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A Neutron Diffraction Study of Perdeutero-α-glycylglycine

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A neutron diffraction study of perdeuterated α-glycylglycine has confirmed the dimensions, conformations and relative positions of the peptide molecules found by other workers in a recent X-ray structure analysis of the hydrogenic compound. The unit-cell dimensions of perdeutero-α-glycylglycine are $a=9{\cdot}425$, $b=9{\cdot}559$, $c=7{\cdot}827$ Å, $\beta=124{\cdot}85°$ for space-group $P2_1/c$. The deuterium atoms have been located. The C(methylene)–D, N(amino)–D and N(peptide)–D bonds have mean lengths 1·085, 1·03 and 1·02 Å. All four crystallographically independent hydrogen (^{2}H) bonds are non-linear, the N–D···O angles ranging from 148 to 163°. The peptide proton and C_α atom lie +0·145 Å and −0·066 Å respectively from the C_α'–CO–N plane.

Introduction

The X-ray structure analysis of α-glycylglycine has recently been reported (Biswas, Hughes, Sharma & Wilson, 1968; Hughes, 1968). The results of the X-ray analysis were made available to us prior to publication, and references to them throughout this paper are indicated by 'BHSW'. So far as we are aware, the present study is the first in which three-dimensional neutron diffraction data have been used to provide precise information on the locations of the hydrogen atoms in a peptide structure. The neutron diffraction data were recorded for perdeuterated rather than hydrogenic α-glycylglycine in order to avoid the problems associated with incoherent neutron scattering by ^{1}H. The symbol D will be used for deuterium except when it is wished to contrast ^{1}H and ^{2}H.)

Preparation of perdeuterated peptide crystals

The compound $NH_2CD_2CONHCD_2COOH$ was synthesized by a standard route (Greenstein & Winitz, 1961; Goldschmidt, 1950) from commercially available NH_2CD_2COOH of 98% isotopic purity. The perdeuterated dipeptide $ND_2CD_2CONDCD_2COOD$ was then obtained by repeated recrystallization from D_2O of 99·79% isotopic purity, each stage being followed by careful evaporation *in vacuo*. The extent of replacement of ^{1}H by ^{2}H for each group in the product was determined by comparing the infrared absorptions of the deuterated and hydrogenic compounds in KBr pellets. Beer's Law calculations showed that the extent of deuteration was 94% for the amino, 95% for the amide and 92% for the methylene groups, respectively. The analytical figures were between 3 and 5% lower than those subsequently computed by treating the site occupancies as variables in the structure refinement. A discussion of this difference is given later.

Large crystals of perdeutero-α-glycylglycine were difficult to grow. The compound was dissolved in D_2O. The solution was slowly evaporated at 20 °C and under reduced pressure, until crystallization commenced. The temperature of the solution was then gradually raised. When it reached 66 °C, all the solid had just dissolved. A seed, stuck to the end of a glass fibre, was introduced. By cooling the saturated solution to 20 °C under reduced pressure during a 24-hour period, a single crystal measuring 3·4 × 3·5 × 0·8 mm and weighing 15·6 mg was grown. The major crystal form was {100}, the next most prominent form being {001}.

Experimental

The unit-cell data are shown in Table 1. The Bragg angles of thirteen reflexions were measured by Bond's (1960) method on a modified Siemens diffractometer (Mayer & Walker, 1963), using Cr *Kα* X-radiation. A linear least-squares program was used to fit the unit-cell dimensions to the thirteen values of *θ*. The errors introduced by neglecting the Lorentz–polarization and refractive index corrections (Bond, 1960) were less than the standard deviations of the unit-cell parameters found by the least-squares calculation. The unit-cells of α-glycylglycine and perdeutero-α-glycylglycine differ by about 0·02% in *a* and *c* (Table 1). The apparently larger discrepancies reported by BHSW have been removed as the result of new X-ray measurements in this laboratory (see Hughes, 1968).

The neutron intensity data were measured on a three-circle single-crystal diffractometer at the HIFAR reactor. (HIFAR is a reactor at the Australian Atomic Energy Commission establishment at Lucas Heights,

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Table 1. *Comparison between unit-cells of α-glycylglycine and perdeutero-α-glycylglycine*

$C_4H_8N_2O_3$	$C_4D_8N_2O_3$
F.W. 132·1	140·2
Monoclinic	
$a = 7{\cdot}812 \pm 0{\cdot}0024$	$a = 9{\cdot}4251 \pm 0{\cdot}0002$ Å
$b = 9{\cdot}566 \pm 0{\cdot}0030$	$b = 9{\cdot}5586 \pm 0{\cdot}0006$
$c = 9{\cdot}410 \pm 0{\cdot}0029$	$c = 7{\cdot}8271 \pm 0{\cdot}0003$
$\beta = 124{\cdot}60 \pm 0{\cdot}017°$	$\beta = 124{\cdot}853 \pm 0{\cdot}002°$
$Z = 4$	4
$D_x = 1{\cdot}512$	1·606 g.cm^{-3}
Space group $P2_1/a$	$P2_1/c$

