Crystal and Molecular Structure of *N*-Acetyl-L-glutamine

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The crystal and molecular structure of the title compound has been determined by direct methods from diffractometer data. Crystals are orthorhombic, with $Z = 4$ in a unit cell of dimensions: $a = 13.811(10)$, $b = 5.095(5)$, $c = 12.914(10)$ Å, space group $P2_12_12_1$. The structure was refined by least-squares to R 3·31% for 868 observed reflections. There is significant non-planarity of the peptide group and its nitrogen atom is significantly pyramidal. There is no correlation between the double-bond character and reactivity of the C–N bond of the terminal amide group in glutamine and acetamide.

THE investigation of the structure of *N*-acetyl-L-glutamine was undertaken as part of a project to determine the structures of simple peptides and other compounds containing the peptide linkage. We wished to obtain information regarding the nature of the distortion of the peptide linkage which was, until recently, assumed to be planar. However significant nonplanarity has now been observed in various crystal structures, [1-4] and information obtained about the deformation of the peptide group induced by inter- and intra-molecular interactions. In constructing models of polypeptide chains or in fitting such models to electron-density maps of protein crystals, the strictly planar peptide unit incorporates restrictions which are easily relaxed in the actual molecules. We wished to see how the distortion of the peptide geometry varies in different structures. The amide group in glutamine is more susceptible to hydrolysis and attack by nitrous acid than acetamide and other primary amides,[5,6] and we wished to investigate whether any correlation exists between the chemical property of the amide group and its geometry as found in acetamide and glutamine.

EXPERIMENTAL

Large needle-shaped crystals elongated along *b*, were obtained by slow evaporation of an aqueous solution.

Crystal Data.—$C_7H_{12}N_2O_4$, $M = 188.2$. Orthorhombic, $a = 13.811$, $b = 5.095$, $c = 12.914$ (all ± 0.01) Å, $U = 908.7$ Å^3, $D_m = 1.382$ g cm^{-3} (by flotation), $Z = 4$, $D_c = 1.384$ g cm^{-3}. Space group $P2_12_12_1$ (No. 19; D_2^4) from systematic absences. Mo-K_α radiation, $\lambda = 0.7107$ Å; μ(Mo-K_α) = 1.2 cm^{-1}.

Unit-cell data were obtained on the diffractometer with Mo-K_α radiation. Intensity data were collected on a Hilger and Watts diffractometer (by F. W.). Of 970 reflections with $2\theta \leqslant 50°$ measured, 868 had intensity $I > 3\sigma(I)$. Since the size of the crystal was 0.15 × 0.30 × 0.20 mm no absorption correction was applied. After Lorentz and polarization corrections, intensities were placed on an absolute scale by a Wilson plot.

The structure was solved by the symbolic addition procedure for noncentrosymmetric crystals.[7] Tangent formula [8] iterations on 198 reflections for which E was $\geqslant 1.26$ were performed using the GAASA [9,10] system of programmes. All non-hydrogen atoms were located in an E map for a set of phases with R_K 17%. Four cycles of block-diagonal least-squares refinement of the positional and the thermal parameters of the non-hydrogen atoms reduced R to 12.6% from the initial value of 29.8%. A further two cycles of refinement with anisotropic temperature factors for all non-hydrogen atoms reduced R to 7.4%. At this stage the hydrogen atoms were located from a difference-Fourier map and were introduced in the refinement with isotropic thermal parameters. Two cycles of refinement reduced R to 4.2%. Up to this stage the least-squares programme originally due to Shiono [11] and modified by Reddy [12] for the IBM 360 44 was used.

