rather long (3·5 Å in NH_4Br, 3·17 Å in strychnine-hydrobromide). This is possibly due to repulsion of the bromine ion by the CH_2 groups numbered C_2 and C_4, which in the chair form of the cyclohexane ring come much closer to bromine than the corresponding atoms in strychnine.

We are very grateful to Prof C. H. MacGillavry for her encouragement and never failing interest in the work. We are also much indebted to Mr A. Kreuger for his kind help in many ways, especially in the experimental work and the drawings. The experimental part of this work was carried out with the help of X-ray apparatus put at the disposal of this Laboratory by the Organization Z. W. O.

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The Crystal Structure of N,N′-Diglycyl-L-Cystine Dihydrate*

By Harry L. Yakel, Jr. and Edward W. Hughes

Gates and Crellin Laboratories, California Institute of Technology, Pasadena, California, U.S.A.

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The crystal structure of N,N′-diglycyl-L-cystine dihydrate has been determined by means of X-ray methods. The unit cell is monoclinic with $a = 12{\cdot}26$, $b = 4{\cdot}84$, $c = 17{\cdot}17$ Å, and $\beta = 124°24'$ and contains two molecules. The space group is $A2$.

The structure of the molecule was deduced from Patterson projections and sections and refined by Fourier and least-squares methods. The bond distances and angles are similar to those found in related structures. Several unusual hydrogen bonds are reported, however. The possible effect of the very long hydrogen bond formed by the amide group on bond distances and planarity of that group is discussed. The structural features of the disulfide bridge in this molecule are also presented in some detail.

1. Introduction

Previous determinations of the crystal structures of simple amino acids, peptides, and related compounds have illustrated several structural features of these molecules which have been used as criteria in the deduction of the configurations of polypeptide chains. For example, the structures of N-acetylglycine (Carpenter & Donohue, 1950) and β-glycylglycine (Hughes & Moore, 1949) have suggested that resonance in the amide groups of peptide molecules forces these groups to assume a planar configuration and also results in a shortening of the C–N bond and a lengthening of the C=O bond from their normal values. All of the amino acid and peptide structures investigated to date have emphasized the importance of the role played by hydrogen bonding in determining the packing of these molecules. Therefore, by analogy, one might expect the packing of the polypeptide chains in proteins to be governed in part by similar considerations.

An interesting series of compounds from the point of view of possible application to protein structures is that consisting of cystine and peptides containing cystine. The $-CH_2-S-S-CH_2$-group (the disulfide bridge) common to the members of this series supposedly constitutes one of the more important means by which the polypeptide chains of proteins are held together. The fact that the peptide chain at one end of the bridge may be identical with that at the other end, a doubling back of the chain having taken place, also indicates that the configuration may provide a convenient method for holding polypeptide chains in a folded configuration. In an effort to determine the structural characteristics of the disulfide bridge an X-ray crystal structure investigation of the peptide N,N′-diglycyl-L-cystine dihydrate, $C_{10}N_4O_6H_{18}S_2.2H_2O$, has been completed. The details and results of this investigation are given in the following sections.

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* Contribution No. 1811 from the Gates and Crellin Laboratories.

2. Determination of unit-cell parameters, space group, and experimental intensities

The N,N′-diglycyl-L-cystine used in this work was prepared by Greenstein (1939). Needle-like crystals were grown from a water solution by the slow addition of *n*-propanol. Microscopic examination showed them to be strongly birefringent with extinctions parallel and perpendicular to the needle axis and with a biaxial interference figure. The efflorescence observed on heating the crystals indicated that they were hydrated.

A crystal mounted with the needle axis vertical was used to obtain oscillation and Weissenberg photographs. Copper $K\alpha$ radiation ($\lambda = 1{\cdot}542$ Å) was employed throughout. A zero-layer Weissenberg photograph taken about the needle axis, in conjunction with the optical data, showed the crystals to be monoclinic with the b axis coincident with the needle direction. Unit-cell parameters were measured from the equators of oscillation pictures taken about b_0 and c_0 with a 5-cm. camera. The results of these determinations are:

$$a = 12{\cdot}26 \pm 0{\cdot}01,\ b = 4{\cdot}84 \pm 0{\cdot}01,\ c = 17{\cdot}17 \pm 0{\cdot}01 \text{ Å},\ \beta = 124^\circ\ 24' \pm 15'.$$

The value $1{\cdot}56 \pm 0.03$ g.cm.$^{-3}$ for the density of the crystals was obtained by a flotation technique. The cell dimensions and this experimental density lead to the value 2·12 and 2·00 respectively for the number of molecules of $C_{10}N_4O_6H_{18}S_2 . nH_2O$ per cell, with $n = 1$ or 2. It is concluded that $n = 2$.

