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The Crystal Structure of *N*-Acetyl-α-D-glucosamine

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The crystal structure of *N*-acetyl-α-D-glucosamine ($C_8H_{15}O_6N$) has been determined by three-dimensional Patterson superposition techniques. The crystals are monoclinic, space group $P2_1$, with unit-cell dimensions $a=11{\cdot}25$, $b=4{\cdot}82$, $c=9{\cdot}72$ Å, $\beta=113{\cdot}7°$.

The glucopyranose ring is in the chair form and its structure is close to that found in other sugars. The amide group is planar and the plane is approximately perpendicular to that of the puckered ring. The dimensions of this group are similar to those found for the amide group in peptides. The packing of the molecules is determined by an almost complete scheme of hydrogen bonding.

There appears to be a small amount of the β sugar present in the structure which is chiefly in the α configuration.

Introduction

N-Acetylglucosamine (2-acetamido-2-deoxy-D-glucose, $C_8H_{15}O_6N$) is a major constituent of many important biological polysaccharides such as chitin, bacterial cell wall mucopolysaccharides, blood group substances and hyaluronic acid. The enzyme lysozyme, whose structure has recently been solved in this laboratory (Blake, Koenig, Mair, North, Phillips & Sarma, 1965), is known to hydrolyse both chitin (Berger & Weiser, 1957) and the bacterial cell wall polysaccharide (Salton, 1952), releasing oligosaccharides of *N*-acetylamino sugars. It has been shown that *N*-acetylglucosamine inhibits both these reactions (Wenzel, Lenk & Schutte, 1962) and this property has been exploited in the crystallographic investigation of the active site of lysozyme. *N*-Acetylglucosamine has been diffused into lysozyme crystals and the site to which it is bound located by difference Fourier techniques at 6 Å resolution (Johnson & Phillips, 1965). It is hoped that when this work is repeated at high resolution the precise nature of the interactions of the inhibitor with the protein will be apparent. In order to aid the interpretation, the structure of *N*-acetylglucosamine itself has been investigated.

The structures of at least ten derivatives of D-glucose are known and the conformation of the glucopyranose ring is now well established. Ramachandran, Ramakrishnan & Sasisekharan (1963) have shown that the conformation is remarkably uniform and may be considered as a standard structure. In view of the importance of *N*-acetylglucosamine in many naturally occuring polysaccharides it was also of interest to compare the structure with the standard structure and with the structures of sugars in general.

A preliminary account of this work has already appeared (Johnson & Phillips, 1964).

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Experimental

Colourless needle-shaped crystals were grown by evaporation at room temperature of a solution of *N*-acetylglucosamine in methyl alcohol and water. The crystals tended to cluster together at the bottom of the glass vessel but it was possible to separate suitable specimens with the use of a sharp needle. The dimensions of the unit cell were obtained from measurements on zero level Weissenberg photographs with Cu $K\alpha$ radiation of wave length 1·542 Å, the films being calibrated by superimposing powder lines from a sodium chloride specimen. The density of the crystals was measured by flotation in a mixture of chloroform and carbon tetrachloride. The crystallographic data are listed as follows:

N-Acetylglucosamine, $C_8H_{15}O_6N$: Molecular weight 221

Monoclinic

$a=11{\cdot}25\pm0{\cdot}03$ Å

$b=4{\cdot}82\pm0{\cdot}01$ Å

$c=9{\cdot}72\pm0{\cdot}02$ Å $\quad\beta=113{\cdot}7\pm0{\cdot}1°$

$V=483$ Å^3 $\quad D_m=1{\cdot}55\pm0{\cdot}02$ g.cm^{-3}

$Z=2$ $\quad D_x=1{\cdot}53$ g.cm^{-3}

$\mu=11{\cdot}7$ cm^{-1} for Cu radiation.

Systematic absences were observed only for the $0k0$ reflexions when k was odd. Since *N*-acetylglucosamine is optically active and the unit cell contains only two molecules, the space group is $P2_1$.

Relative X-ray intensities were estimated from equi-inclination Weissenberg photographs, taken with Cu $K\alpha$ radiation on multiple film packs, by visual comparison with a series of timed exposures of a single reflexion. Of the 825 reflexions available for measurement, representing 68% of the total data available within the copper sphere, 188 reflexions had intensities

which were too weak to measure. These were assigned fractional values of the minimum observable intensity in accordance with the proposals of Hamilton (1955). Intensities were corrected for Lorentz and polarization factors but no corrections were made for absorption or extinction. The scaling factors between individual Weissenberg levels were determined experimentally using the Stadler double slit method (Stadler, 1950).