New South Wales.) The neutron wavelength was 1·09 Å with a 0·5% second-order contamination. The flux at the specimen was approximately 6×10^6 neutrons cm^{-2}sec^{-1}. The diffractometer was monitor-controlled and used in the θ–2θ mode, step-scanning through 5° in 2θ for each reflexion. Predicted neutron scattering intensities (computed from BHSW's structure with the addition of deuterium atoms at reasonable sites) were used to vary the monitor counts and step sizes in such a way as to optimize the statistics for the intensity measurements within the total time available for the experiment. For instance, reflexions with low predicted intensities were recorded with a large monitor count and small scanning steps over a period of half an hour per reflexion. Strong reflexions were recorded in 5′ steps at a rate of about seven per hour.

All 1710 independent reflexions with sin θ smaller than 0·77 were recorded. The crystal was shown to be free of severe extinction effects by measuring the intensity of the fairly strong reflexion 0,12,0 for different path lengths through the crystal. Corrections for incoherent scattering and absorption were negligible. An absolute scale was determined instrumentally by measuring the intensity of the 200 reflexion from a standard KBr crystal, which had been shown to be free from extinction and whose thermal parameters were known (Dr A. Pryor, personal communication).

Analysis of the data

The estimated standard deviation $\sigma(I)$ of an observed integrated intensity I with background B was calculated from

$$\sigma^2(I) = I + B + (\beta I)^2.$$

The first two terms arise from the counting statistics, and the third expresses the observer's confidence in the data. Additional terms to increase the standard deviations of very weak and of extinction-affected reflexions (Craven & Sabine, 1966) were not used. Repeated observations of several reflexions showed that a value of 0·03 for β was appropriate.

The smallest peaks which could be distinguished visually on chart-recorder traces of the counting rate *versus* θ had integrated intensities equal to approximately 0·05 times the background. The reflexions recorded as observable therefore included some whose intensities were considerably lower than $3\sigma(B) = 3B^{1/2}$, which is frequently regarded as the threshold of observability. Unobservably weak reflexions were assigned intensities equal to one-third of the minimum observable intensity in their own data-group (Hamilton, 1955). They were arbitrarily given variances

$$\sigma^2(I_{\text{unobs}}) = 8B$$

[instead of $4I^2_{\text{min}}/45 \simeq 4(0{\cdot}05B)^2/45 \simeq B^2/4500$ required by Hamilton's expression (*q.v.*)] so as to reduce their weights in the subsequent refinement.

The adequacy of these expressions for the standard deviations was later indicated by the small difference between the unweighted and weighted residuals, and by the reasonable agreement between the actual and statistically expected distributions of the structure-factor discrepancies (see *Refinement*, below).

Refinement of the structure

A Fourier synthesis of neutron scattering density was computed with coefficients whose signs were based on BHSW's coordinates for the oxygen, nitrogen and carbon atoms. Six deuterium atoms appeared in the map, and the remaining two were located in a second cycle of computation. The structure factors at this stage had a residual $R = 0{\cdot}364$ and a weighted residual $R' = 0{\cdot}396$.

The structure was refined by full-matrix least-squares in seventeen cycles. The function minimized was $\sum w(|F_{\text{obs}}| - |F_{\text{calc}}|)^2$ for 1684 structure-factors (of which 156 were from unobservably weak reflexions). This number excluded several reflexions affected by extinction. Scattering lengths for all atoms were taken from Bacon (1962). In addition to the atomic positional and thermal parameters, the site occupancies of the deuterium atoms (effectively the D/H scattering lengths) and the scale-factor were treated as refinement variables.

At the end of the isotropic refinement, the residuals R and R' were 0·195 and 0·211, respectively. These values decreased to 0·096 and 0·110 during the first cycle with anisotropic Debye factors, and to 0·066 and 0·068 (0·060 and 0·064 for observable reflexions only) during the subsequent refinement.

