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The Crystal Structure of L-Glutamine

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The crystal structure of L-glutamine has been determined by means of electron-density projections on the (001) and (010) planes. The (001) projection was solved by direct sign-determining methods which are described. A direct indication is obtained from a map of $(\varrho_o - \varrho_c)$ of the positions of all the hydrogen atoms. Bond lengths have been measured with a standard deviation of 0·024 Å and are compared with those in related structures. A correlation between the double-bond character of the C–N bond of the amide group and the reactivity of this group in glutamine and acetamide is pointed out. Cohesion of the crystal is provided by five hydrogen bonds per molecule (the maximum number possible) extending in three dimensions and in each case linking a nitrogen and an oxygen atom.

1. Introduction

Glutamine, the γ-amide of glutamic acid, is of great biological importance. It occurs in the free state and as part of the protein structure in plants and animals, and plays an essential part in many metabolic processes. Its physiological roles have been extensively studied (Archibald, 1945). Considered simply as a chemical compound it has unusual properties, and its crystal-structure analysis was undertaken mainly with a view to providing information which might help to throw light on these characteristics. The amide group is uniquely labile, being rapidly hydrolysed in buffered solutions and being decomposed by nitrous acid in conditions under which all other amides are unchanged (Plimmer, 1925; Taylor, 1930). Hydrolysis leads to the formation of the five-membered lactam ring compound pyrrolidone-carboxylic acid. Neither this reaction nor the highly reactive nature of the amide group in general have been accounted for satisfactorily and it is desirable that the configuration and dimensions of the molecule should be determined as accurately as possible before an attempt is made to explain these chemical features. The analysis may also be regarded as a contribution to the more general study of amino-acid structures. The method by which the structure was solved is of considerable interest in itself as no assumptions were made as to the configuration of the molecule. This direct method is described in some detail.

2. Experimental

The material used was kindly supplied by Dr R. G. Westall of the Cambridge Low Temperature Research Station. It was in the form of a white powder and contained less than 1% impurity. Recrystallization from water at a temperature between 0 and 5° C. produced semitransparent needles up to 1·5 mm. long and with maximum mean diameter of cross section 0·2 mm. Elongation was in the direction of the short crystallographic axis.

Crystallographic and physical data

L-Glutamine $C_5H_{10}O_4N_2$; m.p. 184–185° C.; orthorhombic,

$$a = 16{\cdot}01,\ b = 7{\cdot}76,\ c = 5{\cdot}10\ \text{Å}\ (\text{all}\ \pm 0{\cdot}3\%)\,.$$

Absent spectra: $h00$, $0k0$, $00l$ when h, k, l respectively is odd. Space group $P2_12_12_1$. Four molecules per unit cell. Density (calc.) = 1·52 g.cm.$^{-3}$, density (obs.) = 1·54 g.cm.$^{-3}$. Absorption coefficient for Cu $K\alpha$ radiation μ = 12·2 cm.$^{-1}$.

Cu $K\alpha$ radiation was used throughout, and complete

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sets of $hk0$ and $h0l$ intensities on a relative scale were arrived at by taking Weissenberg photographs about the c and b axes and comparing the observed reflexions visually with an intensity scale. The crystal used for recording the b-axis data was cut to a length of 0·5 mm. in the c direction. After being corrected for Lorentz and polarisation factors the intensities were converted to an approximately absolute scale by comparing the calculated average intensity $\sum_{j=1}^{N} f_j^2$ with that observed for five zones of the appropriate reciprocal lattice section (Wilson, 1942).

3. Determination of the structure projected on (001)

It may be assumed that the molecule will lie fairly flat as seen along the short c axis of the crystal. Attempts were made to account for the magnitudes of the largest structure factors of low order by projecting a model of the molecule, which satisfied this condition, on to structure-factor graphs (Bragg & Lipson, 1936); but after numerous incorrect structures had been postulated this method of attack was given up. The great difficulty was the flexibility of the molecular model about the four single bonds of the chain, which makes it possible for the molecule to assume many different configurations in projection on (001), while remaining relatively flat. The structure was eventually determined in a way which did not require any assumptions about the configuration of the molecule.

Unitary structure factors, $U(hk0)$, were derived by means of the result

$$|U(hk0)| = \left[\frac{\mathcal{I}(hk0)}{\overline{\mathcal{I}}} \sum_{j=1}^{N} n_j^2\right]^{\frac{1}{2}},$$

where

n_j = fraction of electrons in the unit cell associated with the jth atom,

$\mathcal{I}(hk0)$ = relative intensity of the ($hk0$) reflexion

and

$\overline{\mathcal{I}}$ = average intensity of reflexions having nearly the same value of $\sin\theta$ as the ($hk0$) reflexion (Wilson, 1942; Kasper, Lucht & Harker, 1950).

Harker–Kasper inequalities (Harker & Kasper, 1948) were then used to relate the signs of thirteen structure factors. Of these, three were proved to be positive, two could be assumed to be positive, and those of the others were given in terms of $S(620)=a$ and $S(060) = b$, where $S(hkl)$ denotes the sign of $F(hkl)$. Most of the inequality relations involved $|U(620)| = 0{\cdot}55$, and it was really the occurrence of this exceptionally large structure factor which made it possible to obtain useful information from inequalities.

It can be shown on the basis of a relation between the structure factors of an electron-density distribution and the structure factors of the square of this distribution (Sayre, 1952) that the result

$$S(hkl) = S(h'k'l')S(h+h',k+k',l+l')\,, \qquad (1)$$

which can be proved from the inequality relations to be true when the corresponding unitary structure factors are sufficiently large, is *probably* true in other circumstances (Cochran, 1952). An approximate calculation leads to the conclusion that (1) is very probably satisfied when $\sqrt{(3n)}\,.\,\overline{U} > 1$, n being the symmetry number and $\overline{U}$ the r.m.s. value of the three unitary structure factors $U(hkl)$, $U(h'k'l')$ and $U(h+h',k+k',l+l')$. In the practical case we are considering, $n = 4$ and the limit is therefore about $\overline{U} = 1/\sqrt{12} \simeq 0{\cdot}3$. Another derivation of (1) by Zachariasen (1952) appears to lead to a more precise relation between the average magnitude of the unitary structure factors and the probability that (1) is satisfied, but this result was not known to us until later.