Determination of the structure

Since the crystal contained a short *b* axis (4·82 Å) it was thought that the solution of the centrosymmetric (010) projection would prove relatively straightforward. Several attempts were made, all of which were unsuccessful. These included direct methods using Vand & Pepinsky's (1956) modification of Cochran & Douglas's (1955) method, and trial and error methods which involved the use of the two-dimensional Patterson synthesis, optical transforms and a minimum residual test for the best translation (Bhuiya & Stanley, 1964). In retrospect it was found that these methods had indicated structures which were almost correct but had failed to provide solutions near enough to the correct structure for the conventional methods of refinement to take over. In the structure of sugars there are a number of places where six-membered rings almost occur and this tends to confuse the interpretation when trial and error methods are used.

The structure was eventually solved by the application of the minimum function (Buerger, 1959) to the three-dimensional Patterson synthesis, which was suitably sharpened by the sharpening function employed by Jacobson, Wunderlich & Lipscomb (1961) for cellobiose:

$$M(S)=(1/\hat{f})^2(k+S^2/4)\exp(-aS^2),$$

where $k=1/6$ and $a=1{\cdot}36$ and the other symbols have their usual meaning.

In the application of the minimum function to non-centrosymmetric space groups it is essential to choose a single Harker peak for the first interaction, as this fixes both the symmetry element and the origin. A search was made for suitable peaks by moving a projection of the standard glucopyranose ring (Ramachandran, Ramakrishnan & Sasisekharan, 1963), plotted out on twice the scale, over the Harker section in the orientation deduced from the two-dimensional work. It was expected that the 'centre' of the molecule would be represented by a fairly large and diffuse peak due to the pseudo-centrosymmetric nature of the molecule (Patterson, 1949). From stereochemical considerations there appeared to be only three positions at which the centre could be placed so that all atoms lay on peaks. These are marked *A*, *B* and *C* in Fig. 1. Two of these (*B* and *C*) could be discarded from the knowledge that reflexions 100 and 001 were small. With the centre of the molecule on peak *A*, two atoms lay on small peaks which were either single or low-multiple Harker peaks (peaks 1 and 2 in Fig. 1).

Minimum functions were plotted graphically, using the following three peaks:

(1) Harker peak 1 to give minimum function $^1M_2$ with atom 1 placed arbitrarily at $y=0$.

(2) Harker peak 2 to give minimum function $^2M_2$ with atom 2 placed at $y=2/12$ from information derived from the peaks around the origin in the Patterson synthesis.

(3) The peak due to the interaction between atoms 1 and 2 in the same molecule to give the minimum function $^3M_2$.

Three sections only of each minimum function ($y=$ 0,1/12,2/12) were calculated since it was expected that the greater part of the molecule lay nearly in one plane. The three minimum functions were combined to give a fourfold minimum function $^{1,2,3}M_4$, which is shown in Fig. 2 for the section $y=0$. The map showed several spurious peaks, possibly owing to the multiple nature of the starting peaks, but from the knowledge of the model it was easy to place ten of the fifteen atoms on the appropriate peaks. The great advantage of the minimum function over the trial and error methods was that the atoms could now be placed with a greater precision.

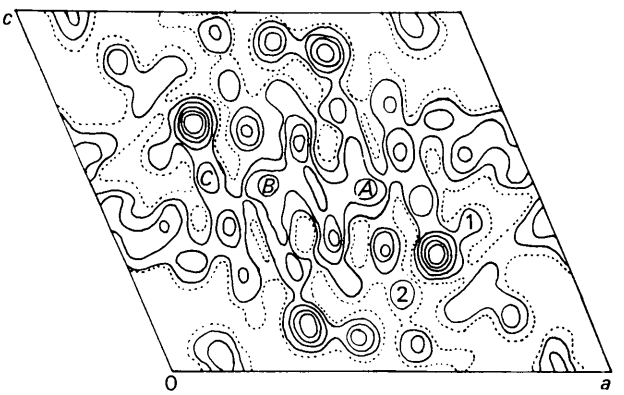

Fig. 1. The Harker section of *N*-acetylglucosamine. The lowest contour is shown dotted and is at −25 arbitrary units. The remaining contours are at intervals of 50.