Further refinements were carried by full-matrix least-squares methods at Zurich. Three cycles with weights defined as [13]: $w = (1/\sigma^2 F_o) \cdot \exp[r \cdot (\sin 2\theta/\lambda^2)]$, with $r = 10$ for the first two cycles and $r = 5$ for the third cycle gave R 3.7% for 868 observed reflections. At this stage the positional and isotropic thermal parameters of hydrogen atoms were refined using 553 reflections for which $\sin\theta/\lambda \leqslant 0.5$ Å^{-1} and using non-hydrogen atoms only for the structure-factor calculations. After the final cycle of refinement of positional and anisotropic parameters of non-hydrogen atoms, taking into account the contributions of the hydrogen atoms for the structure factors, R for 868 observed reflections was 3.31%. The scattering factors for non-hydrogen atoms were taken from ref. 14 and for hydrogen from ref. 15. Final atomic parameters are listed in Table 1. Observed and calculated structure factors and interatomic distances < 3.6 Å are listed in Supplementary Publication No. SUP 21613 (8 pp., 1 microfiche).*

DISCUSSION

Bond lengths and torsion angles corresponding to the final co-ordinates are listed in Table 2 (mean σ in bond

* For details of Supplementary Publications see Notice to Authors No. 7, *J.C.S. Perkin II*, 1975, Index issue.

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[11] R. Shiono, 1970, personal communication.
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lengths involving non-hydrogen atoms is 0.004 Å and in angles 0.25°); the atom numbering system is shown in the Figure. Observed bond lengths within the side-chain are in general agreement with those reported for L-glutamine,[16] but the value (1.513 Å) for C(5)–C(6) is shorter than that expected for a C(sp^3)–C(sp^3) bond. The angle C^β [C(3)–C(5)–C(6)] is 111.6(2)°, which is smaller than that usually found (*ca.* 114°) in other amino-acids and peptides.[17-19]

The carboxy-group which is planar (Table 3) exists in this structure in the un-ionized form. The C(4)–O(2)

TABLE 1

Positional parameters

(*a*) Non-hydrogen atoms

Atom	*x*	*y*	*z*
O(1)	0.094 7(1)	−0.175 3(5)	−0.471 4(2)
C(1)	0.072 7(2)	0.135 2(8)	−0.333 3(2)
C(2)	0.130 0(2)	0.004 0(6)	−0.417 4(2)
N(1)	0.218 5(2)	0.093 9(5)	−0.434 1(2)
C(3)	0.274 8(2)	0.004 5(5)	−0.522 2(2)
C(4)	0.378 9(2)	−0.060 7(5)	−0.491 0(2)
O(2)	0.417 4(1)	0.014 9(5)	−0.412 9(2)
O(3)	0.420 9(1)	−0.205 2(6)	−0.561 1(2)
C(5)	0.274 5(2)	0.207 7(6)	−0.609 9(2)
C(6)	0.172 7(2)	0.273 5(7)	−0.644 3(2)
C(7)	0.168 9(2)	0.460 8(6)	−0.735 1(2)
O(4)	0.234 8(2)	0.476 0(5)	−0.798 3(2)
N(2)	0.088 3(2)	0.602 6(6)	−0.741 8(2)

Anisotropic thermal parameters ($\times 10^4$) of non-hydrogen atoms

Atom	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
O(1)	275(7)	575(11)	535(10)	−144(16)	54(14)	−97(18)
C(1)	317(11)	770(19)	371(11)	−5(14)	70(18)	−43(25)
C(2)	252(9)	443(12)	313(10)	−27(19)	−32(16)	36(21)
N(1)	207(7)	346(9)	305(8)	−8(14)	1(13)	−29(14)
C(3)	212(9)	305(9)	356(9)	7(17)	−4(16)	−35(18)
C(4)	221(9)	351(11)	364(10)	12(15)	3(17)	−32(19)
O(2)	293(7)	656(12)	507(9)	98(17)	−112(14)	−220(20)
O(3)	290(8)	721(13)	478(9)	187(17)	−63(16)	−205(20)
C(5)	253(9)	422(11)	400(11)	−2(19)	30(18)	54(19)
C(6)	278(10)	493(14)	486(12)	5(20)	−5(19)	176(23)
C(7)	314(10)	368(11)	409(11)	−24(18)	15(18)	72(20)
O(4)	419(9)	629(12)	592(10)	67(20)	150(17)	238(21)
N(2)	424(11)	567(13)	501(11)	137(20)	71(20)	198(21)

