

Structure and Conformational Aspects of the Nitrates of Amino Acids and Peptides. I. Crystal Structure of Glycylglycine Nitrate*

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Glycylglycine nitrate, $\text{H}_3\text{N}^+-\text{CH}_2-\text{CO}-\text{NH}-\text{CH}_2-\text{COOH}\cdot\text{NO}_3^-$, crystals are monoclinic, with the following unit-cell parameters at $22 \pm 3^\circ\text{C}$: $a = 8.459$ (1), $b = 9.909$ (1), $c = 9.501$ (1) Å (Cu $K\alpha_1$, $\lambda = 1.54051$ Å) and $\beta = 90.46$ (1)°. The space group is $P2_1/c$, and there are four ion pairs in the unit cell. With a GE XRD-6 diffractometer, 1749 reflections were measured to the limit $2\theta = 160^\circ$. The complete structure was determined by the multi-solution technique and refined by using the least-squares method to an R of 0.044. Interesting structural features are: (a) the peptide plane and the plane of the carboxyl group are inclined at 16.5° ; (b) the values of the torsional angles ψ_1 , φ_2 , ψ_7^1 and ψ_7^2 are, respectively, 148.9 , 165.6 , -176.9 and 3.3° ; (c) the atoms defining the peptide group are nearly planar, the $\text{C}^\alpha-\text{C}-\text{N}-\text{C}^\alpha$ torsion angle ω having a value of 174.5° ; (d) the peptide molecules are in an extended conformation arranged in antiparallel sheets with an inter-sheet hydrogen-bonding distance of 3.12 Å; and (e) the nitrate group does not quite possess its classical D_{3h} symmetry. The three N–O bonds are of length 1.241 , 1.229 and 1.266 Å and the nitrogen atom of the nitrate group is 0.007 Å away from the plane through the oxygen atoms. A correlation was observed between the N–O bond distances and the hydrogen bonding of the oxygen atoms: the oxygen with the N–O bond of 1.229 Å does not take part in any hydrogen bonding while the oxygen with the N–O bond distance of 1.266 Å is bonded to the OH group in the peptide by a strong hydrogen bond of length 2.65 Å.

Introduction

Accurate crystal structure determinations of amino acids and simple peptides play an important role in indicating the preferred conformations of large peptides and proteins. Many peptides and amino acids have been studied either as free compounds or as hydrohalides. Our investigations on the nitrates of amino acids and peptides stem from the work of Rustgi & Box (1973), who measured the e.s.r. and e.n.d.o.r. spectra of several of these substances. They found that the nitrate ion acts as a mediator of radiation damage, scavenging electrons efficiently to form NO_3^{2-} free radical ions (private communication). In order to understand this process from the structural point of view and to examine the environment of the nitrate ions, crystal structure studies on several nitrates of amino acids and peptides were initiated.

Experimental

Good single crystals of glycylglycine nitrate (hereafter referred to as GGNO_3) were obtained by slow evaporation from heavy-water solutions of glycylglycine titrated against nitric acid in equimolar proportions. These crystals were used both for the present X-ray study by us and e.s.r. studies by Rustgi & Box (1973). The crystals obtained were large, clear and had well

formed faces. These large crystals were ground to small sizes suitable for diffraction studies. They are monoclinic and the systematically absent reflections are: $0k0$ with k odd; $h0l$ with l odd and no absences in hkl . These absences are consistent with the space group $P2_1/c$. The unit-cell parameters at $22 \pm 3^\circ\text{C}$ are: $a = 8.459$ (1), $b = 9.909$ (1), $c = 9.501$ (1) Å and $\beta = 90.46$ (1)°; $Z = 4$, Cu $K\alpha_1 = 1.54051$ Å, $\rho_{\text{obs}} = 1.67$, $\rho_{\text{calc}} = 1.63$ g cm $^{-3}$, $\mu = 13.61$ cm $^{-1}$. The unit-cell constants were obtained (and their e.s.d.'s estimated) from a least-squares refinement of 46 large-angle reflections.

Complete three-dimensional intensity data were obtained with Cu $K\alpha$ radiation. The stationary crystal-stationary counter technique with balanced Ross filters (Furnas & Harker, 1955) and a 5° take-off angle were used for obtaining the intensities. 1749 non-equivalent reflections were measured to the limit $2\theta = 160^\circ$ with Cu $K\alpha$ radiation, of which 1623 reflections, whose intensities were twice the background in that ($\sin \theta/\lambda$) range, were considered observable. The standard deviations of the intensities were obtained from counting statistics (Evans, 1961). The crystal of dimensions $0.45 \times 0.17 \times 0.30$ mm was mounted with the b^* axis along the φ axis of the goniostat. The difference in absorption as a function of φ was measured for the axial reflections and was used for correcting approximately for the anisotropy of absorption. No other absorption corrections were carried out and the data were processed in the usual way.

While measuring the anisotropy of absorption as a function of φ , it was discovered that for the 040 reflection, at $\varphi = 159.48$, 170.34 , 359.48° (with $\varphi = 0$ along the a^* axis), sharp and intense multiple reflections

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(Renninger, 1937) were observed. The intensity of the 040 reflection at these special settings of φ was found to be as high as five times the intensity at other φ settings. The φ scan of the intensity near these special φ settings exhibited a very asymmetric angular profile about the peak position. In addition, there were also five other φ settings where the intensity of the 040 reflection increased by a factor ranging from two to three. This phenomenon was observed not only for the 040 reflection but also for the systematically absent 010 reflection.