In practice one can only hope that the result is true in particular cases, and work out the consequences. For example, $U(120)=0{\cdot}30ab$ and $U(18,2,0)= 0{\cdot}42a$ were established by inequalities. Since $|U(17,4,0)| = 0{\cdot}30$, it was assumed that

$$S(17,4,0) = S(1\bar{2}0)S(18,2,0) = -ab.a = -b\,.$$

The signs of three further structure factors were assumed for similar reasons, and this gave a probable sign to almost all structure factors for which $|U|>0{\cdot}30$. The signs of a number of smaller structure factors were then established in much the same way, but generally the result was not regarded as reliable unless a few confirmations of sign were obtained. For example,

$$S(220) = S(060)\,S(280) = b.(+1)$$

and

$$S(220) = S(\bar{1}20)\,S(140) = -ab.(-a)$$

both lead to $S(220) = b$. When the sign of a structure factor appeared to be confirmed by a number of such checks, this term was used to establish further sign relationships. Contradictions of (1) were of course apparent at a fairly early stage of the process, but reasonable self-consistency was obtained, especially if $a = -1$ was assumed. Eventually the signs of 35 structure factors were established, almost all dependent, of course, on a and/or b.

In the projection of the structure on (001) all atoms are approximately equal and we may expect their centres to be separated by distances of about 1 Å. It has been shown by Sayre (1952) that when the atoms are equal and resolved,

$$A.g.F.(hk0) = f\sum_{h'}\sum_{k'} F(h'k'0)F(h-h',k-k',0)\,. \qquad (2)$$

A is the area of projection, f the atomic scattering factor and g the atomic scattering factor of a ‘squared atom'. In this instance the result was used in the slightly modified form

$$U(hk0) = \alpha\,(\sin\theta) \sum_{h'}\sum_{k'} U(h'k'0)U(h+h',k+k',0)\,. \quad (3)$$

The justification of this equation from (2) is very easy, and will not be reproduced here. We may note that it involves the assumption of equal resolved atoms, which is not involved in the derivation of (1). In practice $\alpha\,(\sin\theta)$ was regarded as an unknown function whose value was found empirically, as explained later.

The value of

$$T(hk0) = \sum_{h'}\sum_{k'} U(h'k'0)U(h+h',k+k',0) \quad (4)$$

was now calculated for all structure factors having $|U(hk0)| > 0{\cdot}1$, and for a number of others. The only products appearing on the right-hand side of equation (4) were, of course, those involving the 35 structure factors whose signs were thought to be established. The evaluation was made for each of four possible sign combinations (i) $a=-1$, $b=-1$, (ii) $a=+1$, $b=+1$, (iii) $a=+1$, $b=-1$, (iv) $a=-1$, $b=+1$. The $(hk0)$ reciprocal-lattice section was then divided into four regions by means of concentric circles about the origin. The quantity $\alpha\,(\sin\theta)$ was taken to be constant in any one of them, and given by

$$\alpha = \Sigma|U(hk0)|/\Sigma|T(hk0)|\,,$$

the sum being over all terms for which T had been calculated in that particular region. Values of αT and of U were now compared for each of the possibilities (i)–(iv), and the corresponding values of the reliability index $\Sigma|U-\alpha T|/\Sigma|U|$ were found to be 0·45, 0·62, 0·60 and 0·65, showing that if any one of these sign combinations was correct, it was certainly (i). For this choice of signs for a and b there was sufficient agreement between values of αT and of U for the signs of a further 30 structure factors to be taken as known, making 65 known signs in all. (The number of $(hk0)$ reflexions observed with Cu $K\alpha$ radiation, excluding those accidentally absent, was 134.) An attempt was next made to calculate the signs of all remaining structure factors by repeating the calculation of the $T(hk0)$'s, making use of the additional terms which could now be included among the products in equation (4), but the new values of αT did not agree better with values of U than those obtained when only 35 structure factors were used to form the products. A Fourier synthesis was made using the 65 terms whose signs appeared to be reasonably certain; the result (Fig. 1) shows quite clearly the approximate positions of all atoms in this projection.

Now that the structure was known, it became clear that the signs of the smaller structure factors could not be calculated from Sayre's equation because the condition of equal resolved atoms is seriously violated by the coincidence of one oxygen and one carbon atom in projection. Subsequent work showed that of the 65 signs established as described above only three were incorrect. None of the 35 signs established by inequalities or by the relation between the signs of three large structure factors was incorrect. It is interesting to note that when more exact values of $|U|$ were subsequently obtained by comparison of average observed and calculated values, it was found that the original scale of the $|U|$'s had been overestimated, particularly for low-order terms; for example, the correct value of $|U(620)|$ is 0·45, and not 0·55. When the correct values were substituted in the Harker–Kasper inequalities, it became clear that most of the thirteen signs thought to be 'certainties' from inequalities, are merely 'probables' from relation (1). It is therefore clear that the success of the method described above depended on a measure of good luck, as a failure of relation (1) involving large structure factors would have led to an increasing number of incorrect signs as the sign-determining process was developed.

In this connection it is worth noting that the same method has been applied by us to the two-dimensional data of asparagine monohydrate without success. (Cell dimensions of this compound were determined by Bernal (1931).) There is no structure factor as large as the (620) of glutamine and no signs or definite sign relationships could be obtained from Harker–Kasper inequalities. When equation (1) was applied to the largest $|U|$'s it was clear that it must be contradicted in some instances at the initial stage, and no progress could be made.

4. Refinement of the structure

Approximate x and y atomic coordinates were obtained from the electron-density map shown in Fig. 1.