The slow convergence of the refinement is attributable to strong correlations (i) between the scale factor, the thermal parameters and the deuterium site-occupancy factors during the refinement with isotropic Debye factors, and subsequently (ii) between the deuterium site-occupancy factors and the anisotropic Debye parameters for the principal axes of the vibration ellipsoids (0·45), between the x and z coordinates of all atoms (0·65), and between the anisotropic Debye parameters involving the x and ith axes and the ith and z axes (0·7). These high correlations did not, however, affect the stability of the parameters during the final refinement cycles. The final shifts in all parameters were

smaller than half a standard deviation. Among the final 1684 structure factors, there were

16 with $||F_o|-|F_c||\geq 3$ s.d.'s
(0·95%; statistical value 0·2%)

47 with 3 s.d.'s $\geq ||F_o|-|F_c||\geq 2$ s.d.'s
(2·8%; statistical value 4·3%)

325 with 2 s.d.'s $\geq ||F_o|-|F_c||\geq 1$ s.d.
(19·3%; statistical value 27·1%)

The final positional and vibrational atomic parameters are listed in Table 2, and the observed and calculated structure amplitudes in Table 3. A stereoscopic view of one molecule is shown in Fig. 1.

Discussion

Intramolecular bond distances and angles

The intramolecular bond-distances and bond-angles in perdeutero-α-glycylglycine are listed in Table 4(*a*)

Table 2. *Final atomic parameters in perdeutero-α-glycylglycine*

(*a*)Fractional positional coordinates and their standard deviations (in parentheses), all $\times 10^4$

E.s.d. $=[(\sigma_x^2a^2+\sigma_y^2b^2+\sigma_z^2c^2)/3]^{1/2}$.

	$x(\sigma_x)$	$y(\sigma_y)$	$z(\sigma_z)$	E.s.d.
C(1)	3430 (2)	0951 (2)	2401 (3)	0·0020 Å
C(2)	4854 (2)	1705 (2)	2423 (3)	0·0020
C(3)	7659 (2)	1552 (2)	2996 (3)	0·0020
C(4)	8617 (2)	0538 (2)	2503 (3)	0·0020
N(1)	1779 (2)	1694 (1)	1011 (2)	0·0015
N(2)	6179 (2)	0934 (1)	2816 (2)	0·0015
O(1)	4788 (3)	2989 (2)	2178 (4)	0·0027
O(2)	8220 (3)	−0736 (2)	2313 (4)	0·0027
O(3)	9736 (3)	1069 (3)	2335 (5)	0·0032
D(1)	1925 (3)	2744 (2)	1343 (4)	0·0027
D(2)	0879 (3)	1336 (2)	1270 (3)	0·0024
D(3)	1366 (3)	1529 (3)	−0511 (3)	0·0027
D(4)	3744 (3)	0984 (3)	3963 (4)	0·0029
D(5)	3299 (3)	−0126 (2)	1894 (5)	0·0030
D(6)	6101 (3)	−0132 (2)	2807 (4)	0·0027
D(7)	8565 (4)	1949 (4)	4567 (6)	0·0041
D(8)	7244 (4)	2436 (3)	1935 (7)	0·0042

(*b*) Anisotropic vibrational parameters and (in parentheses) their standard deviations, $\times 10^4$

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

	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
C(1)	73 (2)	37 (2)	145 (4)	5 (1)	79 (3)	12 (3)
C(2)	64 (2)	26 (1)	143 (4)	2 (1)	70 (2)	0 (2)
C(3)	85 (3)	36 (2)	248 (5)	−8 (2)	114 (3)	20 (2)
C(4)	70 (2)	38 (1)	130 (3)	2 (1)	74 (2)	6 (2)
N(1)	70 (2)	39 (1)	155 (3)	0 (1)	79 (2)	5 (1)
N(2)	75 (2)	31 (1)	198 (3)	1 (1)	91 (2)	0 (1)
O(1)	105 (3)	23 (2)	298 (7)	3 (2)	132 (4)	3 (3)
O(2)	126 (4)	33 (2)	196 (5)	5 (2)	126 (4)	1 (2)
O(3)	124 (4)	80 (3)	295 (7)	−12 (3)	166 (5)	0 (3)
D(1)	112 (4)	40 (2)	258 (7)	6 (2)	108 (4)	2 (3)
D(2)	85 (3)	65 (2)	235 (6)	11 (2)	108 (4)	5 (3)
D(3)	112 (4)	90 (3)	159 (5)	3 (2)	82 (4)	5 (3)
D(4)	133 (4)	141 (4)	172 (6)	10 (3)	106 (4)	44 (4)
D(5)	134 (4)	38 (2)	363 (9)	1 (2)	158 (5)	7 (3)
D(6)	108 (4)	32 (2)	285 (7)	7 (2)	126 (4)	7 (3)
D(7)	162 (6)	173 (5)	386 (11)	−91 (4)	185 (7)	−187 (6)
D(8)	194 (6)	63 (3)	611 (6)	34 (3)	277 (8)	91 (5)