Complete diffraction data showed Laue symmetry $2/m$ and centering on A. Thus $A2$, Am, and $A2/m$ are possible space groups for the crystal. The general positions of these space groups are fourfold or eightfold; hence the peptide molecules would in any case be required to lie across symmetry elements. Examination of the usual organic projection formula for the molecule indicated that it was unlikely for the molecule to have either symmetry m or $2/m$ but that it might possess a twofold axis. The space group $A2$ was therefore chosen as probably correct.

Relative intensity data were obtained by visual comparison of multi-film Weissenberg photographs with a calibrated intensity strip. A total of 850 independent hkl intensities were measured, about half of these being recorded more than once; of these, 630 were observably different from zero. After correction of all intensities for Lorentz and polarization factors, the $h0l$ intensities were placed on an absolute scale by applying the averaging method outlined by Wilson (1942). The temperature factor as determined by the same method was $B = 3{\cdot}5$ Å^2.

3. Derivation and refinement of a trial structure

If the molecules of N,N′-diglycyl-L-cystine are lying across the twofold axes of the unit cells, it is obvious that a twofold symmetry axis must pass through the peptide molecule perpendicular to the S–S bond at its midpoint. A Patterson projection down the b axis should therefore contain a peak representing the S–S interaction at a radius of about 2·08 Å (twice the usual covalent sulfur radius as given by Pauling (1940)) and at an azimuth angle corresponding to the orientation of the S–S bond.

Such a Patterson synthesis was made from the 160 non-zero $h0l$ reflections (Fig. 1). The peak representing the S–S interaction is indicated. The rows of strong peaks at $u = \frac{1}{3}$ and $\frac{2}{3}$ suggested that chains of atoms might lie approximately along the lines $x = \frac{1}{3}$ and $\frac{2}{3}$ the unit cell. The large intensity of the 300 reflection had already suggested this prior to the preparation of the Patterson. The nature of the chain was deduced by a comparison of the unit-cell dimensions for N,N′-diglycyl-L-cystine dihydrate with those for β-glycylglycine (Hughes & Moore, 1949). If the differences in space group and molecular symmetry are taken into account one observes that the c axes and β angle of the two crystals are nearly identical. This was interpreted as meaning that the chains present in the N,N′-diglycyl-L-cystine dihydrate structure are diglycine chains and are probably oriented with their long directions parallel to c, as in β-glycylglycine. Secondly, the b axis of N,N′-diglycyl-L-cystine dihydrate is about 0·2 Å longer than that reported for β-glycylglycine, suggesting that the planes of the chains are tipped further out of the a–c plane in the former. Lastly, the a axis is 3·3 Å longer than half that for β-glycylglycine. This difference was ascribed to the fact that, in the direction of a, pairs of diglycine chains in N,N′-diglycyl-L-cystine are separated from each other by $-CH_2-S-S-CH_2-$ groups, whereas in β-glycyl-glycine the molecules are all separated only by van der Waals forces.

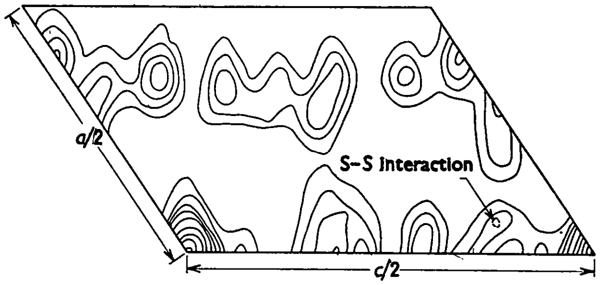
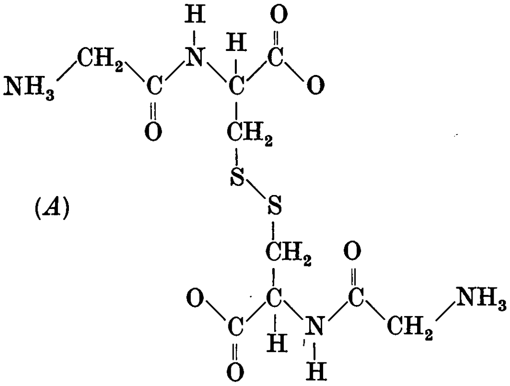