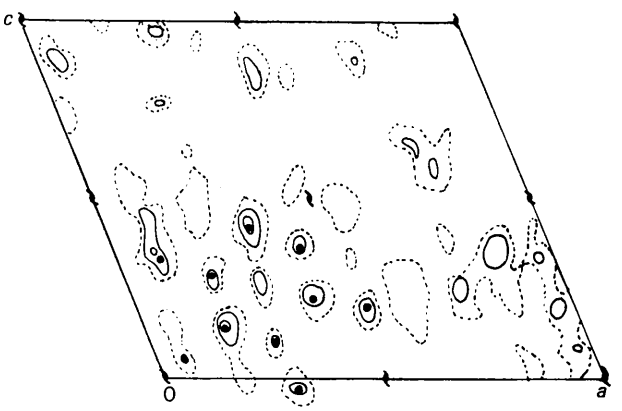

Fig. 2. The section $y=0$ of the minimum function $^{1,2,3}M_4$ for *N*-acetylglucosamine. The positions of ten atoms are indicated by full circles.

A Fourier synthesis of the (010) projection was calculated on the basis of the signs determined by the ten atoms with the coefficients suitably weighted (Woolfson, 1956). The remaining five atoms were easily located in this synthesis and a structure factor calculation with the total fifteen non-hydrogen atoms gave an agreement of 53·4%. After a few cycles of refinement in two dimensions, the third coordinates were estimated from a trial model and the minimum functions, and the refinement continued by an isotropic least-squares procedure until the agreement reached 18·4%. At this stage a difference Fourier synthesis indicated that the mode of vibration of the atoms was anisotropic, and the refinement was continued with an anisotropic least squares program written by G.A. Mair for the Elliott 803 computer. The program uses the block diagonal approximation and the coefficients were weighted by

$$w = 1/\left\{1 + \frac{(KF_o - b)^2}{a^2}\right\},$$

where a and b are constants selected to discriminate against weak reflexions and against very strong reflexions.

In about five cycles the agreement reached 11·4% and the difference Fourier synthesis showed a fairly even distribution of electron density apart from a positive region of 0·86 e.Å^{-3} at a distance of 1·2 Å from the C(1) position. This feature is discussed in more detail below. The positions of eleven of the fifteen hydrogen atoms were calculated from stereochemical considerations and all were found to lie on positive regions of between 0·15 and 0·4 e.Å^{-3}, although there were several other positive regions of the same height which could not be interpreted. It was not possible to fix the positions of the three hydrogen atoms attached to the methyl carbon of the acetyl group (presumably because of a free rotation about the C–C bond) and the hydrogen atom attached to O(4) which was not involved in a hydrogen bond. Inclusion of the eleven hydrogen atoms in the structure factor calculation gave a final agreement of 10·4%, excluding the unobserved reflexions and reflexions 011 and 110 which appeared to be affected by extinction. An agreement analysis is shown in Table 1 for observed and calculated structure factors. A complete list of observed and calculated structure factors may be obtained from the author. Atomic scattering curves used were those of Berghuis, Haanappel, Potters, Loopstra, MacGillavry & Veenendaal (1955) for carbon, Freeman (1959) for nitrogen and oxygen and McWeeny (1951) for hydrogen.

Table 1. *Agreement summary**

636 observed reflexions ($1{\cdot}98 \le |F_o| \le 65{\cdot}78$)
$R = 10{\cdot}4\%$

Category	Limits	Number
(1)	$\|\Delta F\| \le 1{\cdot}0\ \|F_{min}\|$, or $\|\Delta F\|/\|F_o\| \le 0{\cdot}2$	585
(2)	$1{\cdot}0\ \|F_{min}\| < \|\Delta F\| \le 2{\cdot}0\ \|F_{min}\|$, or $0{\cdot}2 < \|\Delta F\|/\|F_o\| \le 0{\cdot}3$	47
(3)	$2{\cdot}0\ \|F_{min}\| < \|\Delta F\| \le 3{\cdot}0\ \|F_{min}\|$, or $0{\cdot}3 < \|\Delta F\|/\|F_o\| \le 0{\cdot}4$	4
	$\|F_{min}\|$ = Minimum $\|F_o\| = 1{\cdot}98$	

* The agreement summary is similar to that described by Ahmed & Barnes (1963) in which the observed reflexions are divided into three categories according to selected limits for $|\Delta F| = ||F_o| - |F_c||$ and $|\Delta F|/|F_o|$. For 78% of the data, each reflexion falls into the same category on the basis of either criterion. Only 4 reflexions occur in category (3), which corresponds to fairly large discrepancies.