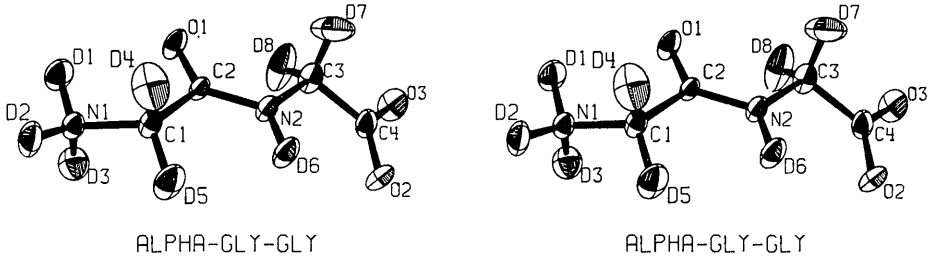
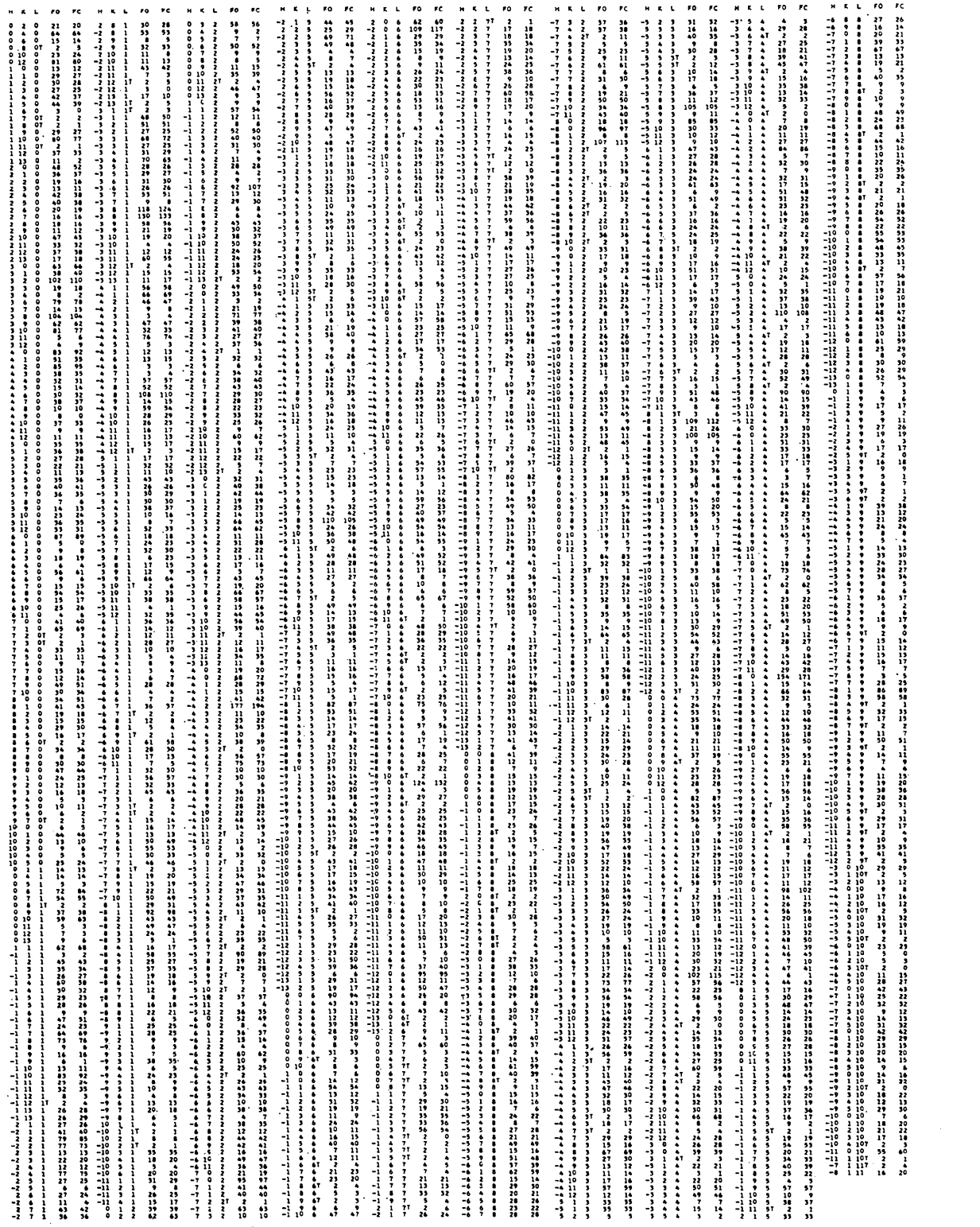


Fig. 1. Stereoscopic drawing of α-glycylglycine, showing thermal vibrational ellipsoids calculated from neutron diffraction data.