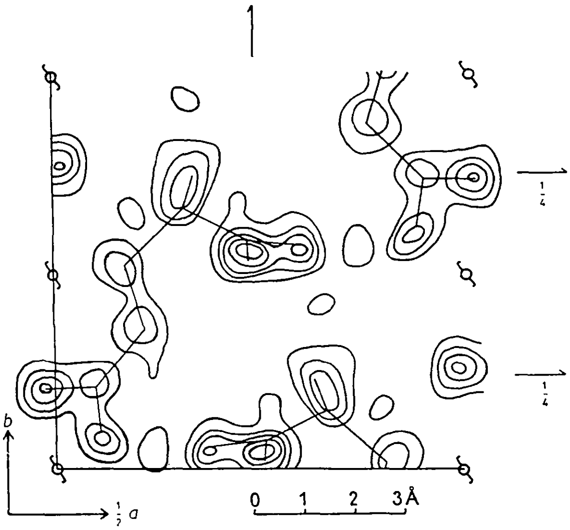

Fig. 1. First electron-density projection on (001) with the final position of the molecule indicated. In this synthesis only those 65 terms were included whose signs appeared reasonably certain from sign relationships and Sayre's equation.

The signs of additional structure factors were then calculated in the usual way, and doubtful signs were checked on X-R.A.C.* by the application of the non-negativity criterion (Eiland & Pepinsky, 1950). It did not prove possible to determine all the signs in this way, and refinement was continued by the calculation of successive (F_o-F_c)-syntheses (Booth, 1948; Cochran, 1951). Progress was slow and when the value of $R=\Sigma|F_o-F_c|\div\Sigma|F_o|$ had fallen from 39% to 27% in three stages it was decided to include in the calculation of F_c the contributions of the five hydrogen atoms attached tetrahedrally to the atoms of the carbon chain. Their positions were determined at first approximately from the model, and later, towards the end of refinement, accurately, assuming tetrahedral configuration about carbon atoms and a C–H distance of 1·05 Å. The marked change in the next difference map showed that the omission of their contribution to ϱ_c had seriously affected the placing of C_3, as, in projection, the two hydrogens attached to this atom fall close together on the same side of it. The change in the apparent position of C_3 due to the inclusion of these two hydrogens is estimated to be 0·07 Å. The remaining five hydrogens were then allowed for, assuming that three were bonded to the amino nitrogen and two to the amide nitrogen and that each lay on one of the straight lines joining these nitrogens to oxygens with which they could form hydrogen bonds. Convergence towards final coordinates from this stage was rapid and the final value of R was 12·0%. If $|F_o-F_c|$ is made zero where F_o is observed zero, $R=10·0\%$. Exclusion of the ten hydrogens from final calculations would raise $\Sigma|F_o-F_c|\div\Sigma|F_o|$ for those reflexions for which $\sin\theta < 0·60$ from 8·3% to 13·2%.

In the calculation of final F_c values, additional isotropic temperature factors for O_1, O_2 and N_1 were assumed, with an anisotropic factor for O_3, all estimated approximately from difference maps. These factors are listed in Table 5. The final electron-density map is shown in Fig. 2.

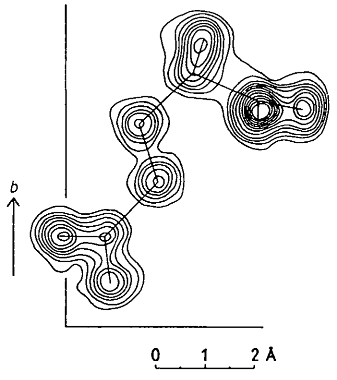

Fig. 2. Final electron-density projection of one molecule on (001). Contours at an interval of 1·0 e.Å^{-2}, starting at 2·0 e.Å^{-2}.

5. z-Coordinates

Inspection of the model placed on the known (001) projection showed that there were only four possible non-equivalent configurations for the molecule. Packing considerations pointed strongly to one of these, and when the correct translation of the whole molecule in the c direction had been found by trial, good structure factor agreement for $h0l$ reflexions showed the postulated z coordinates to be correct to a first approximation. Refinement was carried out in the same way as for the (001) projection and again inclusion of hydrogen atoms had a considerable effect on final 'heavy'-atom coordinates. O_2, O_3, N_1, N_2 were found to have larger-than-average isotropic temperature factors, and O_1 an anisotropic factor the values for which are listed in Table 5. The final value of R was 14·6% (13·5% if zero terms are neglected).

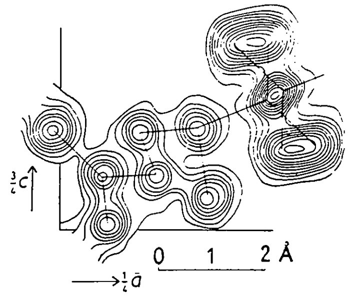

Fig. 3. Final electron-density projection of one molecule on (010). Contours at an interval of 1·0 e.Å^{-2}, starting at 2·0 e.Å^{-2}.

The final electron-density map is shown in Fig. 3. The two carboxyl groups which coincide in this projection are related by a screw axis perpendicular to the plane of projection and are separated by $\frac{1}{2}y$, i.e. 3·88 Å.

x, y and z coordinates derived from final difference maps are listed in Table 1, the x coordinates being weighted means from the c and b projections. Values

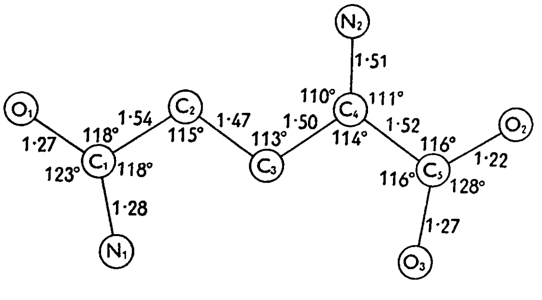

Fig. 4. Intramolecular bond lengths and bond angles. The diagram is simplified for convenience, bonds being drawn approximately equal in length, and the molecule being made to appear planar.