A separate investigation is in progress to sort out the interactions that produced these multiple reflections. It is possible that the intensities of other reflections like $h0l$ might also be affected by such multiple reflections, but we think that any error due to this cause must be small, because the structure refined very well and there are no significant differences between the $|F_o|$'s and $|F_c|$'s. Also the interatomic distances observed in this structure agree well with other studies on similar peptides.

Structure determination

The structure was determined by the multisolution technique of Germain, Main & Woolfson (1971). The reflections used to define the origin and the starting set are presented in Table 1 along with their E values.

Table 1. *Origin-fixing reflections, starting set and their E values*

1. Origin-fixing reflections			
<i>h</i>	<i>k</i>	<i>l</i>	<i>E</i>
5	8	1	4.25
0	6	7	3.51
5	1	$\bar{6}$	2.95
2. Starting set of reflections			
<i>h</i>	<i>k</i>	<i>l</i>	<i>E</i>
5	2	$\bar{6}$	3.67
2	9	$\bar{5}$	3.29
2	6	2	2.65
8	3	$\bar{2}$	2.60
5	5	$\bar{1}$	2.46

Table 2. *Final positional and thermal parameters*

Values are $\times 10^4$; standard deviations given in parentheses refer to the last digit.

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

	<i>x</i>	<i>y</i>	<i>z</i>	<i>b</i> ₁₁	<i>b</i> ₂₂	<i>b</i> ₃₃	<i>b</i> ₁₂	<i>b</i> ₁₃	<i>b</i> ₂₃
O(1)	4910 (2)	- 864 (1)	2542 (1)	156 (2)	54 (1)	141 (1)	6 (3)	145 (3)	5 (2)
O(2)	8693 (1)	1071 (1)	- 976 (1)	132 (2)	82 (1)	101 (2)	- 3 (3)	129 (3)	- 14 (2)
O(3)	7302 (2)	2759 (1)	- 49 (1)	156 (2)	63 (1)	92 (2)	10 (3)	82 (3)	- 6 (2)
O(4)	624 (2)	1031 (1)	6229 (2)	157 (2)	87 (2)	130 (2)	- 84 (3)	116 (3)	- 83 (3)
O(5)	2060 (2)	2793 (1)	5904 (1)	154 (2)	98 (2)	122 (2)	- 68 (3)	127 (3)	- 4 (3)
O(6)	201 (2)	2840 (1)	7442 (1)	150 (2)	89 (2)	95 (2)	31 (3)	107 (3)	- 57 (3)
N(1)	2400 (2)	371 (1)	3789 (1)	84 (2)	53 (1)	76 (2)	- 2 (3)	51 (3)	4 (2)
N(2)	5903 (2)	1128 (1)	1843 (1)	99 (2)	53 (1)	76 (2)	- 12 (3)	80 (3)	- 8 (2)
N(3)	971 (2)	2219 (1)	6507 (1)	87 (2)	70 (2)	64 (2)	- 10 (3)	29 (3)	- 7 (2)
C(1)	3897 (2)	1128 (2)	3623 (2)	88 (2)	60 (2)	67 (2)	- 14 (3)	42 (3)	- 10 (3)
C(2)	4946 (2)	367 (2)	2613 (2)	88 (2)	55 (2)	62 (2)	- 3 (3)	26 (3)	- 6 (3)
C(3)	7073 (2)	536 (2)	919 (2)	102 (2)	69 (2)	83 (2)	9 (3)	78 (3)	- 1 (3)
C(4)	7683 (2)	1586 (2)	- 74 (2)	85 (2)	66 (2)	64 (2)	- 9 (3)	34 (3)	- 17 (3)

Table 3. *Final positional coordinates ($\times 10^3$) and thermal parameters ($\times 10^2$) for hydrogen atoms*

	<i>x</i>	<i>y</i>	<i>z</i>	<i>B</i>
H(1)	165	58	299	621
H(2)	177	74	458	721
H(3)	252	- 43	387	593
H(4)	440	118	455	384
H(5)	361	198	328	276
H(6)	587	189	193	440
H(7)	800	11	150	392
H(8)	661	- 21	36	365
H(9)	924	169	- 150	737

Since five reflections were used in the starting set, there are $2^5 = 32$ possible combinations for starting phases, thus yielding 32 solutions. The figures of merit ranged in value from 1.1203 to 0.8291. The structure was derived from the second E map obtained by using solution 9 which had a figure of merit of 1.1202. This map clearly revealed the positions of all the 13 non-hydrogen atoms. A structure factor calculation for this trial structure yielded an R of 0.18.

Refinement of the structure

The coordinates and thermal parameters of the C, N and O atoms were refined by several cycles of least-squares calculations, employing a block-diagonal approximation. Blocks of 9×9 and 4×4 were employed for atoms with anisotropic and isotropic thermal parameters, respectively. Six cycles of refinement, with individual isotropic thermal parameters, reduced the R value to 0.16. This was followed by eight cycles of refinement, with individual anisotropic thermal parameters, reducing the R value to 0.08. An electron density difference map at this stage clearly revealed the locations of all the nine hydrogen atoms. Four cycles of refinement, in which the positional and individual isotropic thermal parameters of the hydrogen atoms were also allowed to vary, brought the R index to 0.044. The $(1/f_c)$ weighting scheme was used in the earlier stages of refinement and the $(1/\sigma)$ weighting scheme (Evans, 1961) was used in the final stages. The ade-

tors is given in Table 4. The refinement was carried out by minimizing $[\sum w(|F_{\text{obs}}| - (1/k)|F_{\text{calc}}|)^2]$. Reflections too weak to be observed were given zero weight during the refinement and for the R -index calculation. Atomic scattering factors for all non-hydrogen atoms were those in *International Tables for X-ray Crystallography* (1968). For the hydrogen atoms, the values given by Stewart, Davidson & Simpson (1965) were used.