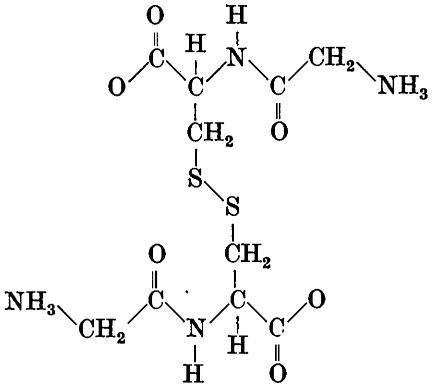
Fig. 1. The $h0l$ Patterson projection for N,N′-diglycyl-L-cystine dihydrate.

The picture of the peptide molecule derived from these considerations permitted two possible orientations of the diglycine chains relative to the disulfide bridge. These orientations are shown schematically in the accompanying sketch. A distinction between the two was made on the basis of a structure-factor calculation which gave better agreement with observation for model (B). For this calculation the waters of hydration were placed near the twofold screw axis at $x = 0, z = \frac{1}{4}$.

A Fourier projection was made which included 57 of the 160 non-zero $h0l$ reflections. Significant shifts in the atomic positions were indicated by this projection. Further efforts at refinement of this structure by the usual methods failed. Inclusion of additional terms with signs calculated from the revised trial structure fitted to the first Fourier in accord with the notions outlined above produced a distorted projection; it was obvious that there were some important terms with wrong signs.

To obtain some further control over the signs, use was made of the facts, already established, that there were no atoms near the line $x = \frac{1}{2}$, that only the S atom, of known position, and the water O atom, of approximately known position, were near the line $x = 0$. Thus $\varrho(\frac{1}{2}, z) = 0$ and $\varrho(0, z) = \varrho_S(0, z)+\varrho_O(0, z)$ = known function of z.


(A)


(B)

Now

$$\varrho(x, z) = \frac{1}{A}\sum_h\sum_l F_{h0l} \cos 2\pi(hx+lz) ,$$

so for $x = \frac{1}{2}$, we have

$$0 = \sum_h\sum_l F_{h0l} \cos 2\pi(h/2+lz) ,$$

and if we multiply by $\cos 2\pi l'z dz$ and integrate from $z = 0$ to $z = 1$ we have

$$\sum_h (-1)^h F_{h0l'} = 0 \text{ for each value of } l'.$$

A similar treatment for $x = 0$ yields

$$\sum_h F_{h0l'} = F_{l'}(\mathrm{S, O})$$

where $F_{l'}(\mathrm{S, O})$ can be calculated for each l' from the S and H_2O positions and the scattering factors of S and O. $F_{l'}(\mathrm{S, O})$ is the amplitude of the l'th Fourier component of $\varrho(0, z)$.

Solving the two sums simultaneously for a given value of l' gives $\sum_h F_{h0l'} = \frac{1}{2}F'_{l'}(\mathrm{S, O})$ for h even or h odd separately, and l' constant.

There are thus small groups of F's each of which must add up to some predictable value. If there is only one large F in a group the sign of the F is at once fixed. If there are two or three large F's a relationship is established between their signs.

Similar relationships can be worked out for any trial structure for which the electron density can be established with certainty over important localities, such as symmetry axes or planes. This method of controlling signs, which was proposed by one of us (E.W.H.) at the Second Congress of the International Union for Crystallography in 1951, is the same as the method set forth in more general terms by Goedkoop, MacGillavry & Pepinsky at the same Congress.

On application of these restricting equations to the $h0l$ data it was found that only 45 of the signs were established with certainty and that some of these were different from those derived from the first trial structure. A third Fourier projection was then prepared (Fig. 2). It suggested that the planes of the diglycine groups are tipped at a much larger angle to the $h0l$ plane than are the molecules in β-glycylglycine and that the groups are not nearly so planar as expected. With this revision of the trial structure the refinement proceeded smoothly, the new signs at each stage being checked for consistency by means of the above equations. The 4th, 5th and 6th (final) Fouriers contained 78, 134 and 160 terms respectively. The final projection is reproduced in Fig. 3. As there are a number of poorly resolved atoms in this projection, the x and z parameters were further refined by least squares (Hughes, 1941). All non-diagonal terms were included in these calculations with the $h0l$ data.