U_{ij} are defined by the expression $B = \exp[-2\pi^2(U_{11}h^2a^{*2} + U_{22}k^2b^{*2} + U_{33}l^2c^{*2} + 2U_{12}hka^*b^* + 2U_{13}hla^*c^* + 2U_{23}klb^*c^*)]$.

(*b*) Hydrogen atoms

Atom	*x*	*y*	*z*	B/Å^2
1H[C(1)]	0.013(2)	0.232(6)	−0.363(3)	7.5
2H[C(1)]	0.044(2)	−0.002(6)	−0.286(2)	7.3
3H[C(1)]	0.109(2)	0.232(6)	−0.288(2)	4.6
H[N(1)]	0.242(2)	0.225(6)	−0.393(2)	3.7
H[C(3)]	0.249(2)	−0.160(5)	−0.547(2)	1.8
H[O(3)]	0.482(3)	−0.235(8)	−0.548(3)	6.4
1H[C(5)]	0.306(2)	0.374(2)	−0.587(2)	4.3
2H[C(5)]	0.314(2)	0.140(6)	−0.668(2)	3.2
1H[C(6)]	0.143(2)	0.119(7)	−0.666(2)	4.5
2H[C(6)]	0.130(2)	0.338(7)	−0.587(3)	4.8
1H[N(2)]	0.041(2)	0.601(5)	−0.697(2)	3.6
2H[N(2)]	0.082(2)	0.718(6)	−0.794(2)	4.6

TABLE 2

Bond lengths (Å), bond angles (°), and torsion angles

(*a*) Bond lengths

C(1)–C(2)	1.501(4)	C(1)–1H(C1)	1.04(3)
O(1)–C(2)	1.249(4)	C(1)–2H(C1)	1.01(3)
C(2)–N(1)	1.322(3)	C(1)–3H(C1)	0.92(3)
N(1)–C(3)	1.452(3)	N(1)–H(N1)	0.91(3)
C(3)–C(4)	1.529(3)	C(3)–H(C3)	0.96(3)
C(4)–O(2)	1.204(3)	O(3)–H(O3)	0.87(4)
C(4)–O(3)	1.303(4)	C(5)–1H(C5)	0.99(3)
C(3)–C(5)	1.535(4)	C(5)–2H(C5)	0.99(3)
C(5)–C(6)	1.513(4)	C(6)–1H(C6)	0.93(3)
C(6)–C(7)	1.513(4)	C(6)–2H(C6)	1.00(3)
C(7)–N(2)	1.330(4)	N(2)–1H(N2)	0.87(2)
C(7)–O(4)	1.225(4)	N(2)–2H(N2)	0.90(3)

TABLE 2 (*Continued*)