The scattering factor of the deuterons for X-rays is

the same as that for hydrogens. Since the percentage of deuteration is not known, the deuterons were also treated as hydrogens for this X-ray study.

Discussion of the structure

The bond distances and angles in the molecule are shown in Figs. 1 and 2 respectively. The estimated standard deviations of these quantities are indicated in parentheses.

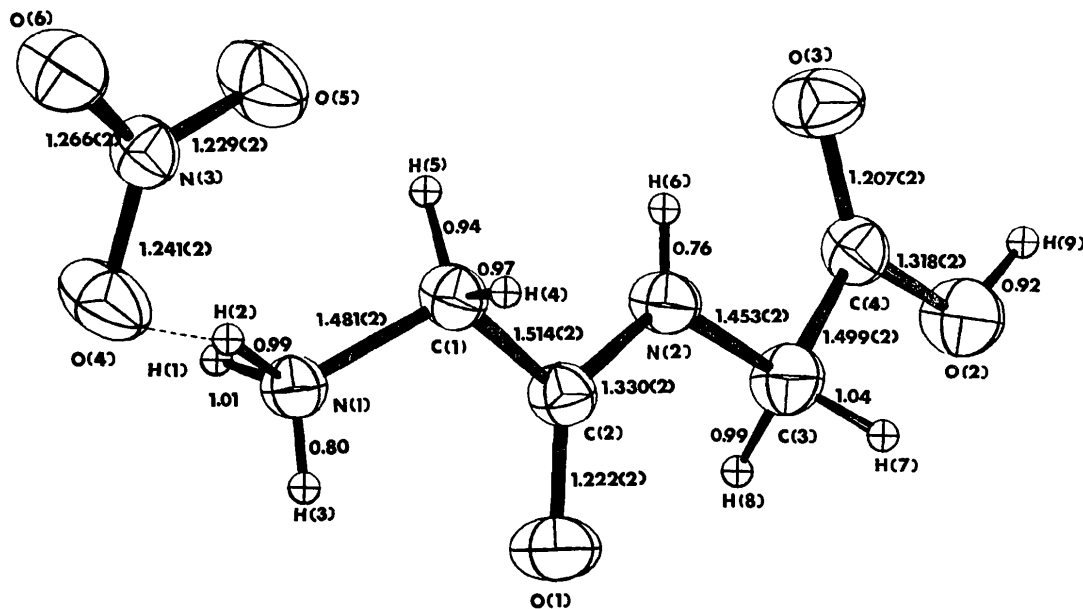


Fig. 1. Bond distances (Å). The e.s.d.'s given in parentheses refer to the last digit. The average e.s.d. for N-H and C-H distances is 0.02 Å.

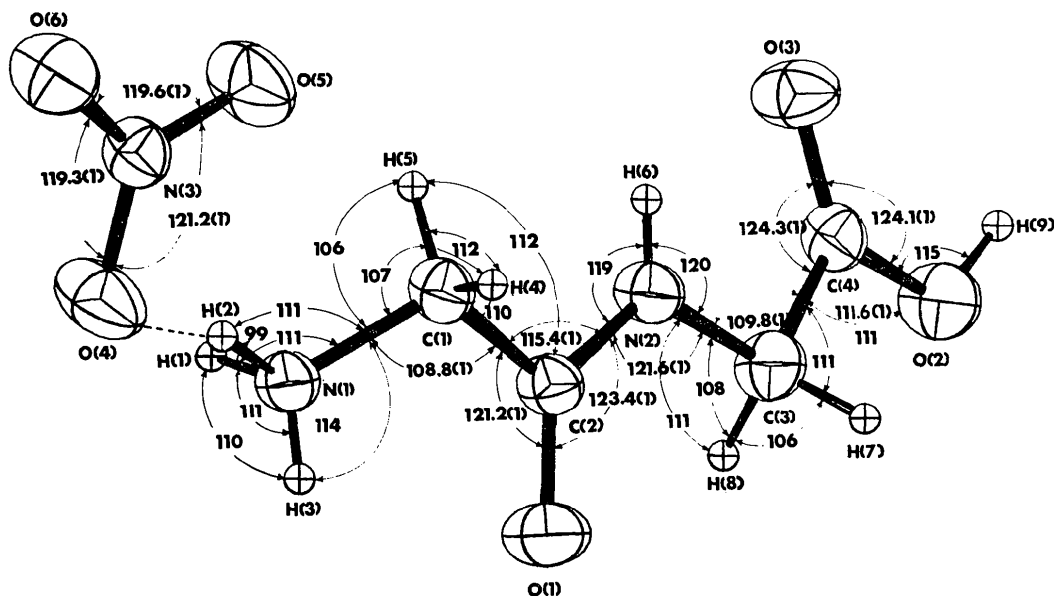


Fig. 2. Bond angles ($^{\circ}$). The e.s.d.'s given in parentheses refer to the last digit.