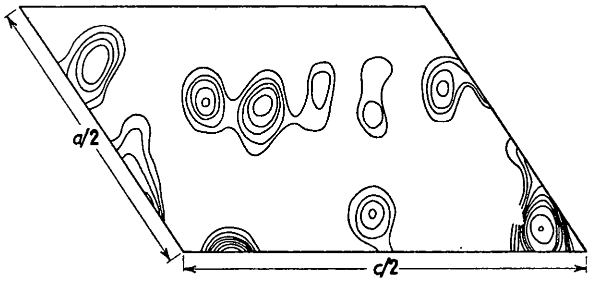


Fig. 2. A preliminary $h0l$ Fourier projection of the N,N′-diglycyl-L-cystine dihydrate structure in which only 45 terms were included.

The estimation of y parameters was made with the aid of the x and z coordinates and the usual bond lengths and angles to be expected in peptides and amino acids (Corey & Donohue, 1950). Several ambiguities concerning the orientation of the S–CH$_2$ bond in the disulfide bridge and the y position of the water oxygen were settled by means of Patterson sections and line Pattersons. The observed general *hkl* intensities used in these series were placed on an absolute scale by comparison with absolute *h0l* intensities or by the use of the statistical method already described.

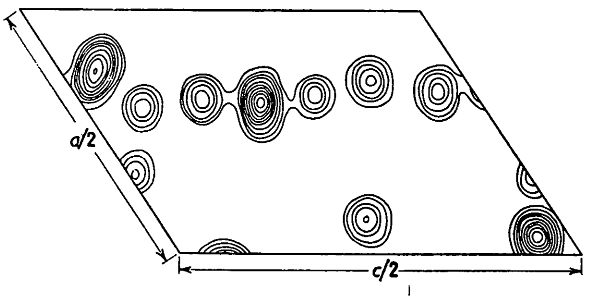


Fig. 3. The final *h0l* Fourier projection of the N,N′-diglycyl-L-cystine dihydrate structure in which all 160 observable *h0l* reflections were included.

The final refinement of the x, y, and z parameters was completed exclusively by least-squares methods. Two one-dimensional least squares (on y coordinates alone) and two three-dimensional least squares were computed. Off-diagonal terms were computed for all the least-squares refinements. The final values for all parameters are listed in Table 1. The probable errors for each parameter were calculated from the relation

$$\text{Probable error } (q_i) = 0\cdot6745\times\text{standard deviation } (q_i)$$
$$= 0\cdot6745\ \{\sum_{hkl} W_{hkl}(\Delta F^2_{hkl})^2 . D^{-1}_{ii}/(m-S)\}\ , \quad (3\cdot1)$$

where W_{hkl} is the square of the weighting factor for the *hkl* reflection, ΔF^2_{hkl} is the difference between observed and calculated intensities, m is the number of observational equations, S is the number of independent variables, and D^{-1}_{ii} is the ith diagonal element of the matrix inverse to the matrix of normal equation coefficients.

Table 1. *Final atomic parameters for* N,N′-*diglycyl*-L-*cystine dihydrate*

Atom	x	Δx (Å)	y	Δy (Å)	z	Δz (Å)
C$_1$	0·3406	0·011	0·0209	0·023	0·0200	0·012
C$_2$	0·2873	0·012	0·8449	0·021	0·0673	0·014
C$_3$	0·3156	0·012	0·8539	0·022	0·2219	0·014
C$_4$	0·3232	0·011	0·0538	0·021	0·2954	0·012
C$_5$	0·1532	0·012	0·7090	0·020	0·0032	0·014
N$_1$	0·3139	0·009	0·9895	0·020	0·1524	0·009
N$_2$	0·3546	0·009	0·8962	0·017	0·3777	0·010
O$_1$	0·3301	0·007	0·4130	0·014	0·4508	0·009
O$_2$	0·3912	0·009	0·2403	0·014	0·0553	0·009
O$_3$	0·2908	0·009	0·6132	0·013	0·2210	0·009
O$_4$	0·0663	0·009	0·5891	0·013	0·2560	0·009
S	0·0316	0·002	0·4989	0·006	0·4563	0·003

The reliability factors for the four layers which contain significant numbers of reflections are given in Table 2. R is defined by the following equation:

$$R = \{\sum_{hkl} (|F_{hkl}|_o - |F_{hkl}|_c) \div \sum_{hkl} |F_{hkl}|_o\} \times 100 \quad (3\cdot2)$$

summed over all observable reflections.