Table 3. *Observed and calculated neutron structure amplitudes* ($\times 10$) *for α-glycylglycine*

Unobservably weak reflexions are denoted by the symbol T.



and (*c*) respectively. Due to the high correlations between the anisotropic thermal and other refinement parameters, dimensions corrected for thermal motion are not reported here. The bond-lengths and angles do not differ significantly from those in the ^{1}H-containing compound (BHSW), which are listed for comparison. The C(1)–D and C(3)–D bond-lengths in the two methylene groups differ by scarcely significant amounts. The mean value of 1·085 ± 0·004 Å represents the most valid estimate of the methylene C–D bond-lengths in this dipeptide.

The three N(1)–D bonds of the $-ND_3^+$ group are systematically longer (mean = 1·032 ± 0·005 Å) than the N(2)–D bond of the peptide group (1·021 Å). The dif-

Table 4. *Dimensions of perdeutero-α-glycylglycine, compared with corresponding dimensions of α-glycylglycine* (BHSW)

Code for symmetry-related atoms

Superscript	atom at			Superscript	atom at		
–	x	y	z	iii	$1-x$	$\bar{y}$	$\bar{z}$
i	$-1+x$	y	z	iv	$1-x$	$\frac{1}{2}+y$	$\frac{1}{2}-z$
ii	$1+x$	y	z	v	$1-x$	$-\frac{1}{2}+y$	$\frac{1}{2}-z$

(*a*) Intramolecular bond-lengths (*l*)

Bond	l	$\sigma(l)$	l(BHSW)
N(1)–C(1)	1·474 Å	0·003 Å	1·491 Å
C(1)–C(2)	1·515	0·003	1·519
C(2)–O(1)	1·238	0·004	1·249
C(2)–N(2)	1·326	0·003	1·319
N(2)–C(3)	1·446	0·003	1·451
C(3)–C(4)	1·518	0·003	1·514
C(4)–O(2)	1·257	0·004	1·260
C(4)–O(3)	1·243	0·004	1·232
N(1)–D(1)	1·027	0·003	
N(1)–D(2)	1·037	0·003	
N(1)–D(3)	1·032	0·003	
C(1)–D(4)	1·081	0·004	
C(1)–D(5)	1·084	0·004	
N(2)–D(6)	1·021	0·003	
C(3)–D(7)	1·088	0·004	
C(3)–D(8)	1·089	0·004	

(*b*) Hydrogen (^{2}H) bonds

Bond (N–D···O)	Equivalent bond	d_{N-D}	$d_{D\cdots O}$	$d_{N\cdots O}$	$d_{N\cdots O}$ (BHSW)
N(1)–D(1)···O(2^{iv})	O(2)···D(1^{v})–N(1^{v})	1·027 Å	1·844 Å	2·785 Å	2·790 Å
N(1)–D(2)···O(3^{i})	O(3)···D(2^{ii})–N(1^{ii})	1·037	1·716	2·724	2·728
N(1)–D(3)···O(2^{iii})	O(2)···D(3^{iii})–N(1^{iii})	1·032	1·829	2·758	2·747
N(2)–D(6)···O(1^{v})	O(1)···D(6^{iv})–N(2^{iv})	1·021	1·958	2·959	2·963

(*c*) Bond-angles *not* involving hydrogen (^{2}H) bonds

Angle	θ	$\sigma(\theta)$	θ(BHSW)
N(1)–C(1)–C(2)	109·5°	0·2°	109·5°
–D(4)	108·2	0·3	–
–D(5)	109·6	0·3	–
C(2)–C(1)–D(4)	107·7	0·3	–
–D(5)	111·8	0·3	–
D(4)–C(1)–D(5)	109·9	0·4	–
C(1)–C(2)–O(1)	120·3	0·2	120·4
–N(2)	116·7	0·2	116·3
O(1)–C(2)–N(2)	123·0	0·2	123·3
C(2)–N(2)–C(3)	121·6	0·2	121·2
N(2)–C(3)–C(4)	113·3	0·2	112·4
–D(7)	110·0	0·3	–
–D(8)	109·2	0·3	–
C(4)–C(3)–D(7)	108·2	0·3	–
–D(8)	108·0	0·3	–
D(7)–C(3)–D(8)	107·4	0·4	–
C(3)–C(4)–O(2)	118·0	0·2	117·9
–O(3)	115·4	0·2	115·5
O(2)–C(4)–O(3)	126·6	0·3	126·7

Table 4 (*cont.*)