Description of the structure

The general features of the molecule are shown in Figs. 3 and 4. The pyranose ring is in the usual chair form with the O(1) oxygen and four hydrogen atoms in axial positions and the remaining oxygen atoms and C(6) in equatorial positions. Atoms O(1) and N(2) are in *cis* positions relative to the plane of the ring. The *N*-acetyl group is planar and the plane is approximately normal to the puckered ring.

The final atomic coordinates of the carbon, oxygen and nitrogen atoms are given in Table 2 together with their estimated standard deviations. As is well known, the absolute configuration of a molecule cannot be determined by X-ray diffraction except in the case of a structure which gives rise to anomalous scattering effects. The absolute configuration of α-D-glucose is known, however, from chemical work which relates

Table 2. *Final atomic coordinates and standard deviations for carbon, oxygen and nitrogen atoms of N-acetylglucosamine*

	x	$(10^4\sigma_x)$	y	$(10^4\sigma_y)$	z	$(10^4\sigma_z)$
C(1)	0·3323	(9)	−0·0147	(31)	0·4078	(14)
C(2)	0·2028	(8)	0·0862	(30)	0·2893	(10)
C(3)	0·1829	(8)	−0·0231	(32)	0·1331	(11)
C(4)	0·2954	(9)	0·0796	(35)	0·0953	(11)
C(5)	0·4213	(9)	−0·0513	(29)	0·2125	(11)
C(6)	0·5440	(10)	0·0395	(33)	0·1954	(13)
C(7)	0·9952	(9)	0·1530	(27)	0·3139	(11)
C(8)	−0·1154	(9)	0·0047	(36)	0·3336	(13)
N(2)	0·0965	(7)	−0·0214	(25)	0·3267	(10)
O(1)	0·3281	(7)	−0·3032	(23)	0·4150	(9)
O(3)	0·0665	(6)	0·1043	(20)	0·0234	(7)
O(4)	0·2785	(7)	−0·0396	(24)	−0·0504	(8)
O(5)	0·4341	(6)	0·0598	(21)	0·3575	(8)
O(6)	0·5541	(7)	0·3270	(23)	0·1948	(9)
O(7)	−0·0083	(7)	0·3978	(21)	0·2816	(9)

α-D-glucose and D-glyceraldehyde to D-tartaric acid, the absolute configuration of which has been established by Peerdeman, van Bommel & Bijvoet (1951). Care has been taken that the parameters listed in Table 2 describe the D form when referred to a right handed system of axes. The hydrogen atom parameters used in the final structure factor calculation are shown in Table 3, together with the isotropic temperature factors which were assigned to each atom on the basis of the isotropic temperature factor associated with the non-hydrogen atom to which it was bonded. The least-squares program uses the expression

$$\exp\left[-(B_{11}h^2+B_{22}k^2+B_{33}l^2+B_{23}kl+B_{31}lh+B_{12}hk)\right]$$

for the anisotropic temperature factor and values of B_{ij} for the non-hydrogen atoms are shown in Table 4. The results indicate that the *N*-acetylglucosamine molecule has considerable thermal anisotropy.

The bond lengths and bond angles were computed from the coordinates given in Table 2 and are shown in Table 5. The carbon–carbon bonds agree well with each other and with the accepted value, the mean being 1·53 Å with the greatest deviation of 0·02 Å. Variations amongst the carbon–oxygen bonds are slightly greater. The mean is 1·44 Å but both (C1)–O(1) and C(6)–O(6) differ from the mean by just over twice their standard deviations. The shortening of the C(1)–O(1) bond is a common feature among sugars and has been attributed by Hordvik (1961) to the partial double bond