Table 2. *Reliability factors for* N,N′-*diglycyl*-L-*cystine dihydrate*

Layer	R (%)
h0l	13·5
h1l	14·9
h2l	14·5
h3l	12·5

The structure-factor calculation used to obtain these reliability factors was made with the parameters corrected by the second three-dimensional least squares and with inclusion of the eleven hydrogen atoms contained in the asymmetric unit. The coordinates of the hydrogen atoms, estimated from the usual C–H, O–H, and N–H bond lengths and angles, are presented in Table 3. The inclusion of these atoms in the structure factors improved the reliability factor by about 1·5%.*

Table 3. *Parameters of hydrogen atoms in* N,N′-*diglycyl*-L-*cystine dihydrate*

Atom	x	y	z
H$_1$	0·145	0·130	0·453
H$_2$	0·132	0·585	0·033
H$_3$	0·349	0·712	0·098
H$_4$	0·324	0·172	0·158
H$_5$	0·382	0·184	0·307
H$_6$	0·245	0·127	0·280
H$_7$	0·443	0·867	0·405
H$_8$	0·310	0·750	0·378
H$_9$	0·348	0·036	0·410
H$_{10}$	0·127	0·594	0·247
H$_{11}$	0·040	0·752	0·256

4. Discussion of the structure

The bond lengths and angles, including those involving hydrogen bonds, calculated with the parameters of Table 1 are given in Table 4. The estimation of probable error in each distance was made by application of the equation

$$\delta R_{ij} = \text{Probable error in } R_{ij} = \sqrt{\sum_i Q_i^2}\ , \quad (4\cdot 1)$$

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* A list of calculated and observed structure factors for 854 observable *hkl* reflections has been deposited as Document No. 4109 with the ADI Auxiliary Publications Project, Photoduplication Service, Library of Congress, Washington 25, D.C., U.S.A. A copy may be secured by citing the Document number and by remitting $2·50 for photoprints, or $1·75 for 35 mm. microfilm. Advance payment is required. Make checks or money orders payable to: Chief, Photoduplication Service, Library of Congress.