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* Computations on X-R.A.C. were carried out under contract N6 o.n.r.-26916, T. O. 16 with the Office of Naval Research.

Table 1. *Atomic coordinates*

Atom	x (Å)	y (Å)	z (Å)
C_1	0·77	1·82	3·55
C_2	1·83	2·93	3·60
C_3	1·46	4·11	4·39
C_4	2·56	5·13	4·49
C_5	3·87	4·56	5·01
N_1	0·89	0·91	2·66
N_2	2·76	5·80	3·15
O_1	−0·14	1·82	4·43
O_2	3·87	4·19	6·17
O_3	4·78	4·38	4·14
H_1	2·09	3·25	2·65
H_2	2·71	2·57	3·97
H_3	1·17	3·78	5·34
H_4	0·58	4·51	3·96
H_5	2·21	5·87	5·11
H_6	0·59	−0·06	2·34
H_7	1·82	0·72	2·05
H_8	1·79	5·80	2·67
H_9	3·25	6·59	3·48
H_{10}	3·20	5·16	2·38

z coordinates are referred to an origin displaced by $-\frac{1}{2}c$ from that shown in Fig. 7.

for H atoms are those calculated from C and N positions, as described above. Intramolecular bond lengths and angles are shown in Fig. 4. Intermolecular contacts of less than 3·5 Å (excluding those in which hydrogen participates) are listed in Table 2.

Table 2. *Intermolecular distances*

N_1–$O_1(B)$	2·94 Å
N_1–$O_3(C)$	2·91
N_2–$O_2(A)$	2·85
N_2–$O_2(C)$	2·79
N_2–$O_1(B)$	2·91
N_2–$O_3(C)$	3·31
N_2–$N_1(A)$	3·46
C_3–$O_1(B)$	3·37

The first-named atom is in each case from a molecule of type A (Figs. 6 and 7).

6. Estimation of accuracy

We shall assume that systematic errors due to termination of the Fourier series have been automatically corrected by the technique of (F_o-F_c)-syntheses (Cochran, 1951) and shall determine the effect of random experimental errors.

The standard deviation in coordinate resulting from random errors is

$$\sigma(x_i)=\frac{\sigma(\partial\varrho/\partial x_i)}{\partial^2\varrho/\partial x_i^2}=\frac{\dfrac{2\pi}{a_iA}\,[\Sigma mh_i^2\Delta F^2]^{\frac{1}{2}}}{2p\varrho(0)}\quad\text{(Cruickshank, 1949),}\qquad(5)$$

assuming that the electron density near the centre of an atom can be expressed as $\varrho(r)=\varrho(0)\exp[-pr^2]$.

From measurements of the curvature of well-resolved carbon peaks in the final projections, p was found to be 4·0.

If in applying equation (5) we make $\Delta F=|F_o-F_c|$ we shall obtain an overestimate of the standard deviation. This was done with the following results:

For the (001) projection $\sigma(x)=0{\cdot}014$ Å, $\sigma(y)=0{\cdot}016$ Å.
For the (010) projection $\sigma(x)=0{\cdot}020$ Å, $\sigma(z)=0{\cdot}021$ Å.

A mean value of the standard deviation of a bond length is obtained by multiplying the r.m.s. value of $\sigma(x_i)$ by $\sqrt{2}$. Taking $\sigma(x)=\sigma(y)=0{\cdot}015$ and $\sigma(z)=0{\cdot}020$, mean standard deviation in bond length $=\sigma=\sqrt{2}\times0{\cdot}017=0{\cdot}024$ Å, corresponding to a probable error of 0·016 Å.

The precise value of the standard deviation of a particular bond will depend upon its orientation with respect to the crystal axes. We can expect slightly larger values for C_5–O_2 and C_4–N_2 which are directed mainly in the c direction, and slightly smaller values for the C–C bonds all of which lie very nearly parallel to the (001) plane.

The reliability of the method of estimating $\sigma(x_i)$ may be checked by comparing the two independent sets of values for the x coordinates obtained from the two projections. If Δx_j is the difference in the x coordinate of the jth atom between the two projections,

$$\{\overline{(\Delta x)^2}\}^{\frac{1}{2}}=0{\cdot}016\text{ Å},$$

which, when compared with the result $(0{\cdot}015^2+0{\cdot}020^2)^{\frac{1}{2}}=0{\cdot}024$ obtained from Cruickshank's formula, indicates that our value of σ is a safe overestimate.

We conclude from this estimate of the standard deviation that the probability of a bond length being in error by as much as 0·060 Å or a bond angle by as much as 4° is certainly less than 1%, and these quantities will be taken as the limits of error (e.g. Cruickshank, 1949).

The standard deviation of electron density is given by

$$\sigma(\varrho_o)=(\sigma(F_o)/A)\{N\}^{\frac{1}{2}},$$

where

$$\sigma(F_o)=\{\Sigma(\Delta F)^2/N\}^{\frac{1}{2}}.$$

By taking $\Delta F=|F_o-F_c|$ we obtain an overestimate of $\sigma(\varrho_o)$:

0·21 e.Å^{-2} for the c projection

and

0·34 e.Å^{-2} for the b projection.

We may reasonably conclude that the standard deviations of electron density are unlikely to exceed 0·20 and 0·30 e.Å^{-2} respectively. The final c-axis projection could therefore be expected to give some indication of the positions of hydrogen atoms. In a map representing the total electron density such small details would be obscured by adjacent heavy-atom peaks, but are revealed if the heavy-atom contributions are subtracted. In Fig. 5 the calculated electron density of only the carbon, nitrogen and oxygen atoms has been subtracted from that observed. The positions of the maxima in this map are seen to agree quite well with positions of nine of the ten hydrogen atoms calculated as described above.

