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The Fundamental Structure of Cycasin, (Methyl-*ONN*-azoxy)methyl β-D-Glucopyranoside

BY M. KAWAMINAMI AND I. KAWANO

College of Liberal Arts, Kagoshima University, Korimoto, Kagoshima 890, *Japan*

A. KOBAYASHI, K. TADERA AND F. YAGI

Faculty of Agriculture, Kagoshima University, Korimoto, Kagoshima 890, *Japan*

AND S. KAWANO

College of General Education, Kyushu University, Ropponmatsu, Chuoku, Fukuoka 810, *Japan*

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Abstract

$C_8H_{16}N_2O_7$ is monoclinic, space group $C2$, with $a = 15{\cdot}422$ (1), $b = 4{\cdot}854$ (1), $c = 15{\cdot}754$ (1) Å, $\beta = 109{\cdot}83$ (1)°, $Z = 4$, $V = 1109{\cdot}4$ (5) Å^3, $M_r = 252{\cdot}22$, $D_m = 1{\cdot}49$, $D_x = 1{\cdot}51$ Mg m^{-3}. The structure was solved by the direct method using three-dimensional X-ray data and refined by the least-squares method to a final $R = 0{\cdot}100$ for 849 observed reflexions. In the crystal, a glucosyl part linked to the neighbouring glucosyl moieties by four different hydrogen bonds forms a double layer parallel to the (001) plane, and the aglycone parts are stretched in a direction perpendicular to the layers and have van der Waals contacts with other molecules.

Introduction

The compound cycasin was isolated as the toxic principle of the Japanese cycad, *Cycas revoluta* Thunb., and its chemical structure was determined as meth-

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ylazoxymethyl β-D-glucoside, $C_8H_{16}N_2O_7$ (Nishida, Kobayashi & Nagahama, 1955). This unusual aliphatic azoxy structure as the natural product was later found to be carcinogenic for rats (Laqueur, Mickelsen, Whiting & Kurland, 1963). The toxic biological properties of cycasin are due to the aglycone liberated on hydrolysis of the glycosidic linkage with β-glucosidase (Nishida, Kobayashi, Nagahama, Kojima & Yamane, 1956; Kobayashi & Matsumoto, 1965). The aglycone is unstable and decomposes spontaneously, but is stabilized by binding to the glucose moiety (and the glucoside). Cycasin is easily crystallized as fine needles from ethyl alcohol. The crystal structure of cycasin is of considerable interest, and we have already reported the crystal data (Kawaminami, Kawano, Tadera & Kobayashi, 1977; Kawaminami, Kawano, Kobayashi & Tadera, 1977). As weak super-reflexions were observed near $k = n + \frac{1}{2}$, cycasin may have a superstructure. But, in this paper, the fundamental structure of cycasin is described.

Experimental

The specimen was extracted from ground kernels of the cycad nuts with aqueous ethyl alcohol and by removal of the co-existing sugars by charcoal chromatography. The colourless crystals were obtained by cooling a hot alcoholic solution slowly to 273 K. They are long hexagonal prisms elongated along the *b* axis. The density was measured by flotation in a mixture of cyclohexane–carbon tetrachloride.

In preliminary experiments using rotating-crystal and Weissenberg photographs, extra reflexions were observed near the position $k = n + \frac{1}{2}$. They were very weak and a slight displacement was observed from the position of $k = n + \frac{1}{2}$ along the b^* axis. The weak reflexions from most of the samples were accompanied by diffuse streaks. It was concluded that the crystal has a fundamental structure with $b = 4{\cdot}854$ Å and that the weak reflexions may be caused by a superstructure along the *b* axis with stacking disorders.

Except for the extra reflexions mentioned above, the systematic absences are found to be $h = 2n + 1$ for $h0l$ and $h00$ reflexions and $k = 2n + 1$ for $0k0$ reflexions. As cycasin contains the glucosyl part and has optical activity, there should be no inversion or mirror symmetry in the crystal. The space group† is, therefore, $C2$. The non-centrosymmetry was determined by Wilson's statistics applied to the $h0l$ and hkl reflexions.