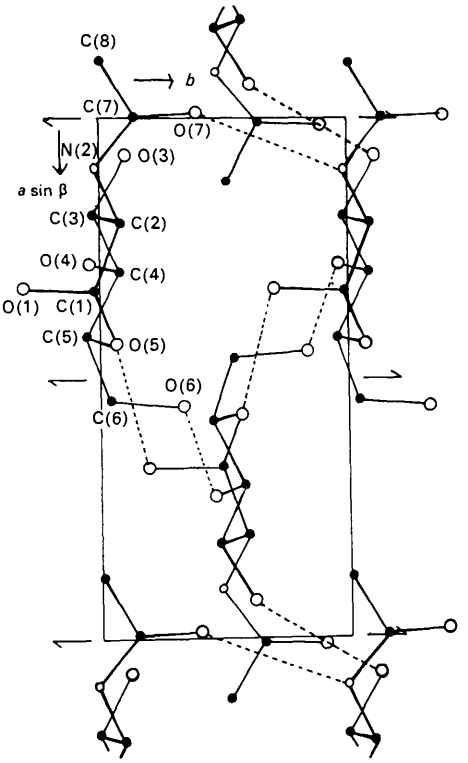

Fig. 4. The (001) projection of *N*-acetylglucosamine. Hydrogen bonds are shown dotted.

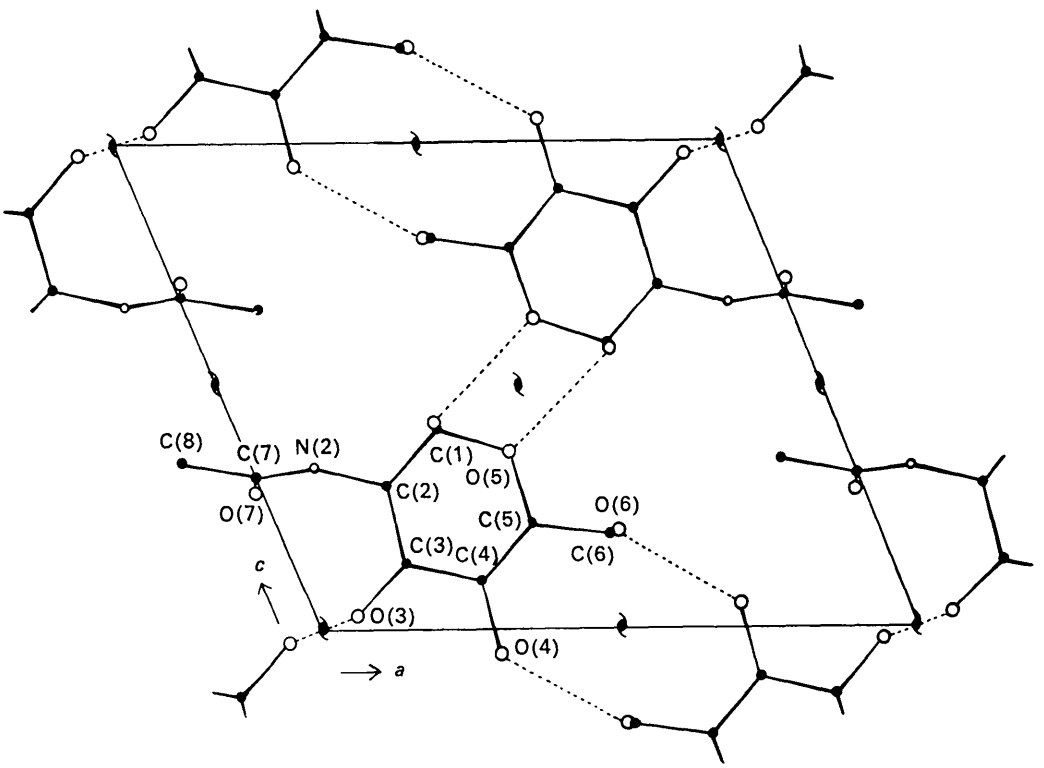

Fig. 3. The (010) projection of *N*-acetylglucosamine. Hydrogen bonds are shown dotted.

nature of the linkage. The shortening of C(6)–O(6) does not occur in other sugars and is difficult to explain. It may be that the standard deviations are over optimistic.

