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The Crystal and Molecular Structure of 1-Acetyl-3-benzamido-4-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyloxy)-Δ^3-2-pyrrolinone

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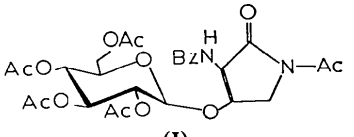
Crystals of the title compound are monoclinic, $a = 19{\cdot}629\ (5)$, $b = 7{\cdot}504\ (2)$, $c = 9{\cdot}830\ (2)$ Å, $\beta = 90{\cdot}53\ (2)°$, $Z = 2$, space group $P2_1$. The structure was solved by direct methods and was refined by full-matrix least-squares procedures to a final R of 0·079 for 1838 reflections with $I \geq 3\sigma(I)$. The structure consists of a pyranose ring in the chair conformation with the four *O*-acetyl substituents in equatorial positions; the pyranose ring is connected to a planar pyrroline ring *via* a β-oxygen bridge. All bond lengths and angles in the molecule are close to the predicted values.

Introduction

From reactions of glycosyl bromide with 2-phenyl-oxazol-5-one (Rosenthal & Brink, 1975) a β-glycoside considered to be 3-benzamido-1-benzoyl-4-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyloxy)-Δ^3-2-pyrrolinone was produced. On refluxing with acetic anhydride and sodium acetate in pyridine, one benzoate group was exchanged for an acetate group giving the title compound (I). The crystal structure analysis was undertaken to verify the initial assignment of structure based upon mass spectrum, NMR and chemical data.



(I)

Experimental

Recrystallization of 1-acetyl-3-benzamido-4-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyloxy)-Δ^3-2-pyrrolinone from hexane–acetone gave colourless rectangular crystals. The crystal chosen for study was mounted with **b** parallel to the goniostat axis and had dimensions of *ca* 0·20 × 0·20 × 0·07 mm. Unit-cell and space-group data were obtained from film and diffractometer measurements. The unit-cell parameters were refined by a least-squares treatment of $\sin^2\theta$ values for 19 reflections measured on a diffractometer with Cu $K\alpha$ radiation.

Crystal data

$C_{27}H_{30}N_2O_{13}$, $M_r = 590{\cdot}54$, monoclinic, $a = 19{\cdot}629\ (5)$, $b = 7{\cdot}504\ (2)$, $c = 9{\cdot}830\ (2)$ Å, $\beta = 90{\cdot}53\ (2)°$, $U = 1447{\cdot}8\ (6)$ Å^3, $Z = 2$, $D_c = 1{\cdot}35\ (1)$ g cm^{-3}, $F(000) = 620$ (20 °C, Cu $K\alpha$, $\lambda = 1{\cdot}5418$ Å, $\mu = 9{\cdot}4$ cm^{-1}). Absent reflections $0k0$, $k \neq 2n$ define the space group $P2_1$ (C_2^2, No. 4).

Intensities were measured on a Datex-automated General Electric XRD 6 diffractometer, with a scintillation counter, Cu $K\alpha$ radiation (Ni filter and pulse-height analyser), and a θ–2θ scan at 2° min^{-1} over a range of $(1{\cdot}80 + 0{\cdot}86 \tan\theta)°$ in 2θ, with 10 s background counts being measured at each end of the scan. Data were measured to $2\theta = 120°$ (minimum interplanar spacing 0·89 Å). Lorentz and polarization corrections were applied, and the structure amplitudes were derived. No absorption correction was applied because of the low value of μ. Of the 2336 independent reflections measured, 498 had intensities less than $3\sigma(I)$ above background where $\sigma^2(I) = S + B + (0{\cdot}05S)^2$ with S = scan count and B = time-averaged background count. These reflections were classified as unobserved and given zero weight in the refinement.