The nitrate group

It is found that in GGNO₃, the nitrate group does not have its classical D_{3h} symmetry. The N–O distances in the NO₃⁻ group are 1.241 (2), 1.229 (2) and 1.266 (2) Å. The N(3)–O(6) bond distance of 1.266 Å is long compared with the average value of 1.24 Å for the N–O bonds quoted in *International Tables for X-ray Crystallography* (1968). Though the three angles around the nitrogen atom are 119.3, 121.2 and 119.6° and only slightly different from 120°, the bond distances clearly indicate that the oxygen atoms of the nitrate group are non-equivalent. The plane through the three oxygen atoms O(4), O(5) and O(6) of the nitrate group is given by $-0.6370X + 0.3206Y - 0.7010Z = -4.1266$ where the coefficients of X , Y , Z are the direction cosines of the normal to the plane relative to a , b and c^* and X , Y , Z are Cartesian coordinates in Å. The nitrogen atom N(3) of the nitrate group is 0.007 Å away from this plane. The least-squares plane of the atoms O(4), O(5), O(6) and N(3) is $0.6370X - 0.3206Y + 0.7010Z = 4.1247$. The r.m.s. deviation of the fitted atoms is 0.0028 Å. The deviations of the atoms O(4), O(5), O(6) and N(3) from the plane are, respectively, 0.0016, 0.0017, 0.0015 and -0.0048 Å. Both these indicate that the nitrate group is only nearly planar. In this structure, it was noticed that the oxygen atoms of the nitrate group participating in hydrogen bonds of differing strength have their respective N–O bonds stretched by differing amounts. In GGNO₃, the atom O(5) of the nitrate group [which is the one nearest to the atom N(3)] is not involved in hydrogen bonding at all and the atom O(6) (which is the most distant one) is involved in the strongest hydrogen bond. A somewhat similar situation exists in urea nitrate also (Harkema & Feil, 1969). Similar stretching of the N–O bonds of the nitrate group has also been observed in

AgCN.2AgNO₃ (Britton & Dunitz, 1965) and the authors have considered this as being due to different strengths of the bonds O–Ag. Further studies on the conformations and disorders of nitrate groups are in progress and will be discussed in a separate communication.

The peptide

The most characteristic properties of peptides are the planarity of the peptide group and the constancy of the dimensions of the peptide unit, independent of different amino-acid constituents. Dimensions of the glycol residues and especially the dimensions of the peptide unit taken from recent accurate structure analyses (Freeman, Paul & Sabine, 1970; Koetzle, Hamilton & Parthasarathy, 1972) and the average dimensions given by Marsh & Donohue (1967) agree quite well with the corresponding bond lengths and angles observed in GGNO₃ (Table 5).

Atoms O(1), N(2), C(1), C(2), C(3) and H(6) define the peptide unit. The least-squares plane of these atoms is given by $0.6806X - 0.0651Y + 0.7297Z = 4.6359$. The atoms C(3), C(4), O(2) and O(3) define the least-squares plane $0.7483X + 0.1965Y + 0.6336Z = 5.1289$. The deviations of various atoms from their respective least-squares planes are given in Table 6 along with a comparison of the same in glycolglycine hydrochloride (Koetzle, Hamilton & Parthasarathy, 1972) and in perdeutero- α -glycolglycine (Freeman, Paul & Sabine, 1970), as calculated by us from their coordinates. In GGNO₃, the peptide plane and the plane of the carboxyl are nearly parallel, the angle of inclination between them being 16.5°, in contrast with glycolglycine hydrochloride (Parthasarathy, 1969) where the peptide plane and the carboxyl planes are nearly perpendicular to one another, their angle of inclination being 78°.

Table 5. *Dimensions of glycol residues*

		Present work	Glycolglycine hydrochloride 1	Perdeutero- α -glycolglycine 2	Marsh & Donohue 3
	N(1)–C(1)	1.481 (2) Å	1.473 (3) Å	1.474 (3) Å	
C ^{α} –C	C(1)–C(2)	1.514 (2)	1.515 (3)	1.515 (3)	1.51 Å
C–O	C(2)–O(1)	1.222 (2)	1.233 (3)	1.238 (4)	1.24
C–N	C(2)–N(2)	1.330 (2)	1.330 (3)	1.326 (3)	1.325
N–C ^{α}	N(2)–C(3)	1.453 (2)	1.440 (3)	1.446 (3)	1.455
	C(3)–C(4)	1.499 (2)	1.508 (3)	1.518 (3)	
	C(4)–O(2)	1.318 (2)	1.316 (4)	1.257 (4)	
	C(4)–O(3)	1.207 (2)	1.204 (4)	1.243 (4)	
	N(1)–C(1)–C(2)	108.8 (1)°	109.6 (2)°	109.5 (2)°	
C ^{α} –C=O	C(1)–C(2)–O(1)	121.2 (1)	120.5 (2)	120.3 (2)	120.5°
C ^{α} –C–N	C(1)–C(2)–N(2)	115.4 (1)	114.4 (2)	116.7 (2)	116.0
O=C–N	O(1)–C(2)–N(2)	123.4 (1)	125.1 (2)	123.0 (2)	123.5
C–N–C ^{α}	C(2)–N(2)–C(3)	121.6 (1)	123.1 (2)	121.6 (2)	122.0
N–C ^{α} –C	N(2)–C(3)–C(4)	109.8 (1)	114.2 (2)	113.3 (2)	111.0
	C(3)–C(4)–O(2)	111.6 (1)	110.8 (2)	115.4 (2)	
	C(3)–C(4)–O(3)	124.3 (1)	124.6 (2)	118.0 (2)	
	O(2)–C(4)–O(3)	124.1 (1)	124.5 (3)	126.6 (2)	

1. Koetzle, Hamilton & Parthasarathy (1972).

2. Freeman, Paul & Sabine (1970).

3. Marsh & Donohue (1967).

This agrees well with the observation made in the earlier studies of peptides that the carboxyl group is either nearly parallel or nearly perpendicular to the adjacent peptide plane (Marsh & Glusker, 1961; Freeman, Robinson & Schoone, 1964).