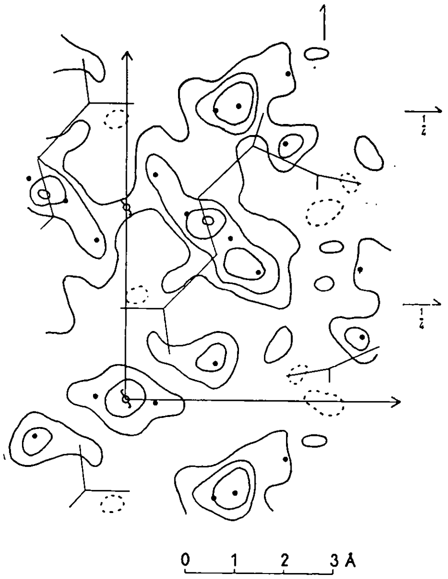


Fig. 5. Projection of electron density on (001) from which the contributions of C, N and O atoms have been subtracted. Calculated positions of H atoms are indicated by dots. Contours at $\frac{1}{3}$, $\frac{2}{3}$, 1, $\frac{4}{3}$ e.Å^{-2}, negative contours broken.

7. Discussion of structure

Bond lengths and angles may be most profitably discussed by comparing them with *accurate* measurements on chemically related compounds. Unfortunately the structure of only one true amide, acetamide, has previously been determined in detail (Senti & Harker, 1940) and the accuracy obtained was not high. On the other hand there have recently been several highly accurate three dimensional analyses of amino acids, namely L-threonine (Shoemaker, Donohue, Schomaker & Corey, 1950); DL-alanine (Donohue, 1950), a redetermination of the structure originally reported by Levy & Corey (1941); and L-hydroxyproline (Donohue & Trueblood, 1952), an independent and more accurate analysis of the structure originally reported by Zussman (1951). There exist, therefore, reliable standards to which the dimensions of the carboxyl and amino groups of glutamine may be referred, but any conclusions drawn from the dimensions of the amide group must at present be somewhat speculative.

The amide group

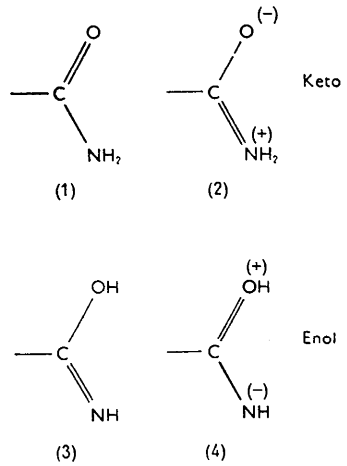
In Table 3 a comparison is made between the bond lengths and angles of the amide group as observed in glutamine and acetamide. While the difference in C–N bond lengths of 0·10 Å is in itself only possibly significant, when considered together with bond angle differences it assumes definite significance. The shorter the C–X bond (where X is N or O), i.e. the greater its double-bond character, the more should the angle ∠ C–C–X exceed the tetrahedral value of 109½°. The amide group of glutamine is symmetrical about C_1C_2 within the limits of experimental error and the evidence indicates strongly that its C–N bond possesses considerably greater double-bond character than the corresponding bond in acetamide, which is much less susceptible to hydrolysis or to attack by nitrous acid. These two reactions both involve the splitting of the C–N bond of the amide group, so the ease with which they take place must depend in some way on the ease with which this bond can be split. We should expect this in turn to depend upon its double-bond character. The observed correlation between the relative reactivites of the amide groups in glutamine and acetamide and the dimensions of this group might therefore have been expected.

Table 3. *Comparison of amide group dimensions in glutamine and acetamide*

Bond	L-Glutamine	Acetamide
C–O	1·27 Å	1·28 Å
C–N	1·28	1·38
Bond angle		
C–C–O	118°	129°
C–C–N	118	109
O–C–N	123	122

We have yet to decide whether the two hydrogen atoms of the group are both attached to the nitrogen or whether the oxygen and nitrogen have one each, i.e. does the keto or the enol form exist? The final difference map from which hydrogens have not been subtracted (Fig. 5) gives an indication of the keto form, but the accuracy of the map is not sufficient for this evidence to be regarded as conclusive. We shall therefore consider hydrogen-bonding possibilities and the likelihood of the appropriate resonance forms occurring in each case. Both N_1 and O_1 have two intermolecular contacts of under 3·0 Å, one of them being $N_1(A)$–$O_1(B)$ (Fig. 6). If it is assumed that each of these contacts represents a hydrogen bond, a hydrogen atom will be expected to lie near the line joining each pair. Considering first N_1, its two oxygen contacts make angles with N_1C_1 of 125° and 145°, the latter being with O_1. The two nitrogen contacts with O_1 make angles with O_1C_1 of 159° and 112°, the latter with N_1. It seems equally likely from these figures either that two hydrogens are attached to N_1 or that there is just one attached to N_1 making an angle near 125° with N_1C_1, and the other attached to O_1 making an angle near 112° with O_1C_1.

Consider now the possible resonance forms for the keto and enol structures:

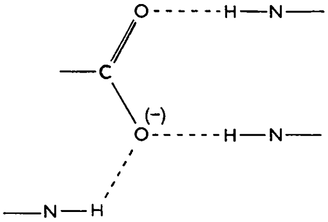


Whichever tautomer exists, the contributing resonance forms will be expected to have approximately equal weight because both the C–O and C–N lengths correspond to approximately 50% double-bond character (Pauling, 1939). The keto form is more likely on this evidence because oxygen has a greater electronegativity than nitrogen, and so one would not expect a form such as (4) to have any great weight. It is worth noting that Senti & Harker (1940) consider that hydrogen-bonding evidence in the acetamide structure points conclusively to the keto form.

It is hoped that when *hk*0 intensities are measured more accurately with a Geiger-counter spectrometer, the projected electron density between N_1 and O_1 of adjacent amide groups will be determined with sufficient accuracy to decide for certain to which of the atoms N_1 or O_1 a hydrogen atom is covalently bound, as has been done in the structure determination of α-pyridone (Penfold, unpublished data), an exactly analogous case.