Structure determination and refinement

The structure was determined by direct methods, 373 reflections with normalized structure factor $|E| \geq 1{\cdot}35$ being used in the symbolic addition procedure for non-centrosymmetric crystals (Karle & Karle, 1966). 16 sets of phases were generated by a computer program which determines phases using the tangent formula (Karle & Hauptman, 1956; Drew, Templeton & Zalkin, 1969; Drew, private communication). The set chosen to generate the E map had the lowest value of Rk (0·24) and the greatest number of phases determined (358). From the E map the positions of 36 of the 42 heavy atoms were indicated in the top 50 peaks. Fixing the y coordinate of atom O(1) at 0·8300 to define the origin in the space group $P2_1$, and refining the remaining positional and isotropic thermal parameters for these 36 atoms gave an R factor of 0·230. A difference Fourier map revealed the positions of the remaining heavy atoms and two further cycles of full-matrix least-squares refinement with anisotropic thermal parameters for all the non-hydrogen atoms reduced R to 0·090. A difference Fourier map at this stage positively revealed the positions of only 16 of the 30 H atoms. The remaining H atoms were given calculated positions. Because of the excessive expense and the nature of the problem, the refinement was concluded after two more cycles, varying only the heavy-atom positional parameters and anisotropic temperature factors, but including the H atom positions and isotropic temperature factors, $U = 63 \times 10^{-3}$ Å^2, in the refinement. The final R value was 0·079 for 1838 reflections with $I \geq 3\sigma(I)$.

The atomic scattering factors and anomalous scattering correction for the C, N, and O atoms were taken from Cromer & Mann (1968), and Cromer & Liberman (1970) respectively, and the scattering factors for the H atoms from Stewart, Davidson & Simpson (1965). The anisotropic thermal parameters

Table 1. *Final positional parameters (fractional ×10⁴) with estimated standard deviations in parentheses*

	x	*y*	*z*
O(1)	2462 (3)	8300	6742 (5)
O(2)	2438 (3)	8305 (10)	3798 (5)
O(3)	3746 (3)	6996 (11)	2761 (5)
O(4)	4063 (3)	3691 (12)	3854 (6)
O(5)	3194 (3)	5947 (10)	6654 (5)
O(6)	3368 (3)	2618 (11)	7897 (6)
O(7)	2739 (4)	11172 (13)	4098 (9)
O(8)	3049 (5)	5920 (16)	1173 (7)
O(9)	5138 (4)	4203 (16)	4410 (8)
O(10)	4195 (5)	2094 (28)	9253 (10)
O(2′)	742 (3)	8638 (10)	10065 (5)
O(3′)	1556 (3)	3482 (11)	10067 (5)
O(4′)	1241 (3)	10214 (10)	5670 (5)
N(1′)	1500 (3)	6368 (11)	9479 (6)
N(2′)	1378 (3)	10750 (11)	7937 (6)
C(1)	2696 (4)	6858 (13)	5925 (7)
C(2)	3000 (4)	7716 (12)	4623 (7)
C(3)	3336 (4)	6195 (14)	3829 (8)
C(4)	3818 (4)	5135 (13)	4706 (8)
C(5)	3423 (4)	4384 (14)	5914 (8)
C(6)	3842 (4)	3291 (15)	6881 (8)
C(7)	2370 (5)	10137 (16)	3593 (9)
C(8)	1794 (5)	10462 (15)	2640 (9)
C(9)	3557 (6)	6639 (16)	1459 (8)
C(10)	4101 (6)	7327 (21)	504 (10)
C(11)	4714 (5)	3471 (18)	3740 (8)
C(12)	4883 (6)	2091 (23)	2708 (8)
C(13)	3622 (5)	2117 (19)	9044 (11)
C(14)	3068 (5)	1436 (20)	9977 (10)
C(2′)	1204 (4)	8038 (13)	9377 (7)
C(3′)	1570 (4)	8985 (13)	8274 (7)
C(4′)	2019 (4)	7866 (13)	7753 (7)
C(5′)	2012 (4)	6118 (13)	8459 (7)
C(6′)	1296 (4)	4929 (15)	10268 (7)
C(7′)	802 (5)	5234 (16)	11396 (8)
C(8′)	1183 (4)	11165 (15)	6638 (8)
C(9′)	900 (4)	13067 (13)	6494 (8)
C(10′)	1083 (5)	14088 (15)	5355 (8)
C(11′)	820 (5)	15805 (16)	5214 (10)
C(12′)	369 (5)	16449 (16)	6158 (11)
C(13′)	174 (5)	15410 (16)	7285 (9)
C(14′)	445 (4)	13764 (14)	7439 (9)

