. A copy may be obtained by application to the author.

The F_o's used to calculate the a-axis projection were derived from a suitably weighted average of the data obtained from three different crystals. The first was chosen small in order to minimize extinction and ab-

sorption effects; the second large, so as to reduce the number of unobserved reflexions, and the third was treated with liquid air (Lonsdale, 1947). The b-axis data were taken from one crystal only. The lower accuracy achieved in the second projection is largely accounted for by this difference in accuracy of the experimental measurements. In addition, the area of projection is smaller, and resolution of the atoms poorer.

$(F_o - F_c)$ syntheses were computed with the final calculated F values, and were used to estimate the probable errors in electron densities and in atomic co-ordinates. The average value of $(\rho_o - \rho_c)$ in the background was: a axis, 0.35 e.A.$^{-2}$; b axis, 0.50 e.A.$^{-2}$. The average value of its first differential corresponded to probable errors in co-ordinate of 0.01 and 0.02 A. in these two projections. (This method of estimating accuracy was suggested by W. Cochran, and is discussed more fully in his recent paper (Cochran, 1951).)

Description of structure

L-Hydroxyproline has the formula

$$\begin{array}{c} H_2C\!\!-\!\!-\!\!-\!\!CHOH \\ | \qquad\qquad | \\ HOOCCH \qquad CH_2 \\ \diagdown \qquad \diagup \\ N \\ | \\ H \end{array}$$
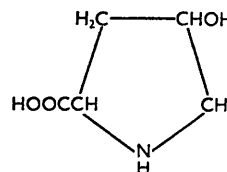
Its cell dimensions are

$$a = 5.01, \quad b = 8.35 \quad \text{and} \quad c = 14.1 \text{ A.}$$

(all ± 1%); space group $P2_12_12_1$; 4 molecules/unit cell. Diagrams showing the structure viewed along the a and b axes are given in the previous paper.

Atomic co-ordinates, intramolecular distances, bond angles and intermolecular distances are given in Tables 1, 2 and 3.

Table 1. *Atomic co-ordinates*

Atom	x/a	y/b	z/c
C_1	0.128	0.228	0.207
C_2	0.119	0.102	0.129
C_3	0.179	0.181	0.036
O_3	0.902	0.221	0.004
C_4	0.317	0.340	0.064
N_5	0.227	0.382	0.161
C_6	0.301	0.190	0.292
O_7	0.486	0.273	0.308
O_8	0.218	0.064	0.337

Table 2. Intramolecular distances and bond angles

Intramolecular distances (probable error 0·03 A.)

C–N	C_1–N_5 1·52A.		C_4–C_3 1·55A.
	N_5–C_4 1·48	C–C	C_3–C_2 1·50
C–O	C_3–O_3 1·49		C_2–C_1 1·52
	C_6–O_7 1·18		C_1–C_6 1·52
	C_6–O_8 1·29		

Bond angles (probable error about 3°)

In five-membered ring	C_1–C_2–C_3 108°	Carboxyl group	C_1–C_6–O_7 118°
	C_2–C_3–C_4 104		C_1–C_6–O_8 112
	C_3–C_4–N_5 107		O_7–C_6–O_8 129
	C_4–N_5–C_1 107	Hydroxyl group	C_4–C_3–O_3 107
	N_5–C_1–C_2 107		C_2–C_3–O_3 101
	N_5–C_1–C_6 109		C_2–C_1–C_6 116

Table 3. Intermolecular distances

Hydrogen bonded	N–H–O_8	2·70 A.
	O_7–H–O_3	2·82
	N–H–$O_8(C)$	3·17

Discussion of results

Carboxyl group

The difference between observed lengths of the two carboxyl C–O bonds, and their asymmetry about the C_1–C_6 bond, may be accounted for by experimental error (maximum error in bond length probably less than 0·1 A.), but these features are more likely to be real since they are similar to those observed in the dicarboxylic acids (Dunitz & Robertson, 1947), β-glycylglycine (Hughes & Moore, 1949), cysteylglycine (Dyer, 1951) and other compounds (see Table 4).

Table 4. Comparison of carboxyl-group features in hydroxyproline, cysteylglycine and β-glycylglycine

Bond	Hydroxy-proline	Cysteyl-glycine	β-Glycyl-glycine
C_6–O_7	1·18A.	1·23A.	1·21A.
C_6–O_8	1·29	1·28	1·27
Bond angle			
O_7–C_6–O_8	129°	126°	124°
C_1–C_6–O_8	112	113	112
C_1–C_6–O_7	118	121	123

Hughes & Moore (1949) pointed out that the oxygen atom forming the longer bond in the carboxyl group also took part in the formation of two hydrogen bonds, whereas the other oxygen atom formed only one. It is worth noting that this feature is also observed in cysteylglycine and in hydroxyproline.