(*b*) Bond angles

O(1)–C(2)–C(1)	121.5(3)
O(1)–C(2)–N(1)	121.6(3)
C(1)–C(2)–N(1)	116.8(3)
C(2)–N(1)–C(3)	121.0(2)
N(1)–C(3)–C(4)	111.5(2)
N(1)–C(3)–C(5)	111.4(2)
C(4)–C(3)–C(5)	110.1(2)
C(3)–C(4)–O(2)	124.5(2)
C(3)–C(4)–O(3)	111.0(2)
O(2)–C(4)–O(3)	124.5(3)
C(3)–C(5)–C(6)	111.6(2)
C(5)–C(6)–C(7)	113.5(2)
C(6)–C(7)–O(4)	122.1(3)
C(6)–C(7)–N(2)	114.9(3)
O(4)–C(7)–N(2)	123.0(3)
C(2)–C(1)–1H(C1)	111.2(1.7)
C(2)–C(1)–2H(C1)	109.6(1.8)
C(2)–C(1)–3H(C1)	114.8(1.9)
1H(C1)–C(1)–2H(C1)	104.4(2.5)
1H(C1)–C(1)–3H(C1)	114.7(2.6)
2H(C1)–C(1)–3H(C1)	101.0(2.6)
C(2)–N(1)–H(N1)	119.2(1.7)
C(3)–N(1)–H(N1)	119.6(1.7)
N(1)–C(3)–H(C3)	109.5(1.5)
C(4)–C(3)–H(C3)	104.3(1.5)
C(5)–C(3)–H(C3)	109.9(1.5)
C(4)–O(3)–H(O3)	113.4(2.5)
C(3)–C(5)–1H(C5)	110.8(1.9)
C(3)–C(5)–2H(C5)	108.8(1.6)
C(6)–C(5)–1H(C5)	107.8(1.9)
C(6)–C(5)–2H(C5)	111.6(1.7)
1H(C5)–C(5)–2H(C5)	106.1(2.5)
C(5)–C(6)–1H(C6)	107.8(2.1)
C(5)–C(6)–2H(C6)	113.6(2.0)
C(7)–C(6)–1H(C6)	106.5(2.1)
C(7)–C(6)–2H(C6)	110.1(2.0)
1H(C6)–C(6)–2H(C6)	104.6(2.9)
C(7)–N(2)–1H(N2)	125.3(1.7)
C(7)–N(2)–2H(N2)	118.9(2.0)
1H(N2)–2H(N2)	115.7(2.6)

(*c*) Dihedral angles

O(1)–C(2)–N(1)–C(3)	5.8(4)
C(1)–C(2)–N(1)–C(3)	−171.8(2)
C(2)–N(1)–C(3)–C(4)	−134.8(2)
C(2)–N(1)–C(3)–C(5)	101.8(3)
N(1)–C(3)–C(4)–O(2)	−18.4(4)
C(5)–C(3)–C(4)–O(2)	105.7(3)
N(1)–C(3)–C(4)–O(3)	162.6(2)
C(5)–C(3)–C(4)–O(3)	−73.2(3)
N(1)–C(3)–C(5)–C(6)	−56.9(3)
C(4)–C(3)–C(5)–C(6)	178.9(2)
C(3)–C(5)–C(6)–C(7)	−176.5(2)
C(5)–C(6)–C(7)–O(4)	26.8(4)
C(5)–C(6)–C(7)–N(2)	−154.4(3)
O(1)–C(2)–C(1)–1H(C1)	−63.0(1.9)
O(1)–C(2)–C(1)–2H(C1)	51.9(1.9)
O(1)–C(2)–C(1)–3H(C1)	164.8(2.1)
N(1)–C(2)–C(1)–1H(C1)	114.6(1.9)
N(1)–C(2)–C(1)–2H(C1)	−130.4(1.9)
N(1)–C(2)–C(1)–3H(C1)	−17.6(2.1)
C(6)–C(7)–N(2)–1H(N2)	3.9(2.1)
C(6)–C(7)–N(2)–2H(N2)	179.8(2.3)
O(4)–C(7)–N(2)–1H(N2)	−177.3(2.1)
O(4)–C(7)–N(2)–2H(N2)	−1.4(2.4)

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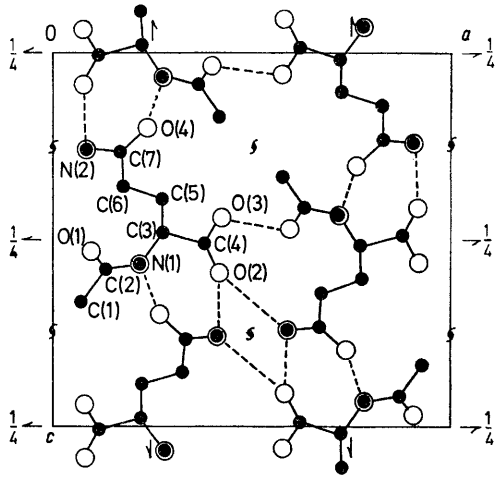
bond is *cis* to C(3)–N(1) with the torsion angle N(1)–C(3)–C(4)–O(2) −18.4°.