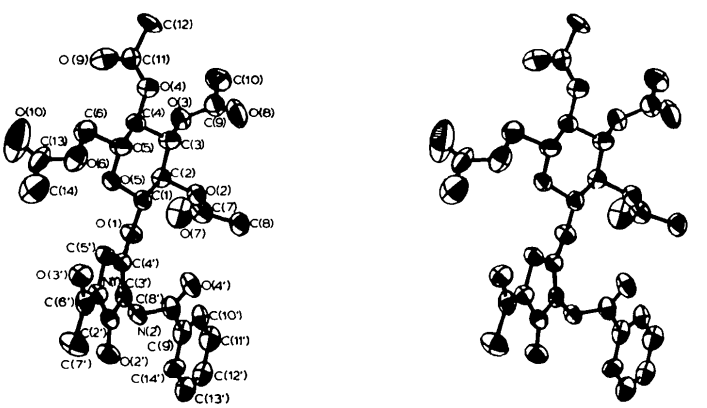


Fig. 1. A general stereoview of the molecule showing the crystallographic numbering scheme.

employed in the refinement are U_{ij} in the expression: $f = f^0 \exp[-2\pi^2(U_{11}h^2a^{*2} + U_{22}k^2b^{*2} + U_{33}l^2c^{*2} + 2U_{12}hka^*b^* + 2U_{13}hla^*c^* + 2U_{23}klb^*c^*)]$ where f^0 is the tabulated scattering factor and f is that corrected for thermal motion. The weighting scheme: $w = 1/\sigma^2(F)$ where $\sigma^2(F)$ is derived from the previously defined $\sigma^2(I)$ gave uniform average values of $w(F_o - F_c)^2$ over ranges of $|F_o|$ and was employed in the final stages of refinement.

An attempt was made to determine the absolute configuration of the molecule through anomalous scattering of the non-hydrogen atoms. The enantiomorph in Table 1, (*A*), and that generated by changing y to $-y$, (*B*), were both refined and Hamilton's (1965) test applied to the resulting *R*-factor ratios. Unfortunately neither (*A*) nor (*B*) was proved to be significantly more correct. (*A*) was therefore chosen in view of the chemical evidence. The final positional parameters are in Table 1.*

Description and discussion of the structure

Fig. 1 shows a general stereoview of 1-acetyl-3-benzamido-4-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyloxy)-Δ^3-2-pyrrolinone, with the crystallographic numbering scheme. Bond lengths and angles are summarized in Table 2. As expected, the six-membered ring is in the chair conformation with equatorial substituents at positions 2, 3, 4 and 5. The range of ring torsion angles [51·7 (7)–69·8 (7)°] is very close to that given by Kim & Jeffrey (1967) as typical of other pyranose rings. The *O*-acetyl groups attached to C(2), C(3), C(4), and C(6) are planar;* the average values for the bond lengths and angles (Table 2) are normal. The terminal atoms on these *O*-acetyl groups exhibit extensive thermal motion, in particular atom C(10), because of the length of these chains and the lack of very strong inter- or intramolecular bonding which might help to constrain these atoms.

Table 2. *Summary of bond lengths* (Å, $\sigma = 0{\cdot}01$ Å) *and angles* (*degrees*, $\sigma = 1°$)

Pyranose		*O*-Acetyl		Mean
C–C	1·50–1·55	O–C	1·29–1·40	1·33
C(5)–O(5)	1·45	C=O	1·14–1·19	1·17
O(5)–C(1)	1·39	C–CH_3	1·48–1·52	1·50
C(1)–O(1)	1·43	C–O–C	116–119	117
C–O	1·43–1·46	O–C=O	122–125	123
Angles in ring	106–112	O–C–CH_3	109–112	110
C(1)–O(1)–C(4′)	116	O=C–CH_3	123–129	127
Pyrroline		**Phenyl**		
C–N	1·37–1·42	C–C	1·35–1·41	
C(2′)–C(3′)	1·49	Angles in ring	119–121	
C(3′)–C(4′)	1·32			
C(4′)–C(5′)	1·48			
Angles in ring	103–112			
C(3′)–N(2′)–C(8′)	120			

The five-membered pyrroline ring (linked to the pyranose ring by a β-oxygen bridge) is planar within experimental error. Bond lengths and angles (Table 2) are close to those in related structures (Boeyens & Kruger, 1970; Fanfani, Nunzi, Zanazzi & Zanzari, 1974; Dupont, Dideberg & Welter, 1975). The benzamido group, the acetyl attached to N(1′), and the phenyl ring are planar, with normal bond lengths and angles.