Carboxyl and amino groups

Dimensions of the carboxyl and amino groups of glutamine are compared with the corresponding data for L-threonine, DL-alanine and L-hydroxyproline in Table 4. The only significant difference from the dimensions of glutamine appears in alanine, in which there is a pronounced lack of symmetry in the carboxyl group. This has been attributed by Donohue (1950) to lack of complete resonance due to the fact that one of the oxygen atoms takes part in two hydrogen bonds while the second oxygen atom takes part in only one, the result being that the form



is favoured.

Table 4. *Dimensions of carboxyl and amino groups in four compounds*

Bond	L-Glutamine	L-Threonine	DL-Alanine	L-Hydroxy-proline
C_5–O_2	1·22 Å	1·25 Å	1·27 Å	1·27 Å
C_5–O_3	1·27	1·24	1·21	1·25
C_4–N_2	1·51	1·49	1·50	—
Bond angle				
C_4–C_5–O_2	116°	116°	113°	115°
C_4–C_5–O_3	116	117	121	119
O_2–C_5–O_3	128	127	125	126

Table 5. *Temperature factors for individual atoms*

	Atom	Additional temperature factor
c-Axis projection	O_1	$\exp[-0{\cdot}5\sin^2\theta]$
	O_2	$\exp[-0{\cdot}5\sin^2\theta]$
	N_1	$\exp[-0{\cdot}4\sin^2\theta]$
	O_3	$\exp[-0{\cdot}4\sin^2\theta-0{\cdot}4(\frac{1}{2}kb^*)^2]$
b-Axis projection	O_2	$\exp[-0{\cdot}5\sin^2\theta]$
	O_3	$\exp[-0{\cdot}5\sin^2\theta]$
	N_1	$\exp[-0{\cdot}4\sin^2\theta]$
	N_2	$\exp[-0{\cdot}4\sin^2\theta]$
	O_1	$\exp[-\{0{\cdot}2+0{\cdot}7\sin^2(\varphi+28°)\}\sin^2\theta]$ (Hughes, 1941)

Similar significant lack of symmetry in the carboxyl group of β-glycyl-glycine (Hughes & Moore, 1949) can be explained in the same way. However, in threonine there is no such lack of symmetry although one oxygen forms two hydrogen bonds and the other only one. In glutamine, with similar disposition of hydrogen bonds, the *shorter* C–O bond contains the oxygen to which two hydrogen bonds are directed, just the opposite to what is observed in alanine and glycylglycine. Although the two C–O bond lengths in glutamine are not significantly different, it is unlikely that the bond which is in actual fact the shorter of the two could have been observed in this determination as the longer bond. It does not seem possible without still more evidence to make any further generalizations about the expected dimensions of carboxyl groups in crystals.

The molecule is assumed to be in the dipolar form with three hydrogens attached to the amino nitrogen which bears a nett positive charge. Evidence is from the contacts of 2·79, 2·85 and 2·91 Å which N_2 makes with three oxygen atoms, making with C_4N_2 angles of 98, 117 and 115°, all sufficiently close to the tetrahedral value to suggest hydrogen-bonding and agreeing closely with the corresponding angles in threonine where there was additional independent evidence of the attachment of three hydrogen atoms. The difference map (Fig. 5) supports the above view but cannot be regarded as conclusive in itself.

The carbon chain

The average C–C single-bond distance of 1·51 Å is low compared with the diamond value of 1·54 Å but agrees with the average found in threonine (1·52 Å), alanine (1·52 Å) and hexamethylene diamine (1·52 Å)

Table 6. *Comparison of observed and calculated structure factors*

In the calculation of F_c, Hartree atomic scattering factors for stationary atoms (James & Brindley, 1931) and coordinates listed in Table 1 were used. Contributions from atoms which have higher-than-average temperature factor were multiplied by the appropriate factor listed in Table 5 and the sum of all atomic contributions was multiplied by the overall observed temperature factor $\exp[-1\cdot00 \sin^2 \theta]$ to give F_c.

h0l terms are referred to an origin displaced by $-\frac{1}{4}a$ from the origin for *hk*0 terms.