(*d*) Bond-angles involving hydrogen (^{2}H) bonds

Angle X–N–D		Angle X–N···O	
C(1)–N(1)–D(1)	110·6°	C(1) ——N(1)···O(2^{iv})	111·2°
–D(2)	109·5	···O(3^{i})	106·9
–D(3)	108·7	···O(2^{iii})	88·1
D(1)–N(1)–D(2)	106·2	O(2^{iv})···N(1)···O(3^{i})	79·9
–D(3)	110·4	···O(2^{iii})	137·5
D(2)–N(1)–D(3)	111·4	O(3^{i}) ···N(1)···O(2^{iii})	131·9
C(2)–N(2)–D(6)	120·0	C(2) ——N(2)···O(1^{v})	106·2
C(3)–N(2)–D(6)	118·0	C(3) ——N(2)···O(1^{v})	132·0
Angle X–O···D or D···O···D		**Angle X–O···N or N···O···N**	
C(2)——O(1)···D(6^{iv})	151·6°	C(2)——O(1)···N(2^{iv})	158·5°
C(4)——O(2)···D(3^{iii})	108·2	C(4)——O(2)···N(1^{iii})	105·8
···D(1^{v})	144·0	···N(1^{v})	151·5
D(3^{iii})···O(2)···D(1^{v})	103·4	N(1^{iii})···O(2)···N(1^{v})	98·7
C(4)——O(3)···D(2^{ii})	156·6	C(4)——O(3)···N(1^{ii})	163·0
Angle N–D···O		**Angle D–N···O or D···O···N**	
N(1)–D(1)···O(2^{iv})	150·7°	D(1)——N(1) ····O(2^{iv})	18·9°
N(1)–D(2)···O(3^{i})	162·8	D(2)——N(1) ····O(3^{i})	10·8
N(1)–D(3)···O(2^{iii})	147·9	D(3)——N(1) ····O(2^{iii})	20·6
N(2)–D(6)···O(1^{v})	158·7	D(6)——N(2) ····O(1^{v})	14·1
		D(1)···O(2^{iv})···N(1)	10·4
		D(2)···O(3^{i})····N(1)	6·5
		D(3)···O(2^{iii})···N(1)	11·5
		D(6)···O(1^{v}) ···N(2)	7·2

ference is consistent with the relative covalent radii of tetrahedrally and trigonally bonded nitrogen atoms (Hahn, 1957; see also Pauling, 1960).

Hydrogen-bonding and intermolecular contacts

BHSW have already described the arrangement and hydrogen bonding of the molecules, and have compared various dimensional and structural features of α-glycylglycine with the corresponding results of an earlier structure analysis of β-glycylglycine. The neutron structure analysis complements BHSW's description by providing details of the involvement of the hydrogen atoms in intermolecular contacts. The hydrogen (^{2}H) bonds are listed in Table 4(*b*), and the bond angles at the donor, acceptor and ^{2}H atoms in Table 4(*d*).

As noted by BHSW, the longest N···O distance, and hence the weakest interaction, occur in the N(peptide)···O(peptide) bond. This bond also has the shortest N–D component. There is no obvious correlation between the three N(1)–D bond-lengths and the corresponding N(amino)···O distances. The angles between the C–N and N–D bonds at the terminal N(amino) atom are close to tetrahedral (106–111°), and at the N(peptide) atom are 120±2°. The actual geometry at the proton donor atoms is therefore much more regular than would be expected from the angles involving the N···O vectors [80–138° at N(1), 106–132° at N(2)]. This implies that the hydrogen (^{2}H) bonds are non-linear. The N–D···O angles range from 148 to 163°, and the N–D bonds lie up to 21° away from their N···O vectors. At the acceptor oxygen atoms, the C–O···N angles have values from 106 to 163°, and the C–O···D angles from 108 to 157°.

A comparison of the N···O bond lengths in the deuterated and hydrogenic crystals [Table 4(*b*)] shows that corresponding bond lengths differ by a 'possibly significant' amount in only one instance, N(1)···O(2^{iii}). There the difference is 0·011 Å. The N(1)···O(2) bonds are almost parallel to the *z* axis, so that two of them in series more than account for the 0·2% increase in *c* on deuteration. The similar increase in *a* must be connected with minor changes in packing and non-bonded contacts. For instance, while N(2)···O(1) remains constant, the angle N(2)···O(1)–C(2) is 157·2° in hydrogenic, but 158·5° in deuterated, α-glycylglycine. The present evidence fails to support the suggestion by Tomita, Rich, de Lozé & Blout (1962), based on fibre diffraction patterns of normal and deuterated polypeptides, that deuteration causes an increase of about 0·025 Å in inter-peptide N–H···O bond-lengths but no significant change in the angular relationships in the hydrogen bonds.