Cell dimensions were determined from double-radius Weissenberg photographs. The intensity data were obtained with a spherically ground crystal ($r = 0{\cdot}2$ mm) on a four-circle diffractometer (Syntex $P\bar{1}$) using monochromated Mo $K\alpha$ radiation at the Faculty of Pharmaceutical Sciences, Kyushu University.

A total of 1808 independent reflexions in the hemisphere $h,k,\pm l$ with $2\theta < 46°$ were collected by the θ–2θ scan technique with a variable scan rate from 4 to 12° min^{-1}, of which 849 with $I - 3\sigma(I) > 0$ were used as observed. The correction for Lorentz and polarization effects was applied. No correction for absorption or extinction effects was made ($\mu = 0{\cdot}143$ mm^{-1}, $\mu r = 0{\cdot}03$)

The structure was solved with *MULTAN* (Germain, Main & Woolfson, 1971). Sixteen sets of *E* maps were calculated for $E > 1{\cdot}0$ (297 reflexions). The peak positions were searched and the structure was developed by block-diagonal least-squares adjustment. Blocked-matrix least-squares refinement of the positional and the thermal parameters* (anisotropic for heavy atoms and isotropic for H) led to convergence to a final *R* of 0·100. The positions of H(1) ~ H(5) and H(10) ~ H(16) were generated geometrically and were mostly fixed throughout the refinement. The positions of H(6) ~ H(9) were taken from a difference Fourier map. Unit weights for the observed reflexions were adopted in the least-squares refinement. The atomic scattering factors and the anomalous-dispersion factors were taken from *International Tables for X-ray Crystallography* (1974). All the calculations have been carried out with the program system *UNICS*-II (Sakurai, Iwasaki, Watanabe, Kobayashi, Bando & Nakamichi, 1974; Kawano, 1980) adapted to a FACOM M-200 computer at the Computer Center, Kyushu University.

Results and discussion

The fractional atomic coordinates are listed in Table 1. The standard deviations of the *y* parameters are larger than those of the *x* or *z* parameters, because of the superstructure along the *b* axis with stacking disorder.

Cycasin is composed of two moieties: the β-D-glucosyl and the methylazoxymethanol moieties. An *ORTEP* (Johnson, 1965) stereo-projection of the molecule is shown in Fig. 1. The β-D-glucose ring conformation is that of a slightly distorted chair with puckering parameters (Cremer & Pople, 1975) of $Q = 0{\cdot}568$, $q_2 = 0{\cdot}099$, $q_3 = 0{\cdot}559$ Å, $\theta = 10{\cdot}1$ and $\varphi = 28{\cdot}4°$. The β conformation of the glucosyl moiety has been ascertained crystallographically by the present

† The published space group and the *b* cell dimension (Kawaminami, Kawano, Tadera & Kobayashi, 1977) are incorrect.

* Lists of structure factors and anisotropic thermal parameters have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 36030 (11 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

Table 1. *Fractional atomic coordinates* ($\times 10^4$, *for* H $\times 10^3$) *and isotropic thermal parameters* (B_{eq} *for non-*H *atoms*) *for cycasin*

The standard deviations are given in parentheses.