Table 3. *Atomic coordinates and isotropic thermal vibrations assigned to hydrogen atoms in the final structure factor calculations for N-acetylglucosamine*

Hydrogen atoms bonded to carbon atoms are indicated by the number corresponding to the carbon atom designation. Hydrogen atoms bonded to oxygen and nitrogen atoms are indicated by the number 1 or 2, respectively, followed by the atom designation.

	x	*y*	*z*	*B*
H(1)	0·3514	0·0853	0·5156	3·3 Å²
H(2)	0·2032	0·3136	0·2885	2·1
H(3)	0·1808	−0·2491	0·1317	3·8
H(4)	0·3018	0·3086	0·1063	4·7
H(5)	0·4106	−0·2768	0·2129	2·9
H(61)	0·6308	−0·0320	0·2933	3·3
H(62)	0·5380	−0·0277	0·0848	3·3
H(11)	0·4099	−0·3515	0·4941	4·0
H(13)	0·0311	−0·0706	0·0450	3·4
H(16)	0·6260	0·3740	0·1510	5·1
H(22)	0·0630	−0·2120	0·3130	3·3

The carbon valence angles in the pyranose ring range from 105 to 110°, which is a slightly greater deviation from the tetrahedral valence angle of 109·5° than is usually encountered. The smallest angle of 105°, for C(4)–C(5)–O(5), appears to be a consequence of the slight lengthening of the C(4)–C(5) bond, but the cause of this is not clear.

The planarity of the amide group is described by a root mean square distance of 0·04 Å of the five atoms from the best least-squares plane

$$0{\cdot}894x - 0{\cdot}987y + 8{\cdot}371z = 2{\cdot}740$$

calculated by the method of Schomaker, Waser, Marsh & Bergman (1959). The greatest deviation from the plane is 0·053 Å for the atom N(2). The dimensions of this group are similar to those of the standard amide group found from an examination of the structures of amino acids, simple peptides and other related substances (Corey & Pauling, 1953). The only significant difference is in the length of the N(2)–C(7) bond which is 0·06 Å longer in *N*-acetylglucosamine.

The packing of the *N*-acetylglucosamine molecules is determined by an almost complete system of hydro-

Table 4. *Anisotropic thermal parameters for the carbon, oxygen and nitrogen atoms of N-acetylglucosamine*

	B_{11}	B_{22}	B_{33}	B_{12}	B_{13}	B_{23}
C(1)	0·0034	0·0275	0·0160	0·0049	−0·0220	0·0054
C(2)	0·0025	0·0492	0·0059	−0·0002	−0·0127	0·0054
C(3)	0·0017	0·0461	0·0068	−0·0045	0·0067	0·0019
C(4)	0·0032	0·0602	0·0038	−0·0013	0·0063	0·0003
C(5)	0·0027	0·0305	0·0068	0·0077	0·0037	0·0060
C(6)	0·0037	0·0458	0·0134	−0·0072	−0·0020	0·0077
C(7)	0·0039	0·0128	0·0070	0·0123	0·0021	0·0019
C(8)	0·0035	0·0576	0·0127	0·0013	−0·0038	0·0100
N(2)	0·0024	0·0378	0·0098	−0·0162	−0·0051	0·0051
O(1)	0·0043	0·0450	0·0121	−0·0063	−0·0065	0·0044
O(3)	0·0026	0·0441	0·0053	−0·0051	0·0035	0·0007
O(4)	0·0042	0·0560	0·0074	−0·0088	0·0076	0·0051
O(5)	0·0026	0·0473	0·0073	−0·0037	0·0021	0·0028
O(6)	0·0070	0·0448	0·0120	0·0012	0·0035	0·0113
O(7)	0·0059	0·0326	0·0105	0·0008	−0·0042	0·0071

Table 5. *Bond lengths and bond angles in the N-acetylglucosamine molecule*

Standard deviations are shown in brackets

C(1)–C(2)	1·53 (0·01) Å	C(1)–O(1)	1·40 (0·02) Å
C(2)–C(3)	1·54 (0·02)	C(1)–O(5)	1·46 (0·01)
C(3)–C(4)	1·54 (0·02)	C(3)–O(3)	1·45 (0·01)
C(4)–C(5)	1·55 (0·02)	C(4)–O(4)	1·47 (0·01)
C(5)–C(6)	1·52 (0·01)	C(5)–O(5)	1·46 (0·01)
C(7)–C(8)	1·51 (0·02)	C(6)–O(6)	1·39 (0·02)
		C(7)–O(7)	1·22 (0·02)
C(2)–N(2)	1·48 (0·01)		
C(7)–N(2)	1·38 (0·02)		