hkl	F_o	F_c
000	—	312
200	18·2	−18·6
400	26·0	32·0
600	4·8	−3·6
800	1·4	1·8
10,0,0	4·2	−6·0
12,0,0	11·4	13·2
14,0,0	10·4	−11·0
16,0,0	7·0	6·2
18,0,0	4·2	6·2
20,0,0	8·0	8·0
110	9·5	−8·3
210	3·9	7·4
310	19·8	17·5
410	44·7	−46·7
510	34·2	32·4
610	8·0	−6·5
710	8·1	−9·9
810	13·6	12·6
910	13·8	−13·1
10,1,0	5·7	−7·3
11,1,0	0	−0·3
12,1,0	4·0	4·8
13,1,0	12·6	−11·8
14,1,0	20·6	20·1
15,1,0	16·3	16·3
16,1,0	1·5	1·3
17,1,0	3·9	−3·5
18,1,0	5·6	5·6
19,1,0	3·8	4·3
20,1,0	0	0·3
020	5·4	−3·4
120	52·5	−53·7
220	44·9	−44·9
320	18·8	19·2
420	9·2	10·3
520	34·5	−32·0
620	54·9	−56·1
720	12·0	−10·7
820	7·6	6·6
920	1·3	−1·0
10,2,0	4·3	0·9
11,2,0	14·9	18·4
12,2,0	1·7	−1·7
13,2,0	0	−0·3
14,2,0	1·5	−1·5
15,2,0	1·6	0·9
16,2,0	0	−0·9
17,2,0	0	0·4
18,2,0	10·1	−10·8
19,2,0	5·9	5·9
20,2,0	2·6	2·4
130	11·6	−11·3
230	29·9	−29·9
330	27·0	−28·0
430	19·1	19·4
530	7·2	6·9
630	3·7	−4·0
730	12·8	13·4
830	6·9	−6·0
930	3·0	−3·6
10,3,0	5·5	5·2
11,3,0	0	0·8
12,3,0	13·2	−13·2
13,3,0	3·7	−3·7
14,3,0	6·3	6·7
15,3,0	0	−0·2
16,3,0	5·3	−4·7
17,3,0	0	−0·6
18,3,0	2·9	−2·7
19,3,0	2·6	2·9
040	15·2	16·6
140	27·5	−27·1
240	6·8	6·4
340	18·2	19·9
440	15·6	15·9
540	22·7	−23·3
640	12·0	11·7
740	13·2	14·2
840	2·1*	0·3
940	0	2·0
10,4,0	5·7	6·2
11,4,0	0	−2·9
12,4,0	10·5	10·2
13,4,0	4·2	−4·6
14,4,0	0	−1·1
15,4,0	3·1	1·4
16,4,0	2·6	−2·1
17,4,0	7·4	−6·9
18,4,0	0	−1·4
150	1·9	−0·9
250	8·4	7·5
350	7·4	8·6
450	6·4	7·3
550	0	0·6
650	5·5	−5·2
750	9·8	9·3
850	3·8	−5·7
950	9·6	8·8
10,5,0	2·5	0·7
11,5,0	2·6	−2·6
12,5,0	4·0	4·7
13,5,0	1·9	−2·1
14,5,0	8·0	7·8
15,5,0	0	−1·1
16,5,0	4·0	−4·8
17,5,0	3·7	5·1
060	21·8	−21·2
160	14·3	−15·4
260	1·4	0·6
360	4·1	2·2
460	13·9	−15·5
560	2·1	−2·4
660	1·8	−2·9
760	10·8	10·8
860	0	1·0
960	0	1·9
10,6,0	0	0·4
11,6,0	3·4	−4·0
12,6,0	0	0·8
13,6,0	4·6	−3·4
14;6,0	4·2	3·7
15,6,0	1·5	2·9
16,6,0	7·2	−7·2
170	9·0	−9·0
270	9·0	9·5
370	2·2	2·4
470	11·6	12·3
570	2·1	−2·3
670	0	−1·6
770	3·1	−2·4
870	3·7	−3·0
970	2·7	−1·5
10,7,0	1·4	1·4
11,7,0	3·6	−2·6
12,7,0	4·7	−3·3
13,7,0	1·5	2·3
14,7,0	1·6	−1·6
080	8·8	9·4
180	3·5	−3·0
280	11·3	10·2
380	0	−2·3
480	5·0	−4·4
580	4·3	5·5
680	2·9	2·5
780	2·7	2·3
880	2·6	−2·0
980	1·2	2·1
10,8,0	0	−0·3
11,8,0	0	−0·2
12,8,0	2·1	−1·1
190	6·1	5·2
290	2·1	3·2
390	3·7	4·0
490	4·0	−4·3
590	3·0	2·5
690	0	−0·3
790	0	0·6
890	0	−0·2
990	3·0	−6·1
0,10,0	3·4	−6·2
1,10,0	0	3·5
101	17·1	−15·9
201	38·5	−42·7
301	35·3	−41·8
401	23·6	21·5
501	2·2	2·2
601	23·7	23·0
701	26·7	−27·5
801	14·4	14·8
901	0	0·6
10,0,1	15·3	14·7
11,0,1	8·3	−8·0
12,0,1	0	−0·3
13,0,1	8·7	−7·4
14,0,1	0	−0·5
15,0,1	5·8	5·1
16,0,1	13·6	−12·5
17,0,1	2·2	−1·4
18,0,1	5·0	5·1

Table 6 (*cont.*)

hkl	F_o	F_c
19,0,1	8·2	−6·9
20,0,1	2·3	2·3
002	14·8	−18·6
102	7·9	8·3
202	14·0	−16·2
302	24·6	−25·3
402	7·7	−5·3
502	7·9	−6·2
602	4·0	−1·3
702	20·0	−22·7
802	4·1	−4·4
902	10·0	9·1
10,0,2	8·7	10·9
11,0,2	9·1	−10·2
12,0,2	5·7	5·7
13,0,2	12·9	14·6
14,0,2	3·5	−3·2
15,0,2	2·2	1·9
16,0,2	1·6	0·4
17,0,2	5·8	−6·2
18,0,2	0	0·7
19,0,2	2·2*	−0·2
103	10·4	10·0
203	11·6	−10·0
303	5·1	2·2
403	15·3	−17·2
503	4·3	3·4
603	5·7	−4·2
703	0	−1·5
803	2·2	−1·1
903	11·5	−11·0
10,0,3	6·9	8·2
11,0,3	3·7	3·2
12,0,3	3·7	−4·2
13,0,3	6·4	−3·1
14,0,3	7·7	−7·1
15,0,3	4·7	−6·7
16,0,3	2·3	−1·1
17,0,3	0	−0·3
18,0,3	0	0·5
004	5·8	−5·0
104	5·0	5·8
204	4·9	6·2
304	8·4	7·3
404	10·2	10·2
504	5·5	4·2
604	16·1	18·5
704	6·4	5·9
804	4·2	4·7
904	5·5	4·6
10,0,4	9·4	8·6
11,0,4	5·1	4·9
12,0,4	9·0	−8·2
13,0,4	4·5	−4·5
14,0,4	2·2	2·7
15,0,4	0	−1·1
16,0,4	6·8	7·0
105	6·5	−4·9
205	4·4	3·7
305	15·0	−18·5
405	2·2	1·5
505	2·7	−2·5
605	5·0	−4·2
705	3·5	−3·1
805	0	0
905	0	−0·9
10,0,5	4·9	−4·6
11,0,5	1·7	−1·0
12,0,5	0	−1·1
13,0,5	4·6	−4·4
006	7·0	7·0
106	9·8	−10·3
206	0	−0·3
306	1·7*	−0·3
406	1·8	2·8
506	2·0	2·6
606	3·0	4·3
706	2·3	−1·7
806	0	−0·3

* Omitted from the final synthesis because of uncertain sign.