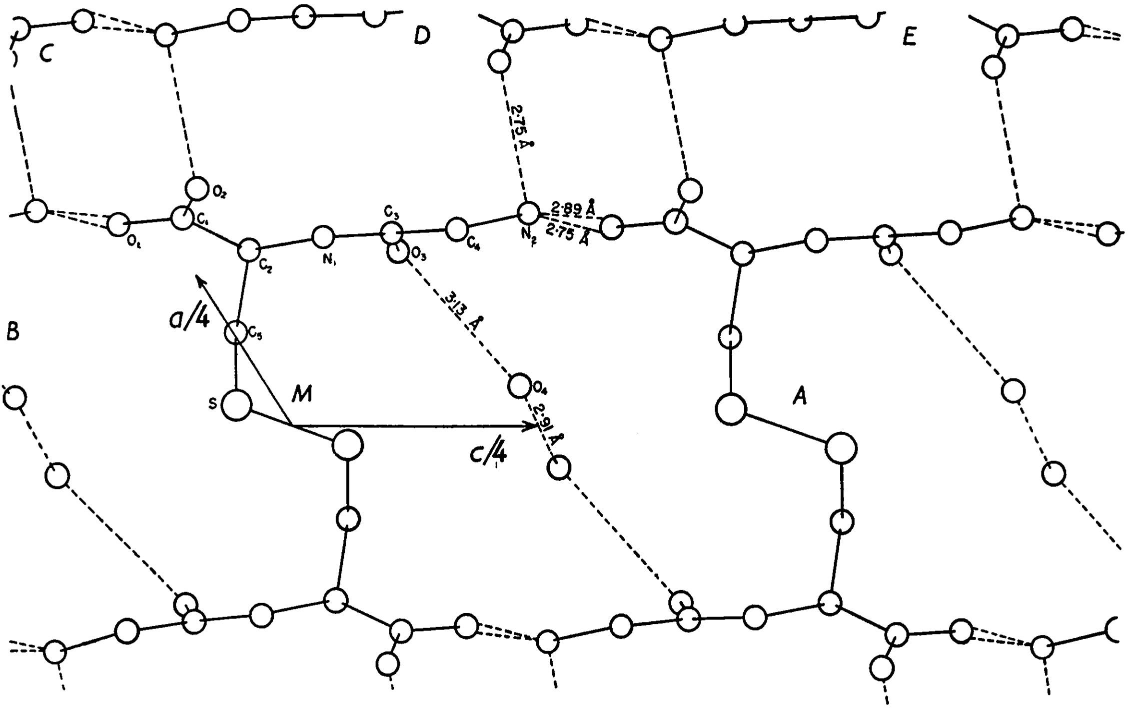

Fig. 4. A view of the N,N′-diglycyl-L-cystine dihydrate structure looking down the *b* axis. Dashed lines represent hydrogen bonds.

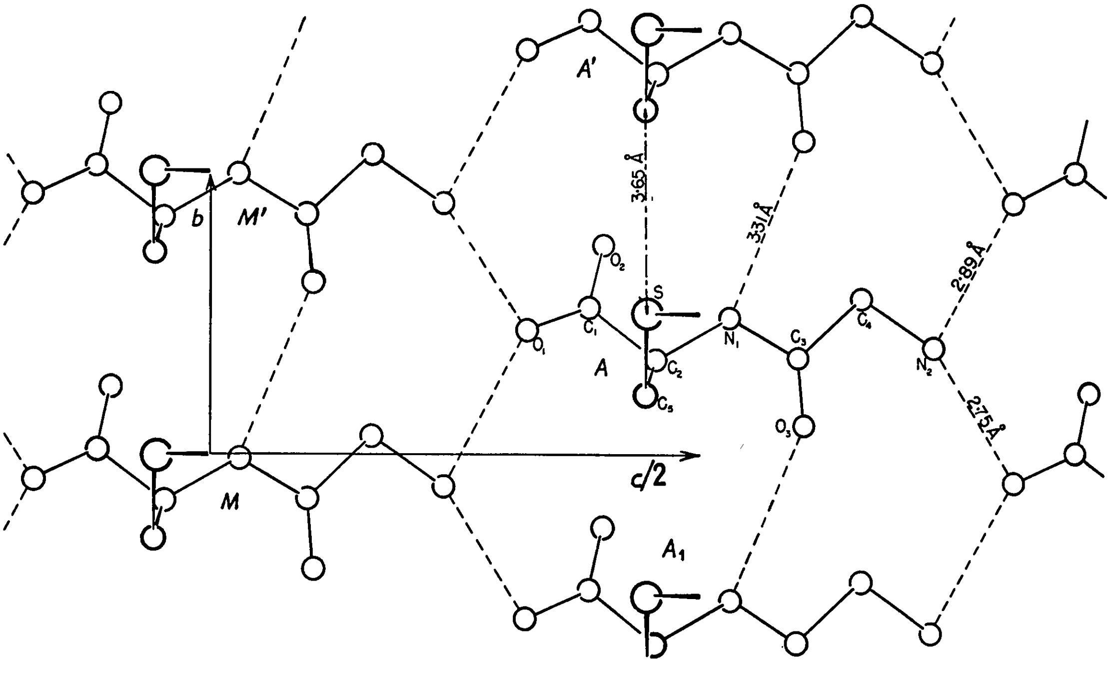

Fig. 5. A view of the N,N′-diglycyl-L-cystine dihydrate structure looking down a direction perpendicular to the 100 plane. Only half of each molecule is shown for the sake of simplicity. The two water molecules are omitted for the same reason. Dashed lines represent hydrogen bonds.

where

$$Q_i = (\partial R_{ij}/\partial q_i)\,\delta q_i$$

and δq_i is the probable error of the parameter q_i. The probable errors in the bond angles were calculated indirectly from an expression similar to (4·1) which gives the error in the cosine of the angle.

Two projections of the final structure are shown in Figs. 4 and 5. The numbering system used to denote atoms in Tables 1 and 4 is also illustrated in both diagrams. The prime superscript used for O_4 indicates the oxygen atom related to O_4 by the twofold screw axis.

The distances and angles reported in Table 4 are in general agreement with those found in previous amino acid and peptide structures with several exceptions to be noted below.