C(2)–C(1)–O(1)	108·3 (0·7)°	C(5)–C(4)–O(4)	106·0 (0·6)°
C(2)–C(1)–O(5)	107·9 (0·7)	C(4)–C(5)–C(6)	113·8 (0·8)
O(1)–C(1)–O(5)	108·0 (0·7)	C(4)–C(5)–O(5)	105·5 (0·6)
C(1)–C(2)–C(3)	110·6 (0·6)	C(6)–C(5)–O(5)	105·0 (0·5)
C(1)–C(2)–N(2)	108·9 (0·7)	C(5)–C(6)–O(6)	111·5 (0·7)
C(3)–C(2)–N(2)	108·1 (0·6)	C(8)–C(7)–N(2)	113·2 (0·6)
C(2)–C(3)–C(4)	108·6 (0·6)	C(8)–C(7)–O(7)	123·3 (0·7)
C(2)–C(3)–O(3)	108·1 (0·6)	N(2)–C(7)–O(7)	123·6 (0·7)
C(4)–C(3)–O(3)	105·9 (0·7)	C(2)–N(2)–C(7)	118·9 (0·7)
C(3)–C(4)–C(5)	106·8 (0·7)	C(1)–O(5)–C(5)	116·5 (0·5)
C(3)–C(4)–O(4)	107·7 (0·6)		

gen bonding. Of the five available hydrogen atoms per molecule, four are utilized in the formation of hydrogen bonds. Three of these, O(1)–H(11)···O(5′), O(3)–H(13)···O(3′) and O(6)–H(16)···O(4′) are shown in Fig. 3. In addition there is a weak bond between the CO and NH groups of adjacent amide groups which is shown in Fig. 4. Hydrogen bond lengths are given in Table 6. Thus the intermolecular binding is strong in all directions, accounting for the hardness of the crystals and the absence of cleavage planes.

Table 6. *Hydrogen bond lengths in N-acetylglucosamine*

N(2)–H(22)···O(7′)	3·00 Å
O(1)–H(11)···O(5′)	2·78
O(3)–H(13)···O(3′)	2·78
O(6)–H(16)···O(4′)	2·84

The dimensions of the glucopyranose ring of the *N*-acetylglucosamine molecule are in close agreement with those found for other sugars. This is shown in Table 7 where in part (*a*) are given the mean coordinates, and their probable errors, of the glucopyranose unit as computed by Ramachandran, Ramakrishnan & Sasisekharan (1963) from the structures of eight sugars published before 1963, and in part (*b*) are given the corresponding parameters for *N*-acetylglucosamine, together with their deviations from the standard structure. The two sets are referred to the same orthogonal system of coordinates in which the atom C(3) is taken to be at the origin, C(3)–C(5) to be the *X* axis and C(1), C(3), C(5) to be in the *XY* plane. The *Z* axis is chosen normal to this plane so that *XYZ* form a right handed system. The maximum deviation of an atom of *N*-acetylglucosamine from the mean structure is 0·22 Å, while most of the deviations are less than 0·1 Å. A greater deviation is to be anticipated for N(2) since C–O and C–N bond lengths differ. In view of the fact that some of the structures included in the calculations of the standard structure are of rather low precision, the agreement appears to be satisfactory.

Table 7. *A comparison of the N-acetylglucosamine molecule with the standard structure for the glucopyranose ring proposed by Ramachandran et al.* (1963)

(*a*) Coordinates (Å) proposed by Ramachandran *et al.*

	x	*y*	*z*
C(1)	1·34 (0·04)	2·08 (0·02)	—
C(2)	0·05 (0·03)	1·44 (0·03)	0·47 (0·06)
C(3)	—	—	—
C(4)	1·24 (0·02)	−0·76 (0·03)	0·42 (0·06)
C(5)	2·50 (0·03)	—	—
O(5)	2·45 (0·02)	1·33 (0·03)	0·48 (0·04)
O(2)	−1·06 (0·02)	2·17 (0·04)	0·00 (0·10)
O(3)	−1·15 (0·03)	−0·62 (0·05)	0·50 (0·06)
O(4)	1·25 (0·03)	−2·06 (0·03)	−0·13 (0·14)
C(6)	3·80 (0·04)	−0·64 (0·06)	0·49 (0·09)

(*b*) Coordinates proposed for *N*-acetylglucosamine

	x	*y*	*z*
C(1)	1·28 (0·06)	2·18 (0·10)	—
C(2)	0·01 (0·04)	1·46 (0·02)	0·44 (0·03)
C(3)	—	—	—
C(4)	1·28 (0·04)	−0·68 (0·08)	0·57 (0·15)
C(5)	2·48 (0·02)	—	—
O(5)	2·42 (0·03)	1·39 (0·06)	0·44 (0·04)
N(2)	−1·19 (0·13)	2·14 (0·03)	−0·16 (0·16)
O(3)	−1·16 (0·01)	0·66 (0·04)	0·57 (0·07)
O(4)	1·25 (0·00)	−2·06 (0·00)	0·09 (0·22)
C(6)	3·85 (0·05)	−0·55 (0·09)	0·52 (0·03)