A systematic search of interatomic vectors shows that all non-bonded contacts between adjacent molecules are longer than the van der Waals distances proposed by Leach, Nemethy & Scheraga (1966). The shortest D···D contact is 2·43 Å [between D(2) and the D(7) at $(-1+x, \frac{1}{2}-y, -\frac{1}{2}+z)$], and the shortest contact between a D and a non-hydrogen atom is 2·52 Å [between D(3) and the C(4) at $(\bar{x}, \bar{y}, \bar{z})$]. The three shortest intramolecular contacts between D atoms not bonded to the same atom are all between 2·31 and 2·33 Å [D(2)···D(4), D(3)···D(5), D(5)···D(6)].

These values suggest an effective van der Waals radius of 1·15 Å for deuterium.

Planarity of the peptide group

Projections along the bonds of the backbone of the molecule are shown in Fig. 2. As noted by BHSW, the α-glycylglycine zwitterion is not planar: the N(amino) atom, N(1), alone lies 0·6 to 0·7 Å from planes fitted to various combinations of atoms in the peptide group (Table 5). Even when allowance is made for the underestimation of the e.s.d.'s of the atomic positions and for the uncertainties in the equations of the least-squares planes in Table 5, it is clear that only two groups of atoms are planar – the carbonyl group C(1)C(2)O(1)N(2) and the carboxyl group C(3)C(4)O(2)O(3) (planes 1 and 6). The configuration of the bonds about the N(peptide) atom, N(2), is flattened tetrahedral (planes 4 and 5). As a result, the atoms normally thought of as constituting the peptide group, C(1)C(2)O(1)N(2)D(6)C(3), cannot be described

Table 5. *Planes of best fit in perdeutero-α-glycylglycine*

Each plane is represented by $lx+mY+nZ+p=0$, where X, Y, Z are orthogonal coordinates in Å obtained from the fractional coordinates x, y, z by the transformations $X=ax+cz\cos\beta$, $Y=by$, $Z=cz\sin\beta$. Deviations from these planes are enclosed in brackets for atoms which were not included in the least-squares calculation.

Plane	1	2	3	4	5	6
			Least-squares coefficients			
l	0·1077	0·0875	0·1195	0·1434	0·1483	−0·1563
m	−0·1494	−0·1150	−0·1042	−0·1272	−0·0554	0·1369
n	−0·9829	−0·9895	−0·9874	−0·9815	−0·9874	−0·9782
p	1·4144	1·4274	1·3035	1·2343	1·1215	2·5979
			Deviations from planes of best fit (in Å)			
N(1)	(0·666)	(0·706)	(0·640)	—	—	—
C(1)	−0·005	−0·014	−0·056	—	—	—
C(2)	0·017	0·005	0·014	0	0·012	—
O(1)	−0·006	0·024	0·048	—	—	—
N(2)	−0·006	−0·066	−0·030	0	−0·037	—
D(6)	(0·145)	0·051	0·074	(0·126)	0·015	—
C(3)	(−0·066)	(−0·133)	−0·049	0	0·011	$-0{\cdot}000_3$
C(4)	—	—	—	—	—	0·001
O(2)	—	—	—	—	—	$-0{\cdot}000_4$
O(3)	—	—	—	—	—	$-0{\cdot}000_4$
$N(2^{iv})$	(−0·529)	(−0·383)	(−0·359)	—	—	—
$O(1^{v})$	(0·313)	(0·174)	(0·150)	—	—	—