	x	y	z	B_{eq}/B (Å^2)
C(1)	2537 (10)	40 (37)	2602 (10)	3·0 (5)
C(2)	2873 (10)	613 (36)	1809 (9)	2·9 (5)
C(3)	2362 (10)	3054 (36)	1266 (9)	2·7 (5)
C(4)	1316 (10)	2819 (42)	1010 (10)	3·5 (5)
C(5)	1054 (10)	1931 (34)	1828 (11)	3·2 (5)
C(6)	26 (11)	1221 (49)	1535 (11)	4·6 (6)
C(7)	2987 (11)	−2495 (35)	3954 (10)	3·5 (5)
C(8)	4387 (12)	1311 (55)	5970 (11)	5·6 (7)
N(1)	3656 (8)	−518 (37)	4510 (8)	3·8 (5)
N(2)	3727 (8)	−734 (38)	5330 (9)	4·3 (6)
O(1)	2978 (7)	−2293 (26)	3060 (6)	3·4 (5)
O(2)	3831 (6)	1153 (27)	2138 (7)	3·7 (4)
O(3)	2618 (7)	3296 (25)	483 (6)	3·4 (4)
O(4)	915 (7)	5240 (24)	637 (8)	4·0 (5)
O(5)	1563 (7)	−505 (28)	2255 (7)	3·7 (5)
O(6)	−282 (7)	1175 (29)	2286 (8)	4·4 (4)
O(7)	3276 (8)	−2263 (34)	5649 (7)	5·2 (5)
H(1)	267 (9)	184 (37)	305 (9)	2 (3)
H(2)	275 (9)	−120 (32)	137 (8)	1 (3)
H(3)	259 (6)	490 (24)	165 (6)	−2 (2)
H(4)	105 (11)	133 (44)	50 (10)	3 (4)
H(5)	121 (9)	360 (32)	233 (8)	1 (3)
H(6)	433 (10)	−3 (39)	215 (10)	3 (4)
H(7)	261 (10)	272 (37)	−11 (10)	1 (3)
H(8)	59 (9)	671 (38)	27 (10)	2 (4)
H(9)	−94 (11)	113 (43)	224 (10)	3 (4)
H(10)	−10 (8)	−81 (31)	121 (7)	−1 (2)
H(11)	−36 (8)	281 (34)	106 (8)	0 (3)
H(12)	320 (7)	−457 (26)	421 (6)	−2 (2)
H(13)	231 (8)	−208 (31)	398 (7)	−1 (2)
H(14)	438 (11)	98 (45)	665 (11)	4 (4)
H(15)	414 (10)	347 (41)	577 (10)	3 (4)
H(16)	506 (11)	117 (49)	595 (11)	4 (5)

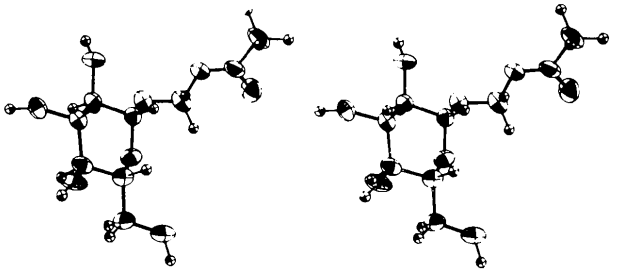

Fig. 1. *ORTEP* view (Johnson, 1965) of cycasin.

work, although it was confirmed from the results of enzymatic analysis and infrared spectrometry (Nishida *et al.*, 1955).

The β-D-glucosyl moiety is comparable with that in other compounds (Ferrier, 1963; Chu & Jeffrey, 1968; Takagi & Jeffrey, 1979). The bond lengths are listed in Table 2 (Fig. 2 shows the atom numbering). The C–C bond lengths in the glucose ring range from 1·52 to 1·54 Å with a mean value of 1·53 Å. The C(5)–C(6) bond length (1·53 Å) has the same value as the C–C bonds in the ring, and is rather longer than values for comparable bonds reported so far.

Table 2. *Bond lengths* (Å) *for cycasin*

The standard deviations are given in parentheses.