The peptide group C(3), N(1), C(2), O(1), C(1), and H[N(1)] is significantly non-planar. The best plane among its constituent atoms is that through C(1), C(2),



Packing of the structure, viewed down the *b* axis

O(1), and N(1). Atom C$^{\alpha}$ [C(3)] at the *C*-terminal end deviates by 0.17 Å from this plane but H[N(1)] lies in it. Non-planarity of the peptide group has been observed in several other simple peptides, *e.g.* α-glycylglycine,[2] L-alanylglycine,[3] glyclyl-L-alanine hydrochloride,[1] and glycyl-L-leucine.[4] In most of these structures the best four-atom plane among the atoms of the peptide group is obtained by omitting the C$^{\alpha}$ atom at the *C*-terminal end. Winkler and Dunitz [20] have observed that the nitrogen atom of the amide group may be slightly pyramidal in character. By use of the CNDO/2 method, it has been demonstrated [21] that in *N*-methylacetamide the peptide group is non-planar with NH and NC$^{\alpha}_2$ bonds significantly out of the plane defined by atoms C$^{\alpha}_1$, C′, O, and N. But a close study of the deviations of the hydrogen linked to peptide nitrogen and C$^{\alpha}_2$ from the remaining four atoms of the peptide group in the structures of simple peptides which have been accurately determined [1-4] reveals that the deviations vary. The non-planarity could be partly due to intermolecular interaction, in particular, of the hydrogen bond involving the hydrogen attached to the peptide nitrogen. From the best plane passing through the atoms of the peptide group, the deviations of hydrogen H[N(1)] and O(4), which is hydrogen-bonded with N(1) in *N*-acetyl-L-glutamine, are 0.01 and 0.095 Å respectively. If there were no pyramidal character at the peptide nitrogen, the deviation of the hydrogen atom would have been −0.1 Å. Thus we may conclude that the pyramidal character observed is partly due to hydrogen bonding. The out-of-plane displacement of H[N(1)] is such that the hydrogen bond is more linear. In fact, a similar situation has been observed in the structure of glycyl-L-leucine.[4]

TABLE 3

Equations of least-squares planes and deviations (Å) of relevant atoms from these planes. *X*, *Y*, *Z* are orthogonal (Å) co-ordinates defined by the *a*,*b*,*c* axes.

Plane (*a*): O(2), C(4), O(3), C(3)

$0.317\,36X + 0.821\,76Y - 0.473\,28Z = 4.413\,30$

[O(2) 0.002, O(3) 0.002, N(1) −0.409, C(4) −0.005, C(3) 0.001, H[O(3)] 0.063]

Plane (*b*): C(1), C(2), O(1), N(1)

$-0.352\,63X + 0.668\,90Y - 0.654\,39Z = 2.920\,21$

[C(3) 0.169, H[N(1)] −0.01, O(1) 0.005, N(1) 0.004, C(2) −0.012, C(1) 0.003, O bonded to H[N(1)] 0.095]

Plane (*c*): C(6), C(7), N(2), O(4)

$-0.427\,47X - 0.713\,69Y - 0.554\,90Z = 2.601\,17$

[C(6) 0.002, N(2) 0.002, 1H[N(2)] −0.03, C(7) −0.006, O(4) 0.002, 2H[N(2)] −0.01, O accepting 1H[N(2)] 0.330, O accepting 2H[N(2)] −0.129]