The carbon-oxygen bond lengths in the carboxyl group are 1.318 and 1.207 Å for C(4)-O(2) and C(4)-O(3) respectively. This result suggests that C(4)-O(3) and C(4)-O(2) do not possess purely double and purely single bond characters respectively, and the angular dimensions of the carboxyl group are in agreement with such a description. H(9) which is covalently bound to O(2) is at a distance of 0.153 Å from the least-squares plane through the carboxyl group.

Molecular conformation

The conformation of the molecule along various bonds and the corresponding torsion angles are shown in Fig. 3. The conformation of a peptide chain is usually described in terms of torsion angles about the bonds of the chain. The torsion angles φ , ψ , and ω were calculated according to the latest IUPAC-IUB (1970) conventions. The values of φ , ψ , and ω for the N-terminal residue, C-terminal residue and the peptide bond for our structure and for perdeutero- α -glycylglycine (Freeman, Paul & Sabine, 1970) as calculated by us from their coordinates and for glycylglycine hydrochloride (Koetzle, Hamilton & Parthasarathy, 1972; Parthasarathy, 1969) are given in Table 7. The allowed ranges of φ and ψ for glycyl residues (Lakshminarayanan, Sasisekharan & Ramachandran, 1967) are consistent with the values observed in our structure. A comparison of the values of $\varphi = 165.6^\circ$ and $\psi = 148.9^\circ$ observed in this molecule with $\varphi = 180^\circ$ and $\psi = 180^\circ$ for a fully extended chain shows that this molecule is almost in an extended conformation. The

value of $\omega = 174.5^\circ$ shows that the peptide is twisted significantly.

Packing and hydrogen bonding

In this structure there are five hydrogen atoms which can take part in hydrogen bonding. These are the three hydrogens on the amino group, one on the peptide nitrogen and one on the carboxylic acid group. The hydrogen-bonding distances and angles are given in Table 8. The hydrogen H(1) on N(1) forms a hydrogen bond of length 2.864 Å with O(6ⁱ), an oxygen atom of the nitrate group. Though this H...O contact distance of 2.06 Å is considerably shorter than the sum of the van der Waals radii of H and O, the N-H...O angle of 136° is far from linearity. Such bent hydrogen bonds have been found in many structures (for a discussion, see Baur, 1964). The hydrogen atom H(2) forms a hydrogen bond with O(4) and this bond is fairly linear, having an angle of 167° . The third hydrogen atom H(3) on N(1) is bonded to the oxygen O(3ⁱⁱ) of the carboxylic acid of another GGNO₃. The distances and angles involved in this bond are: N(1)...O(3ⁱⁱ), 2.863 Å; H(3)...O(3ⁱⁱ), 2.13 Å and N(1)-H(3)...O(3ⁱⁱ), 153° . In addition, H(3) also has a short intermolecular contact of 2.43 Å with O(1). Such short contacts are very commonly observed in many amino acids and peptides. Though we may prefer not to call this interaction the weaker member of a bifurcated hydrogen bond, this interaction does contribute to the stability of the structure. Such interactions have recently been discussed by Koetzle, Hamilton & Parthasarathy (1972).

The hydrogen atom H(6) on the peptide nitrogen N(2) is involved in hydrogen bonding with O(1^{iv}), the oxygen of the peptide group in a neighboring mole-

Table 6. Deviations of atoms from the respective least-squares planes (values $\times 10^3$)

Peptide plane	Present study	Glycylglycine hydrochloride		Perdeutero- α -glycylglycine
		1	2	3
O(1)	5	0 (2)	- 3	- 3
N(2)	43	-23 (-19)	20	20
C(1)	-27	17 (17)	-20	-20
C(2)	14	-14 (-17)	23	23
C(3)	-34	20 (18)	-20	-20
H(6)†	49	-150 (-104)	18	18
R.m.s. deviations of fitted atoms from plane	28	1 (16)	18	18
Carboxyl plane				
C(3)	- 1	- 1 (-1)	- 1	- 1
C(4)	1	3 (3)	1	1
C(2)	- 0	- 1 (-1)	- 1	- 1
O(3)	- 1	- 1 (-1)	- 1	- 1
H(9)†	-153	-83 (107)	1	1
R.m.s. deviations of fitted atoms from plane	1	2 (2)	1	1
Angle between the planes	16.5°	77.9° (103.2°)	23.2°	23.2°