C(1)–C(2)	1·53 (2)	C(1)–O(1)	1·39 (2)
C(2)–C(3)	1·52 (2)	C(1)–O(5)	1·44 (2)
C(3)–C(4)	1·53 (2)	C(2)–O(2)	1·41 (2)
C(4)–C(5)	1·54 (3)	C(3)–O(3)	1·42 (2)
C(5)–C(6)	1·53 (2)	C(4)–O(4)	1·37 (2)
		C(5)–O(5)	1·45 (2)
C(7)–N(1)	1·46 (2)	C(6)–O(6)	1·42 (2)
C(8)–N(2)	1·53 (3)	C(7)–O(1)	1·41 (2)
N(1)–N(2)	1·26 (2)	N(2)–O(7)	1·24 (2)

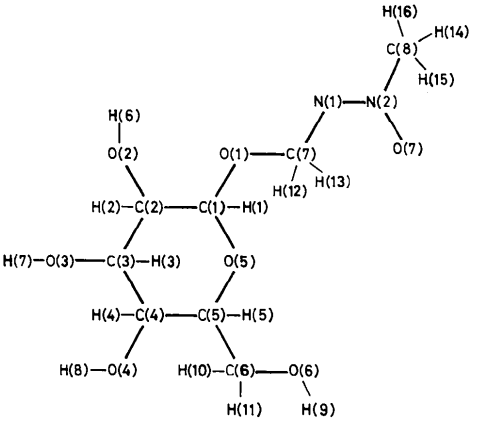

Fig. 2. The atom numbering.

The C–O bond lengths in the ring are 1·44 and 1·45 Å. These values are in good agreement with the reported values. The C(i)–O(i) (i = 1, ..., 4) bond lengths range from 1·37 to 1·42 Å with a mean value of 1·40 Å. They are slightly shorter than the C–O bonds in the ring. The C(6)–O(6) and the C(7)–O(1) bond lengths are of the same order as the above-mentioned C–O bond lengths.

The C–N bond lengths are 1·46 and 1·53 Å. The N=N bond length, 1·26 Å, is in good agreement with the value for the divalent N=N bond found in many compounds (Kennard, Watson, Allen, Isaacs, Motherwell, Pettersen & Town, 1972). The N–O bond length of 1·24 Å is the usual value.

The bond angles are listed in Table 3. Except for the angles around the N(2) atom, they range from 107 to 114° and correspond to generally accepted values. The mean values of the C–C–C, C–C–O and O–C–O bond angles are 111, 110 and 108°, respectively, and both the C–O–C and C–N=N angles average 113°.

The sum of the bond angles around N(2) is 360°. The torsion angles C(7)–N(1)–N(2)–O(7) and

Table 3. *Bond angles* (°) *for cycasin*

The standard deviations are given in parentheses.

C(2)–C(1)–O(1)	109 (1)	C(4)–C(5)–C(6)	111 (1)
C(2)–C(1)–O(5)	109 (1)	C(4)–C(5)–O(5)	111 (1)
O(1)–C(1)–O(5)	108 (1)	C(6)–C(5)–O(5)	107 (1)
C(1)–C(2)–C(3)	110 (1)	C(5)–C(6)–O(6)	111 (1)
C(1)–C(2)–O(2)	110 (1)	N(1)–C(7)–O(1)	109 (1)
C(3)–C(2)–O(2)	110 (1)	C(7)–N(1)–N(2)	111 (2)
C(2)–C(3)–C(4)	113 (1)	C(8)–N(2)–N(1)	115 (2)
C(2)–C(3)–O(3)	108 (1)	C(8)–N(2)–O(7)	118 (1)
C(4)–C(3)–O(3)	111 (1)	N(1)–N(2)–O(7)	127 (1)
C(3)–C(4)–C(5)	111 (1)	C(1)–O(1)–C(7)	114 (1)
C(3)–C(4)–O(4)	109 (2)	C(1)–O(5)–C(5)	111 (1)
C(5)–C(4)–O(4)	113 (2)		