The geometry of the terminal amide group which exists in the keto-form agrees well with the dimensions reported for acetamide [22] and L-glutamine.[16] The fact that the primary amide in glutamine is more susceptible to hydrolysis and attack by nitrous acid than in acetamide [5,6] was attributed [23] to the larger double-bond character of the C–N bond in glutamine. However the accurate study carried out on glutamine [16] and the present results show that the dimensions of the amide group in both are in agreement. The observed differences in the chemical property cannot be explained in terms of solid-state results. Hydrolysis leads to formation of a five-membered lactam ring compound, pyrrolidonecarboxylic acid, the formation of which cannot be explained in terms of the extended-chain conformation observed for glutamine, and its derivatives in the crystal lattice. It is tempting to propose a curled-up conformation in which the carboxy- and amide group of the glutamine molecule are brought near each other in solution although the predominant conformation would be expected to be extended.

Molecular Conformation.—The notation followed in the description of the conformational parameters of this molecule is that of Edsall *et al.*[24] In the present structure χ^1[N(1)–C(3)–C(5)–C(6)] is −56.9, χ^2[C(3)–C(5)–C(6)–C(7)] −176.4, $\chi^{3,1}$[C(5)–C(6)–C(7)–O(4)] 26.8, and $\chi^{3,2}$[C(5)–C(6)–C(7)–N(2)] −154.4°. The observed conformation is similar to that in L-glutamine hydrochloride.[1] However, in the case of L-glutamine the side-group conformation is different and is approximately a mirror image of the situation in *N*-acetyl-L-glutamine. The value of ϕ [C(2)–N(1)–C(3)–C(4)] is −134.8°. It is worth mentioning that the hydrogen atoms bonded to C(1) are almost staggered with respect to the bond C(1)=O(1).

[20] F. Winkler and J. D. Dunitz, *J. Mol. Biol.*, 1971, **59**, 169.
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Crystal Packing and Hydrogen Bonding.—The Figure shows the packing of the molecules down the *b* axis. The structure is stabilized by intermolecular hydrogen bonds of the types N–H · · · O and O–H · · · O. The hydrogen bond lengths and angles are recorded in Table 4.

TABLE 4

Hydrogen bond lengths (Å) and angles (°)

H Bond	D · · · A	H · · · A
O(3)–H · · · O(1^{I})	2.511	1.64
N(1)–H(N1) · · · O(4^{II})	2.880	1.98
N(2)–1H(N2) · · · O(2^{III})	3.150	2.30
N(2)–2H(N2) · · · O(2^{IV})	2.950	2.05
H Bond	**H–D · · · A**	**D–H · · · A**
O(3)–H · · · O(1^{I})	4.1	173.8
N(1)–H(N1) · · · O(4^{II})	7.9	168.5
N(2)–1H(N2) · · · O(2^{III})	10.6	165.4
N(2)–2H(N2) · · · O(2^{IV})	4.0	174.2

Roman numeral superscripts denote the following equivalent positions relative to the original molecule at x, y, z:

I $\frac{1}{2} + x, -\frac{1}{2} - y, -1 - z$
II $\frac{1}{2} - x, 1 - y, \frac{1}{2} + z$
III $-\frac{1}{2} + x, \frac{1}{2} - y, -1 - z$
IV $\frac{1}{2} - x, 1 - y, -\frac{1}{2} + z$.

The hydrogen-bond distance N(1) · · · O(4) is shorter than the other two hydrogen bond distances between N(2) and oxygen atoms. All the terminal atoms such as O(1), C(1), O(2)—(4), and N(2) tend to exhibit larger anisotropic vibration than the other atoms. The bond lengths C(7)–N(2) and C(2)–N(1) are equal whereas C(7)–O(4) is significantly shorter than C(2)–O(1). The longer value for C(2)–O(1) seems to be due to the fact that O(1) is involved in a much stronger intermolecular hydrogen bond than is O(4). It has been shown recently [25] that hydrogen bonding affects the bond length of the proton-accepting group.

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