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

1. Parthasarathy (1969).

2. Koetzle, Hamilton & Parthasarathy (1972).

3. Freeman, Paul & Sabine (1970).

cule. The distances and angles for this bond are: N(2)–H(6)···O(1^{iv}), 3.116 Å; H(6)···O(1^{iv}), 2.38 Å and N(2)–H(6)···O(1^{iv}), 165°. In our structure, the hydrogen bond from one peptide nitrogen to another peptide oxygen is significantly longer and presumably weaker than the hydrogen bonds from the amino nitrogen. In several peptide structures, it has been found that the peptide nitrogen forms only weak hydrogen bonds; Marsh & Donohue (1967) have suggested a

value of 2.90 Å for such N–H···O hydrogen-bond distances. The value of 2.75–2.80 Å has been suggested by Corey & Pauling (1953) and is normally used in constructing models of α -helices and polypeptide configurations. In GGNO₃, the peptide chain is in an extended conformation. The extended molecules are arranged together in an antiparallel arrangement with hydrogen bonds linking them across and the nitrate group linking the molecules end to end. This struc-

Table 7. Torsion angles (°)*

	IUPAC-IUB designation	Present study	Glycylglycine hydrochloride 1 2	Perdeutero- α -glycylglycine 3
N-Terminal	H(1)–N(1)–C(1)–C(2)	ϕ_1^1	82.0	70.8 (73.2)
	H(2)–N(1)–C(1)–C(2)	ϕ_2^1	168.8	169.7 (165.3)
	H(3)–N(1)–C(1)–C(2)	ϕ_3^1	42.7	51.1 (45.7)
	N(1)–C(1)–C(2)–N(2)	ψ_1	148.9	161.4 (162.1)
C-Terminal	C(2)–N(2)–C(3)–C(4)	ϕ_2	165.6	80.0 (79.6)
	N(2)–C(3)–C(4)–O(2)	ψ_1^2	176.9	176.2 (176.8)
	N(2)–C(3)–C(4)–O(3)	ψ_2^2	3.3	3.3 (3.8)
Peptide bond	C(1)–C(2)–N(2)–C(3)	ω	174.5	176.6 (176.8)

* Because the structures are centrosymmetric, the signs of the torsion angles were made to agree for easy comparison with other structures.

1. Parthasarathy (1969).
2. Koetzle, Hamilton & Parthasarathy (1972).
3. Freeman, Paul & Sabine (1970).

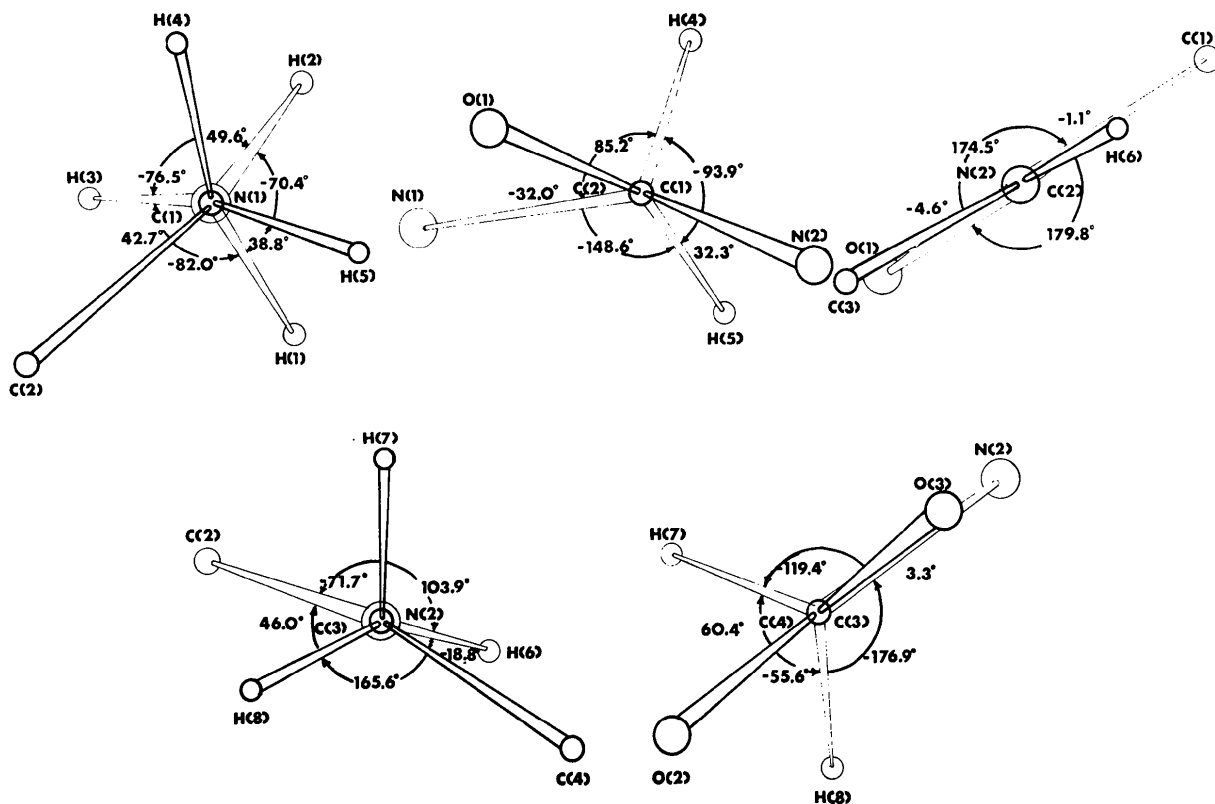


Fig. 3. Projections of the molecule along various bonds and their corresponding torsion angles (°).

tural arrangement is similar to the so-called anti-parallel pleated-sheet arrangement typical of β -proteins.