Table 4. *Bond lengths and angles in N,N′-diglycyl-*L*-cystine dihydrate*

Bond	R_{ij} (Å)	Probable error (Å)
S–S	2·04	0·005
S–C_5	1·87	0·017
C_5–C_2	1·52	0·017
C_2–C_1	1·55	0·024
C_3–C_4	1·55	0·024
C_1–O_1	1·24	0·018
C_1–O_2	1·21	0·023
C_3–O_3	1·21	0·025
C_2–N_1	1·48	0·020
C_3–N_1	1·35	0·021
C_4–N_2	1·46	0·019

Hydrogen bond	R_{ij} (Å)	Probable error (Å)
$N_1 \cdots O_3$	3·31	0·022
$N_2 \cdots O_1$ down	2·75	0·020
$N_2 \cdots O_1$ up	2·89	0·021
$N_2 \cdots O_2$	2·75	0·013
$O_4 \cdots O_4'$	2·91	0·017
$O_4 \cdots O_3$	3·13	0·015

Angle	θ (°)	Probable error (°)
S–S–C_5	103·0	1·1
S–C_5–C_2	105·0	1·4
C_5–C_2–C_1	117·4	2·6
C_5–C_2–N_1	117·6	2·3
C_1–C_2–N_1	109·4	2·3
O_1–C_1–O_2	126·6	3·8
C_2–C_1–O_1	115·0	2·9
C_2–C_1–O_2	118·5	3·4
C_2–N_1–C_3	121·6	3·1
N_1–C_3–O_3	125·3	4·1
C_4–C_3–O_3	120·6	3·6
N_1–C_3–C_4	113·2	2·3
C_3–C_4–N_2	108·8	2·2

Hydrogen-bond angle	θ (°)	Probable error (°)
C_4–$N_2 \cdots O_1$ up	84·5	2·4
C_4–$N_2 \cdots O_1$ down	129·8	2·3
C_4–$N_2 \cdots O_2$	111·8	1·8
C_1–$O_1 \cdots N_2$ up	95·6	2·7
C_1–$O_1 \cdots N_2$ down	143·0	3·0
C_1–$O_2 \cdots N_2$	125·9	2·1
C_3–$O_3 \cdots N_1$	144·0	3·8
C_2–$N_1 \cdots O_3$	140·1	2·8
C_3–$N_1 \cdots O_3$	95·5	3·1
C_3–$O_3 \cdots O_4$	106·0	2·5
$O_3 \cdots O_4 \cdots O_4'$	118·2	2·2

Among the covalent bond distances the 1·87 Å S–C_5 distance appears to be abnormally long compared with the sum of the usual covalent radii, 1·81 Å. This increase in length appears to be outside the limit of error calculated for the bond. Other compounds containing a long S–C bond are 2,2′-diiodo-diethyl-trisulfide (1·86 Å) (Donohue, 1950), cyclohexyl mercaptan (1·87 Å) (Hassel & Viervoll, 1947), and mustard gas (average of C–Cl and C–S equals 1·84 Å) (Schomaker, 1945). A similar lengthening of Se–C bonds seems to occur in the 1,4-diselenane molecule. The N_1–C_3 distance is also slightly longer than that found in other peptides, although if the reported length is assumed to be high by the amount of the probable error, 0·02 Å, the true distance would be nearly normal. A possible explanation for this lengthening may lie in the weakness of the hydrogen bonds formed by the amide group.

Except for the two angles C_5–C_2–C_1 and C_5–C_2–N_1, the angles between covalently bonded atoms are in good agreement with previously observed values. In fact, the angles along the glycylglycine chain are closely similar to the angles reported for β-glycylglycine by Hughes & Moore (1949). It may be significant that the two angles which deviate appreciably from the normal are both angles around C_2, where the disulfide bridge meets the peptide chain. There seem to be no unusually close van der Waals distances which would bend out the C_2–C_1 and C_2–N_1 bonds. Indeed, if these angles were more nearly tetrahedral the long $O_3 \cdots O_4$ hydrogen bond distance would be shortened markedly.

Another interesting feature of the diglycine chains in this structure is the slight bend in the amide group, which corresponds to a rotation of 6° about the N_1–C_3 bond. Assuming that there exists a complete equivalence between double- and single-bonded structures in the amide group, this rotation introduces a strain energy of 0·4 kcal./mole. However, the actual strain energy may be considerably less than this, since complete resonance in the amide group would be favored by the formation of a strong hydrogen bond by the group, a condition obviously not fulfilled in this case. The long C_3–N_1 distance and the short C_3–O_3 distance are also in agreement with the concept of reduced resonance in the amide group.