The most significant intermolecular interaction is a probable hydrogen bond, N(2′)–H···O(3′)(x, $1 + y$, z), N···O 2·95 Å, C–N···O 117, 122°. The packing of the molecules in the crystal lattice is shown in Fig. 2.

* Lists of structure factors, thermal parameters, hydrogen parameters, and mean planes have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 33239 (15 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 13 White Friars, Chester CH1 1NZ, England.

* See deposition footnote.

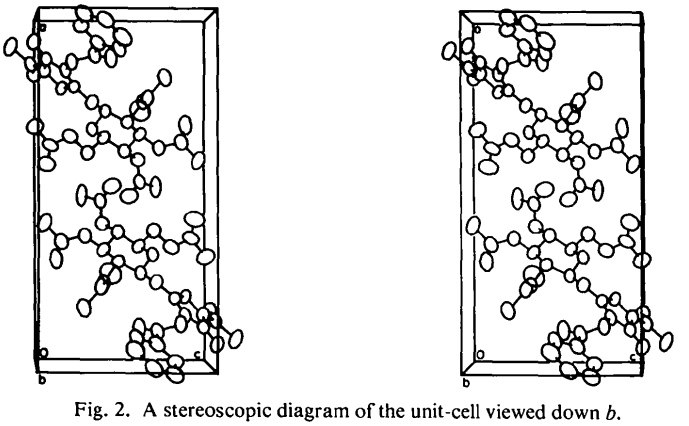


Fig. 2. A stereoscopic diagram of the unit-cell viewed down *b*.

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The Application of *MULTAN* to the Analysis of Isomorphous Derivatives in Protein Crystallography

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The application of *MULTAN* to the analysis of isomorphous derivatives of four proteins (phosphorylase b, phosphoglycerate kinase, hagfish insulin and triose phosphate isomerase) is described. The method leads to the correct structure for almost all the derivatives studied. The best phase set can be selected on the basis of the 'ABSFOM' figure of merit which represents the internal consistency of the phase set. The method is limited by the experimental accuracy of the isomorphous difference.

1. Introduction

The preparation of isomorphous derivatives can be the most time-consuming step in the determination of the crystal structure of a protein. After diffusion of a heavy-atom reagent there should be measurable changes in the diffracted intensities. These changes must be analysed to give an atomic model and the parameters of the model refined. For the first derivative the analysis is conventionally carried out by inspection of the difference Patterson synthesis.

For proteins of high molecular weight multi-site binding may be required to produce intensity changes which are statistically significant. Multi-site binding will necessarily occur when non-crystallographic symmetry elements are present. Unfortunately the difference Patterson map becomes increasingly complex as the number of equivalent positions in the space group and the number of binding sites per asymmetric unit rise.

Table 1. *Abbreviations used in the text*

EMP	Ethyl mercury phosphate
AUCN	Gold cyanide
PCMB	Mercury *p*-chlorobenzoate
BAKERS	The Bakers' dimercurial reagent
ABSFOM, RESID, PSIZERO	Figures of merit for the phase sets generated by *MULTAN* defined in § 2
F_H	The structure factor moduli of the heavy-atom structure
F_P	The structure factor moduli of the native protein
F_{PH}	The structure factor moduli of the isomorphous derivative
ΔF	The isomorphous difference $\|\|F_{PH}\| - \|F_P\|\|$
E_h	The normalized structure factor
R_c	Conventional R factor for least-squares refinement of the centric terms: $R_c = \sum(F_o - F_c)/\sum F_o$, where F_o is the observed and F_c the calculated structure factor amplitude. F_o is the isomorphous difference, ΔF, in this paper
B	Atomic temperature factor