The hydrogen atom H(9) of the carboxylic acid group participates in a strong linear hydrogen bond to the oxygen atom O(6ⁱⁱⁱ) of the nitrate group (see Table 8).

Table 8. *Hydrogen-bond distances (Å) and angles (°)*

<i>D-H...A</i>	Distance		Angle
	<i>D...A</i>	<i>H...A</i>	<i>D-H...A</i>
N(1)-H(1)···O(6 ⁱ)	2.864	2.06	136 (2)
N(1)-H(2)···O(4)	2.849	1.87	167 (2)
N(1)-H(3)···O(3 ⁱⁱ)	2.863	2.13	153 (2)
O(2)-H(9)···O(6 ⁱⁱⁱ)	2.645	1.73	177 (2)
N(2)-H(6)···O(1 ^{iv})	3.116	2.38	165 (2)

D is the donor and *A* is the acceptor atom. The superscript notation for the coordinates of symmetry-equivalent atoms is:

None	<i>x</i>	<i>y</i>	<i>z</i>
i	<i>x</i>	$\frac{1}{2}-y$	$-\frac{1}{2}+z$
ii	$1-x$	$-\frac{1}{2}+y$	$\frac{1}{2}-z$
iii	$1+x$	<i>y</i>	$-1+z$
iv	$1-x$	$\frac{1}{2}+y$	$\frac{1}{2}-z$

The glycylglycine molecules are packed in a head-to-tail fashion along the *a* direction. The nitrate groups occupy positions between the amino ends of one pair of glycylglycine molecules and the carboxyl ends of another pair of such molecules. The glycylglycine molecules related by the screw axis are linked to each other along the *b* direction by hydrogen bonds between the amino and carboxyl ends.

The nitrate ions exhibit an interesting mode of packing (Fig. 4). Since the nitrogen atom is not quite in the plane of the three oxygens, the nitrate ions can be visualized as very flattened pyramids. These flattened pyramids are stacked along the *c* axis such that their apices point alternately up and down along the *c* direction. These stacks are located in a cylindrical cavity along the *c* axis. The cavities are formed by glycylglycine molecules, half of them pointing their amino groups and the other half their carboxylate groups into this cavity. All intermolecular contacts other than hydrogen bonds are larger than the sum of their respective van der Waals radii.

Table 9. *Bond lengths (Å) before and after thermal correction*

Bond	Present work		Glycylglycine hydrochloride (neutron data)*	
	Uncorrected	Corrected for thermal motion	Uncorrected	Corrected for thermal motion
N(1)-C(1)	1.481	1.486	1.473	1.475
C(1)-C(2)	1.514	1.521	1.515	1.522
C(2)-O(1)	1.222	1.230	1.233	1.238
C(2)-N(2)	1.330	1.332	1.330	1.331
N(2)-C(3)	1.453	1.458	1.440	1.445
C(3)-C(4)	1.499	1.504	1.508	1.517
C(4)-O(2)	1.318	1.322	1.316	1.320
C(4)-O(3)	1.207	1.216	1.204	1.209

* Koetzle, Hamilton & Parthasarathy (1972).

Analysis of thermal motions

The thermal parameters of the non-hydrogen atoms in the peptide were fitted to a rigid-body model by the method of Schomaker & Trueblood (1968). The axis of maximum libration is approximately along the peptide chain with a calculated r.m.s. amplitude of 8.0°. The r.m.s. difference between the observed and calculated U_{ij} values is 0.003 Å². The libration-corrected distances obtained from this analysis are compared in Table 9 with those for glycylglycine hydrochloride (Koetzle, Hamilton & Parthasarathy, 1972).

The U_{ij} 's for the oxygen atoms of the nitrate group are given in Table 10 along with the direction cosines for the N-O bonds. It is found that the directions of the minimum thermal vibrations for the oxygen atoms are along the respective N-O bonds while the corresponding directions for the maximum vibration are in the plane of the nitrate group and are perpendicular to the N-O bonds. This suggests a rotational libration of the nitrate group about an axis normal to its plane.

Table 10. *Description of thermal motion for the nitrate group*

Atom	U_{ij}	Direction cosines ($\times 10^4$) with respect to		
		<i>a</i>	<i>b</i>	<i>c</i> *
N(3)	0.16	6271	-0056	-7789
	0.19	-4931	-7770	-3914
	0.20	-6030	6295	-4900
O(4)	0.17	-2161	-8816	-4196
	0.19	7671	1126	-6315
	0.31	6040	-4584	6520
O(5)	0.16	6998	4198	-5779
	0.22	0927	-8556	-5093
	0.29	-7083	3028	-6377
O(6)	0.15	-4823	3723	7929
	0.20	5349	8420	-0699
	0.28	-6937	3904	-6053
Direction cosines of N-O bonds				
	N(3)-O(4)	-2350	-9480	-2127
	N(3)-O(5)	7537	4632	-4655
	N(3)-O(6)	-5197	4863	7019