Other important angles in the glycylglycine chain are the dihedral angle between the planes of the amide group and the carboxyl group, which is 19±2°, and the angle made by the C_4–N_2 bond and the amide plane, which is 15±2°.

Except for the S–C_5 bond already mentioned, the dimensions of the disulfide bridge are not unusual. The C–S–S–C dihedral angle is 101±2°, about 19° greater than the S–S–S–C angle in diiodo-diethyl-trisulfide (Donohue, 1950) and very near the value found in S_8 (Warren & Burwell, 1935). The C_5–C_2 bond makes an angle of 124±3° with the amide plane. The separation produced by the bridge between the two glycylglycine arms af the molecule is about 6·3 Å.

The hydrogen bonds formed by the amino group of the peptide molecule are all normal in that their lengths are of the same order of magnitude as those found in other peptide and amino acid structures. The C_4–$N_2 \cdots X$ angles differ from tetrahedral by as much as 25°, but this is not unexpected (Donohue, 1952). A zwitterion form for the amino group is clearly indicated by the disposition of hydrogen bonds about N_2.

The hydrogen bonds formed by the amide group and water molecule are not normal, however. The N_1–$H \cdots O_3'$ bond in particular is so long (3·31 Å) that the presence of an attractive force between the atoms involved may be questioned. Indeed, if the parameters of the hydrogen atom attached to N_1 are presumed to be correct, the hydrogen–oxygen separation in this bond is 2·53 Å, only slightly lower than the usual van der Waals separation of 2·60 Å. The reason for this abnormal distance is to be found in the van der Waals repulsion of the molecules forming the bond. As shown in Fig. 5, the distance between the sulfur atom of one molecule and the C_5 methylene group of the other is 3·65 Å. The sum of the van der Waals radii for these atoms given by Pauling (1940) is 3·85 Å. The observed value indicates that the true van der Waals radius of sulfur should be less than Pauling's value by 0·2 Å, in agreement with Donohue's work (Donohue, 1950). It must be assumed that the S–C_5 repulsive energy is counterbalanced by the attractive energy of the hydrogen bond at these distances but that on closer approach of the molecules the repulsion would become so great as to lead to an energetically unstable situation. The effects of the abnormal N_1–$H \cdots O_3$ distance on the N_1–C_3 and C_3–O_3 bond lengths and on the planarity of the amide group have already been noted.

The O_4–$H \cdots O_3$ bond is also unusual since it is about 0·4–0·5 Å longer than the oxygen–oxygen hydrogen bonds reported previously. The fact that the O_4–$H \cdots O_4'$ bond is also slightly longer suggests that in this case the oxygen atom of the water molecule prefers to form two relatively weak hydrogen bonds rather than the one strong bond it might form by moving nearer either the carbonyl oxygen of the amide group or another water molecule.

Table 5. *Some van der Waals distances in N,N′-diglycyl-*L*-cystine dihydrate*

(See Figs. 4 and 5 for numbering scheme. Letters in parentheses following atom designation indicate the particular molecule in which the atom is located. Primed and unprimed atoms of the same molecule are related by a twofold axis, or, in the case of the water molecules, by a twofold screw axis.)

Atoms	Distance (Å)
$S(A)$–$C_5(A^1)$	3·65
$O_2(A)$–$C_2(A^1)$	3·24
$O_2(A)$–$C_4(E)$	3·32
$O_3(A)$–$C_4(A_1)$	2·92
O_4–$O_1(A)$	3·19
O_4–$N_2(M)$	3·28

The packing of the molecules in the lattice is reasonably clear from Figs. 4 and 5. As is usual in peptide and amino acid structures, hydrogen bonding is the dominant factor in controlling packing. Parallel to the *b* axis the molecules are held together by strong hydrogen bonds from amino groups to carboxyl groups and weaker bonds involving amide groups and water molecules. Amino–carboxyl hydrogen bonds also firmly connect the molecules in the *a* and *c* directions. A representative list of van der Waals contacts is given in Table 5. It is apparent that the only distances significantly lower than the normal values are those between molecules related by the *b*-axis repeat. This is to be expected, however, since the molecules so related are trying to approach as close as possible to strengthen the $N_1 \cdots O_3$ hydrogen bond.

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