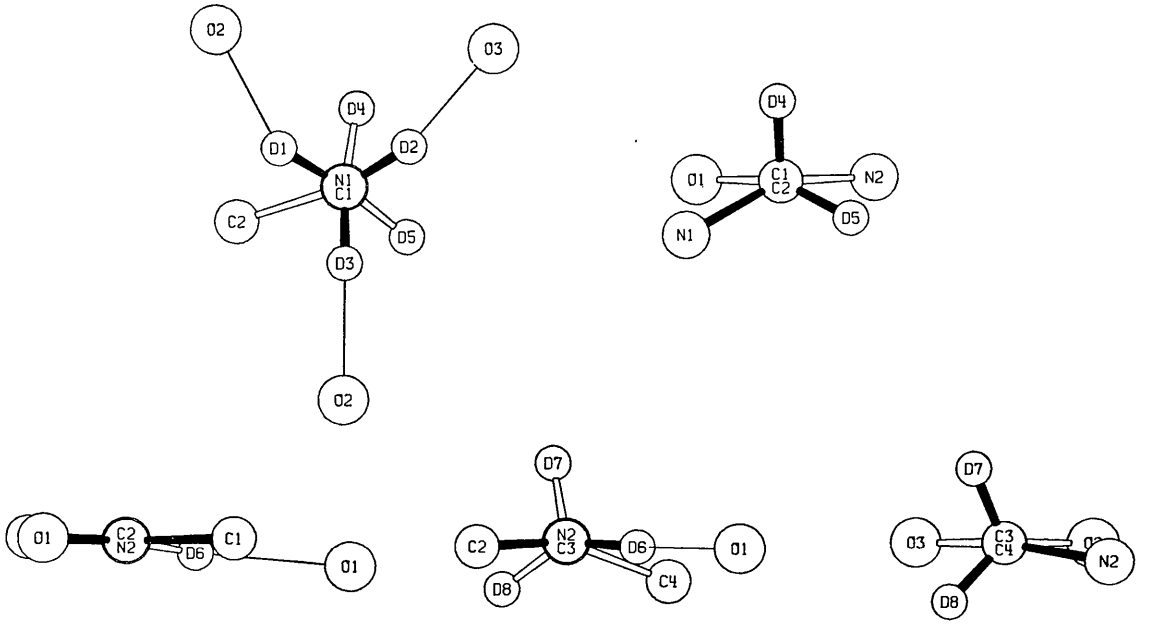


Fig. 2. Projections along bonds of α-glycylglycine. The bond along which the structure is viewed is represented by the two symbols in the central atom of each diagram, the top symbol representing the atom closer to the observer.

as coplanar, whether D(6) and C(3) are included or not (planes 2 and 3.)

It is, however, convenient to adopt the approximation that the peptide group is planar in order to be able to describe the molecule by means of the conventional torsion angles ψ and φ (Edsall, Flory, Kendrew, Liquori, Nemethy, Ramachandran & Scheraga, 1966). The angles ψ and φ are then the angles between the plane of the peptide group and the planes of N(1)C(1)C(2) and N(2)C(3)C(4) respectively. We have also calculated torsion angles ψ_1 (between the planes of N(2)C(3)C(4) and the carboxyl group C(3)C(4)O(2)O(3)] and φ_i [between the planes of D(i)N(1)C(1) and N(1)C(1)C(2)]. These angles are:

$$\psi = 31{\cdot}3°, \qquad \varphi = 23{\cdot}5°,$$
$$\psi_1 = -10{\cdot}3, \qquad \varphi_1 = -49{\cdot}3 = 310{\cdot}7,$$
$$\varphi_2 = 194{\cdot}0, \qquad \varphi_3 = 72{\cdot}1.$$

Comparison with structure of glycylglycine hydrochloride

The structure of glycylglycine hydrochloride has recently been determined by X-ray diffraction (Parthasarathy, 1969). With two exceptions, the bond lengths and angles not involving ^{1}H atoms agree well with those in α-glycylglycine: (i) since the carboxyl group in the hydrochloride is protonated, its two C–O bond lengths are unequal; (ii) the angles which depend on the position of N(2) are significantly different in the two structures (the relevant values in the hydrochloride being C(1)–C(2)–N(2), 114·6°, O(1)–C(2)–N(2), 125·4° and C(2)–N(2)–C(3), 123° with s.d.'s = 0·4°).

The positions of the ^{1}H atoms in glycylglycine hydrochloride were located from the X-ray data. For reasons which have been discussed elsewhere (*e.g.* Hamilton & Ibers, 1968) they lead to average N–H and C–H bond lengths which are respectively 0·04 and 0·05 Å shorter than the lengths of corresponding bonds now found by neutron diffraction. The N(amino)–H and N(peptide)–H bond-lengths in glycylglycine hydrochloride, though determined to lower precisions than those in α-glycylglycine, differ in the same direction and by almost the same average amount (0·13 Å compared with 0·11 Å).

The most important difference between the two structures lies in the relative orientations of their carboxyl and peptide groups. This is shown by a comparison of the torsion angles φ (23·5° in α-glycylglycine, −80° in glycylglycine hydrochloride), which correspond to a twist of about 100° about the N(2)–C(3) bond. Thus the carboxyl and peptide groups are roughly coplanar in one structure and roughly perpendicular in the other. The remaining peptide torsion angles in the hydrochloride are similar to those in the present structure ($\psi = 20{\cdot}2$, $\psi_1 = 3{\cdot}6$, $\varphi_1 = 70{\cdot}8$, $\varphi_2 = 190{\cdot}1$, $\varphi_3 = 309{\cdot}0°$).

The deuterium scattering length

The value used in this work for the coherent scattering length of deuterium was $0{\cdot}65 \times 10^{-12}$ cm (Bacon, 1962). This value led to site occupancy factors 3–5% higher than those found by analysis. There is, however, a severe discrepancy between the published values of b_D. The Neutron Diffraction Commission of the I.U.Cr. (1969) recommends $0{\cdot}621 \times 10^{-12}$ cm. Coppens & Sabine (1969) have found $0{\cdot}66$–$0{\cdot}67 \times 10^{-12}$ cm to be more appropriate. Adoption of the latter figure decreases the discrepancy in the present work.

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