(Binnie & Robertson, 1950) all aliphatics, and it would appear that the standard C–C single bond in such compounds is somewhat shorter than in diamond. Similarly the average value of the ∠ C–C–C angle of 114° may be compared with that in threonine (113°), alanine (111°) and hexamethylene diamine (115½°).

Molecular environment

Figs. 6 and 7 show the structure viewed along the *c* and *b* axes respectively, the origin and boundaries of the cell being chosen to correspond in each case. If all intermolecular contacts of less than 3·0 Å are regarded as hydrogen bonds, there are five N–H---O bonds per molecule, the maximum number possible with only five available hydrogen atoms.

In Fig. 6, $N_2(A)$ and $O_2(A)$ of the same molecule are only apparently bonded together, the bond in reality linking molecules which are one unit cell apart in the direction of projection.

The dominating feature of the intermolecular packing is the hydrogen bonding between amino and carboxyl groups which links N_2 and O_2 atoms into spirals about the screw axes perpendicular to (010). The effects of such bonding will be enhanced by the electrostatic forces which must exist between these oppositely charged groups, and strong cohesion in the *b* direction results. Cohesion in the *c* direction can be accounted for by the hydrogen bonds which link amide groups into spirals about the screw axes perpendicular to (001), while the two remaining hydrogen bonds of type $N_1(A)$–$O_3(C)$ and $N_2(A)$–$O_1(B)$ account for the cohesion in the *a* direction.

The above arguments show that L-glutamine, considered as an amino acid, has a crystal structure which conforms to the general pattern observed in other amino acids. There appears from the structure no stereochemical reason for the tendency of an individual glutamine molecule to condense with itself. It would be most valuable to have structural data on the related compound asparagine (the γ-amide of aspartic acid) and on some non-acidic amides in order to make further comparisons with the chemically abnormal amide group of glutamine. Work is being continued on the structure determination of asparagine monohydrate.

We should like to conclude by thanking Prof. Sir Lawrence Bragg and Dr W. H. Taylor for their continued support and encouragement. Part of this work was done at Pennsylvania State College in the laboratory of Prof. R. Pepinsky, to whom we are greatly indebted for the facilities made available to us. We should also like to thank Dr M. V. Wilkes, Director of the Cambridge University Mathematical Laboratory, for permission to use a Hollerith tabulator for much of the computing work, and Mrs L. Remnant for assistance with the latter. One of us (B.R.P.) acknowledges gratefully the award of postgraduate scholarships by the University of New Zealand and the British Council during the tenure of which this work was carried out, and thanks the Council of Canterbury University College, New Zealand, for granting three years leave of absence.

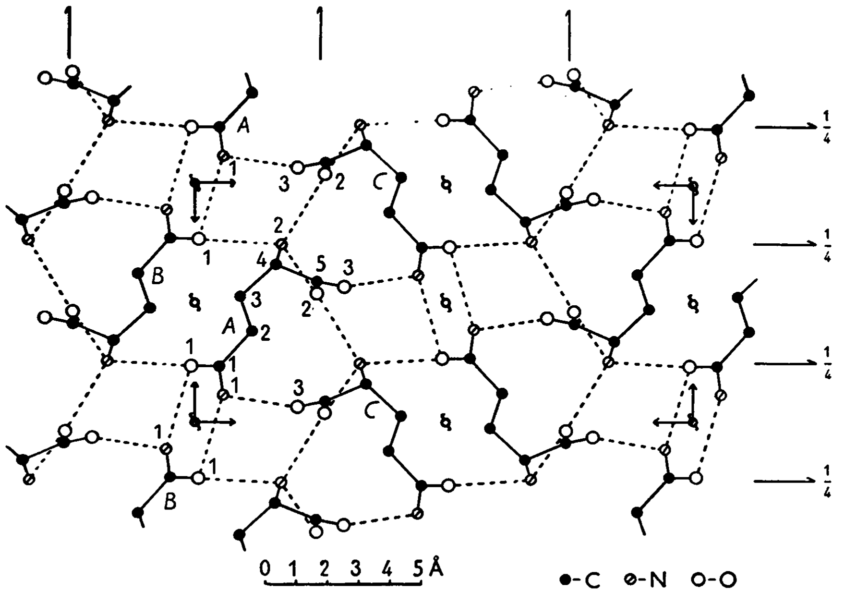


Fig. 6. Structure viewed along *c* axis showing hydrogen bonds (broken lines).

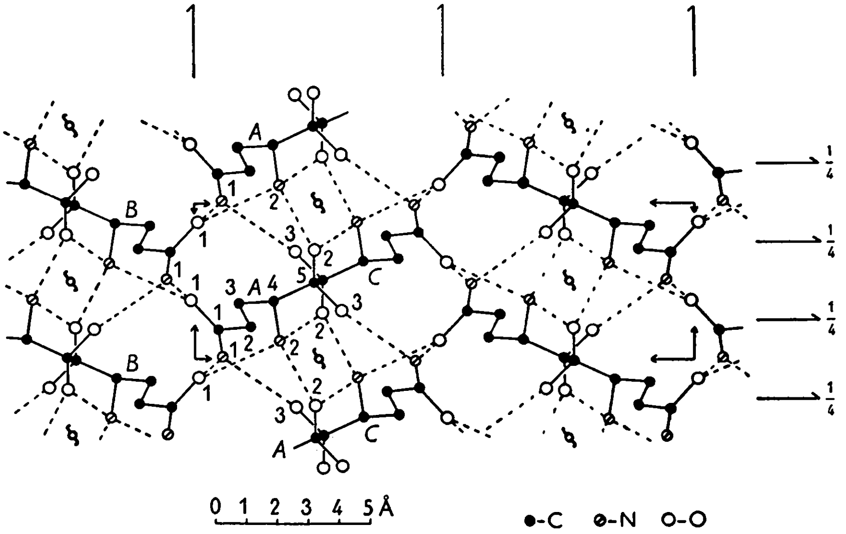


Fig. 7. Structure viewed along *b* axis showing hydrogen bonds (broken lines).

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