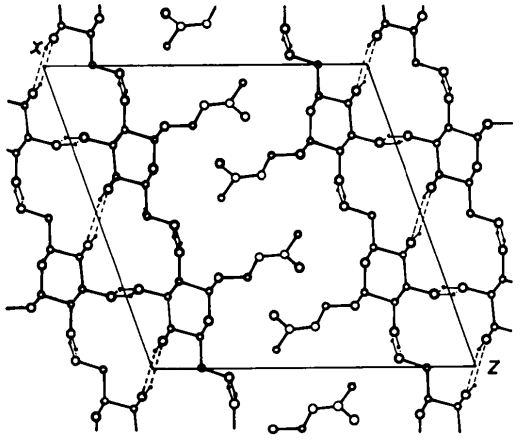

Fig. 3. A view of the molecular packing along the *b* axis showing the hydrogen bonds. The H atoms and bonds (<2·5 Å) are indicated by dots and dashed lines respectively.

C(7)–N(1)–N(2)–C(8) are −4 and −178°, respectively. Furthermore, the torsion angle O(1)–C(7)–N(1)–N(2) is −176°. This means that the aglycone methylazoxymethanol group is nearly planar. Table 4 lists the torsion angles.

The molecular packing projected along the *b* axis is shown in Fig. 3. The arrangement of the four molecules in the unit cell is as follows: the glucosyl parts form a double layer parallel to the (001) plane. The methylazoxymethanol parts extend in a direction perpendicular to the (001) plane and penetrate in alternating fashion into the opposing row of molecules. This packing mode could be expected from the characteristic chemical structure; the hydrophilic glucosyl parts are joined to each other by four different hydrogen bonds [O(2)···H(9), O(3)···H(7), O(4)···H(8) and O(6)···H(6)]. The hydrophobic aglycone parts are in contact with each other at van der Waals distances.

The molecular arrangement in the crystal may explain the four major external surfaces: (100), (010), (001) and (20$\bar{1}$).

As mentioned in the experimental section, the crystal may have a sort of superlattice structure, and a study on the origin of the extra reflexions is now under way.

Table 4. *Torsion angles* (°) *for cycasin*

The standard deviations are given in parentheses.

O(1)–C(1)–C(2)–C(3)	177 (1)	O(3)–C(3)–C(4)–C(5)	168 (1)
O(1)–C(1)–C(2)–O(2)	−62 (2)	O(3)–C(3)–C(4)–O(4)	−67 (2)
O(5)–C(1)–C(2)–C(3)	59 (2)	C(3)–C(4)–C(5)–C(6)	−170 (1)
O(5)–C(1)–C(2)–O(2)	180 (1)	C(3)–C(4)–C(5)–O(5)	−51 (2)
C(2)–C(1)–O(1)–C(7)	159 (1)	O(4)–C(4)–C(5)–C(6)	66 (2)
O(5)–C(1)–O(1)–C(7)	−83 (2)	O(4)–C(4)–C(5)–O(5)	−175 (1)
C(2)–C(1)–O(5)–C(5)	−66 (2)	C(4)–C(5)–C(6)–O(6)	−163 (1)
O(1)–C(1)–O(5)–C(5)	176 (1)	O(5)–C(5)–C(6)–O(6)	75 (2)
C(1)–C(2)–C(3)–C(4)	−50 (2)	C(4)–C(5)–O(5)–C(1)	63 (2)
C(1)–C(2)–C(3)–O(3)	−173 (1)	C(6)–C(5)–O(5)–C(1)	−176 (1)
O(2)–C(2)–C(3)–C(4)	−171 (1)	O(1)–C(7)–N(1)–N(2)	−176 (1)
O(2)–C(2)–C(3)–O(3)	66 (2)	N(1)–C(7)–O(1)–C(1)	−74 (2)
C(2)–C(3)–C(4)–C(5)	46 (2)	C(7)–N(1)–N(2)–C(8)	−178 (1)
C(2)–C(3)–C(4)–O(4)	172 (1)	C(7)–N(1)–N(2)–O(7)	−4 (2)

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