The possibility of the presence of the β sugar

As was mentioned earlier, the final difference Fourier synthesis showed an anomalous peak close to the C(1) position, which is illustrated in Fig. 5. The peak was rather large to be interpreted as a hydrogen atom, yet was too close to C(1) not to be bonded to it. It was thought that the peak might represent a trace of the β sugar in a structure which was largely in the α configuration. In solution the pure derivative of either the α or β form mutarotates to form an equilibrium mixture. The process of crystallization generally favours one derivative, but mixtures of the α and β sugars in the solid state are not unknown (Westphal & Holzmann, 1942).

If it is assumed that the heights of atoms not included in the phase determination approach 50% of their true height in a Fourier synthesis when the ratio of known to unknown atoms is large (Luzzati, 1953), an electron count of 0·86 e.Å^{-3} in a difference Fourier synthesis may be taken to represent 22% of an oxygen atom in the β configuration. Two cycles of least-squares refinement were computed on the basis of an 80% α structure and a 20% β structure with the refinement limited to the α and β oxygen atoms only. The agreement between observed and calculated structure factors fell by only 0·1%, but no large shifts were indicated for the two atoms. The bond length of C(1)–O(β) was 1·12 ± 0·06 Å and the valence angles were all close to those of a tetrahedral carbon atom. Refinement of a hydrogen atom in the position of the peak gave rise to a large negative temperature factor and did not appear encouraging.

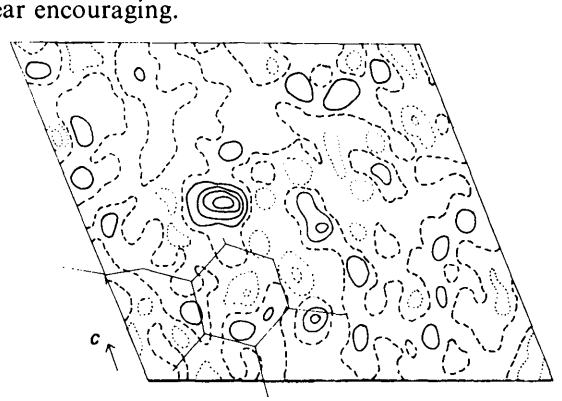

Fig. 5. The section $y=0{\cdot}10$ of the final difference Fourier synthesis showing the anomalous peak. The contours are at intervals of 0·2 e.Å^{-3}. Negative contours are dotted and the zero contour is shown by the broken line.

Optical rotation measurements showed that the crystalline *N*-acetylglucosamine had a lower optical rotation than the pure commercial sugar:

Commercial *N*-acetylglucosamine:

$[\alpha]_D = 82°$ ($C = 1{\cdot}00$ in water)

Once-crystallized *N*-acetylglucosamine:

$[\alpha]_D = 56{\cdot}5°$ ($C = 1{\cdot}06$ in water)

Both substances mutarotated to give a value of $[\alpha]_D = 39{\cdot}5°$ after $3\frac{1}{2}$ hr. Using Kuhn & Haber's (1953) values of $[\alpha]_D = 82°$ for *N*-acetyl-α-D-glucosamine and $[\alpha]_D = -21{\cdot}5°$ for *N*-acetyl-β-D-glucosamine, a simple calculation showed that the lowered value of the optical rotation of the crystalline derivative may be taken to represent a structure containing 75% of the α derivative and 25% of the β derivative, which is in full agreement with the suggestion from X-ray analysis.

Thus the evidence from these two independent experiments give some support to the hypothesis. However, this surprising result must be treated with caution. Refinement of the structure on this basis did not yield a significantly better agreement and neither was the C–O bond length reasonable. It is possible that there could be a slight shift in the β molecule as a whole which probably would not be detected in a crystal chiefly in the α configuration. It is interesting that an atom in the β position makes no short contacts with the other atoms in the structure and there is even the possibility of a hydrogen bond, O(6)–H···O(β) = 2·82 Å. Such an occurrence must be rare and no doubt explains why this phenomenon has not been observed in the structures of other sugars published so far.

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