Hydrogen bonds

In the previous communication (Zussman, 1951) the hydrogen-bond system was discussed, and the possibility of an additional hydrogen bond between N and $O_8(C)$ was mentioned. After refinement this N–O distance is found to be 3·17 A., which is greater than that usually found between hydrogen-bonded nitrogen and oxygen atoms, but is nevertheless too small for a normal van der Waals contact. Similar N–H–O distances have been observed in some other structures, e.g. hydroxyl-amine HCl 3·15 and 3·3A. (Jerslev, 1948), p-amino-

phenol 3·13 and 3·18A. (Brown, 1951). Regarding this as a hydrogen bond it is seen that the structure has its maximum of three hydrogen bonds per molecule. In O_7–H–O_3 the hydrogen must be that of the OH group and is probably closer to O_3. In N–H–O_8 and N–H–$O_8(C)$ one of the hydrogen atoms is that of the imino group, and the other is associated with O_8 of the carboxyl group. This is consistent with the C_6–O_8 bond of the carboxyl group being the longer of the two and hence having more single-bond character.
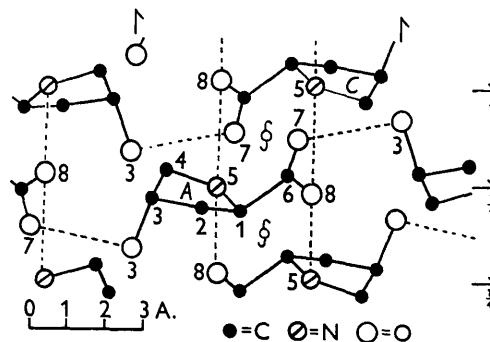
Fig. 3. Part of structure viewed along the b axis, showing hydrogen bonds (broken lines).

Hydroxyl group

The C_3–O_3 bond length, 1·49A., is greater than that generally accepted for a single C–O bond, but is similar to that observed in pentaerythritol (Llewellyn, Cox & Goodwin, 1937) and in p-aminophenol (Brown, 1951).

The angle C_2–C_3–O_3 appears to be considerably smaller than the normal tetrahedral angle.

Five-membered ring

The five-membered ring (average angle 107°) is found to be puckered, C_4 lying out of the plane close to which the other four atoms lie. This puckering is such as to remove the substituents of the ring further from its plane, an effect contrary to that observed with the five-membered furanose ring (Beevers & Cochran, 1947; Furberg, 1950), where the puckering achieved maximum planarity of the molecule as a whole.

At the time of the original publication it became known that the same structure was being studied by J. Donohue & K. N. Trublood, at the California Institute of Technology, using complete three-dimensional syntheses. This highly accurate structure analysis is now completed (private communication from Professor L. Pauling), and it will be of interest to compare the results obtained by these methods with those described in the present paper.

analysis of this structure should be undertaken. I am also indebted to the Department of Scientific and Industrial Research for a maintenance grant during the tenure of which this work was carried out.

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A Chemical Determination of 'Tetrahedral' and 'Octahedral' Aluminium Ions in a Silicate

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Chemical analysis of the material extracted from a magnesian chlorite (penninite) by acid attack at constant temperatures after various time intervals shows that the percentage extractions of Mg, Fe and H_2O follow the same curve when plotted against time; in the case of Al the extraction is closely similar up to about 47 %, but thereafter proceeds very much more slowly. If the limit of the first stage of the reaction is taken to represent the octahedral Al, then the ratio of tetrahedral to octahedral Al in the structure is $53/47 = 1\cdot13$, which agrees closely with that given by the structural formula, namely, $1\cdot16$. X-ray examination shows that the mineral becomes effectively amorphous when the octahedral Al is removed. The retention of the tetrahedral Al suggests that the Si(Al)–O networks are still intact, and this is further confirmed by the fact that they can be reconstituted into a mica-type structure.

1. Determination of Al^{IV} and Al^{VI} in a chlorite*

When the chemical analysis of a silicate mineral, expressed as percentages of oxides, is converted to a structural formula, the accepted procedure is to equate the number of oxygen atoms in the formula to the number in the unit cell and then to derive the corresponding numbers of the other atoms present. When the number of Si atoms is insufficient to fill the tetrahedral positions in the structure, Al atoms or occasionally Fe atoms are assumed to fill the gaps. The remaining Al atoms then go into octahedral positions. The correctness of this procedure is now unquestioned, but (so far as we are aware) no direct measurement of 'tetrahedral' and 'octahedral' Al, i.e. Al^{IV} and Al^{VI}, has been made.

In experiments on the chemical dissolution of chlorites, in which we were attempting to remove the brucitic layer without attacking the mica-type layer, data were obtained giving directly the ratio Al^{IV}/Al^{VI}; the result confirmed almost exactly the ratio deduced from the bulk analysis of the mineral.

The experiments were carried out as follows: about 1 g. of a chlorite, a penninite (or pennine) from Binnental, Switzerland, kindly supplied by Dr F. A. Bannister from the British Museum (specimen No. B.M. 26647), crushed to pass a 100-mesh sieve, was treated with a considerable excess of 1:10 HCl, at thermostatically controlled temperatures, 60, 80 and 100° C., for varying periods of time. After each treatment, the solid residue, dried at 300° C., was weighed, and the products in solution were quantitatively analysed for Al, Mg and Fe, the principal cations other than Si, which is not removed by this treatment. The treatment was then continued with a fresh supply of acid. In later experiments separate samples of 0·2–0·3 g. were used for each acid treatment to avoid any accumulation of experimental errors.

Fig. 1 shows the progress of extraction of Al, Mg, Fe and H_2O, expressed as percentages of their total content in the mineral, when acted on by 1:10 HCl at 100° C. The percentage of water extracted from the mineral was obtained by difference and therefore is less accurate than the other data. Within the limits of experimental error, Mg, Fe and H_2O are extracted at the same rate, and a single curve has been drawn through the observed

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* The notation Al^{IV} and Al^{VI} is convenient for indicating aluminium atoms in fourfold and sixfold co-ordination, i.e. in tetrahedral and octahedral positions (cf. MacEwan, 1951).