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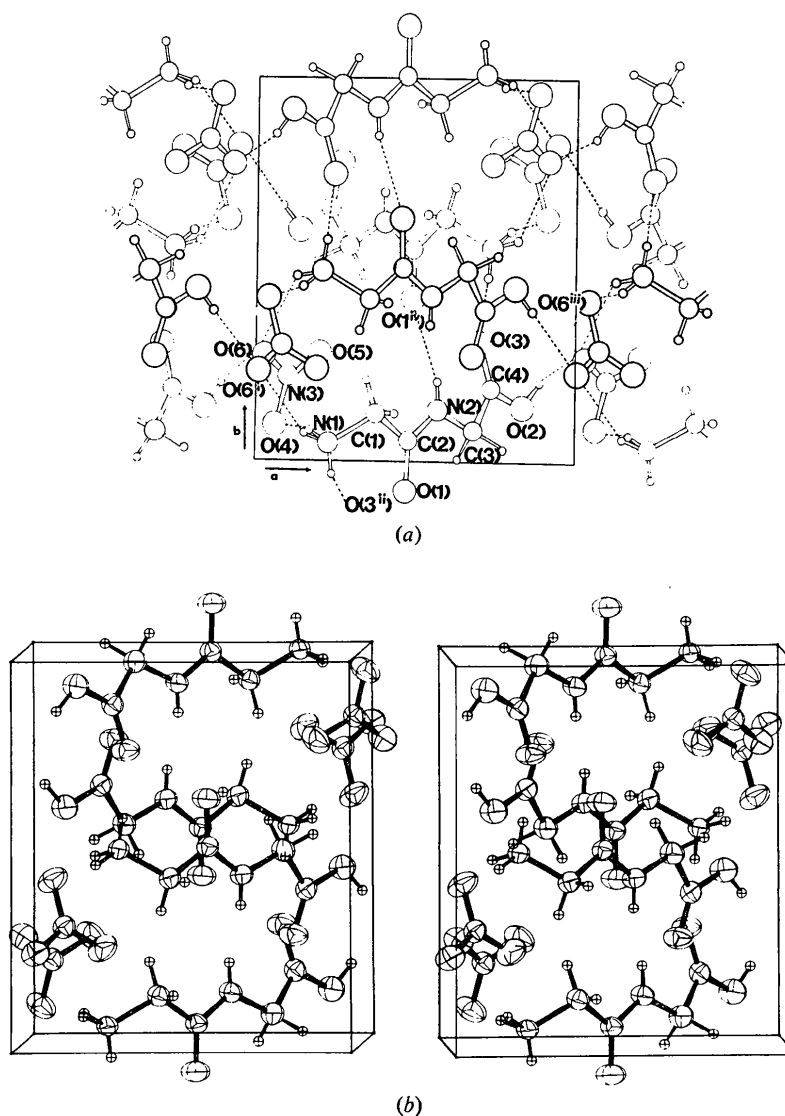


Fig. 4. (a) Packing of the molecules and hydrogen-bonding scheme. (b) A stereoscopic pair illustrating the packing of the molecules and the thermal ellipsoids. The view is nearly along the *c* axis.

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A Model for a Torsional Oscillator in Crystallographic Least-Squares Refinements*

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For an axially symmetric group of n atoms in torsional oscillation about the symmetry axis and with no other modes of motion, the contribution to the structure factor assuming a Gaussian distribution for the angular displacement from the mean angular position is given by a Bessel-function series expression of the same form as the King & Lipscomb [*Acta Cryst.* (1950). 3, 155–158] expression for a hindered rotator except that their factors $I_m(b)/I_0(b)$ are replaced by factors $\exp[-n^2 m^2 \beta^2/2]$, where β^2 is the mean-square angular displacement. Expressions for the real and imaginary parts of the structure-factor contribution and the derivatives required for least-squares refinement are given. With minor changes the formalism of these expressions applies to the King–Lipscomb hindered rotator. With the addition of factors for other modes of motion, the case with $n=1$ has been programmed and successfully applied by Brown & Chidambaram in the paper following this one.

Introduction

A number of authors have discussed the problem of devising a proper structure-factor expression for an atom in librational motion. For such motion the usual six-parameter thermal factor is not appropriate, since it is derived on the assumption of independent rectilinear harmonic motions in three degrees of freedom. For general discussions and leading references see Cruickshank (1956), Johnson (1970*a, b*), Johnson & Levy (1972), and Maslen (1970). This paper describes a model for use in least-squares refinement for an atom having as principal mode of motion a libration of large amplitude about a single axis. The circumstances leading to the development of the model and the details of its application to the methyl hydrogen atom in copper(II) acetate monohydrate were described preliminarily by Brown & Chidambaram (1967) and are detailed in the accompanying paper by Brown & Chidambaram (1973).

Derivation

Bloch (1932) showed that for the linear quantum-mechanical harmonic oscillator at a given temperature the probability distribution function for displacements x is Gaussian. In analogy, we make the assumption that for an atom in torsional oscillation about a single axis the probability distribution function of positions θ about the mean position γ on a circular arc is the Gaussian function

$$P(\theta) = (2\pi\delta^2)^{-1/2} [\exp -(\theta - \gamma)^2/2\delta^2], \quad (1)$$

where δ^2 is the mean value of $(\theta - \gamma)^2$. Normal probability distributions have been used by Kay & Behrendt (1963) in developing a structure-factor expression for an atom in two-dimensional librational motion and also by Maslen (1968, 1970) for the one-dimensional case. Our treatment differs from that of Kay & Behrendt and that of Maslen in that we do not make the approximation that $\sin \theta = \theta$ in order to obtain an expression in closed form; instead, we develop a series expression, following very closely the derivation of King & Lipscomb (1950) for a hindered rotator. The expression is also generalized for the case of a group of n equal atoms with an n -fold axis of symmetry to corre-

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