

The Crystal and Molecular Structure of the Intermediate Compound Obtained in Cyanide Reaction of Pyridoxal

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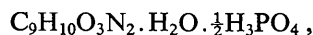
In order to elucidate the molecular structure of the intermediate compound obtained in the cyanide reaction of pyridoxal, an X-ray analysis was carried out of the crystal structure of its hydrobromide ($C_9H_{10}O_3N_2 \cdot HBr \cdot H_2O$). Crystals of the hydrobromide are monoclinic, space group $P2_1/a$ and the lattice parameters are $a=10.15$, $b=17.70$, $c=6.83$ Å, $\beta=110.8^\circ$. There are four molecules in the unit cell. The structure was solved by the heavy atom method using three-dimensional Fourier and difference Fourier syntheses. The refinement was carried out by the block-matrix least-squares calculations including anisotropic temperature factors. The final R value for 1306 non-zero reflexions was 0.095. The molecule is almost planar, only the oxygen atom of the hydroxymethyl group being out of the bicyclic plane. Molecules are bound together through four kinds of hydrogen bonds. The molecular structure was found to be of the aminofuran type, the exact name of the compound being 2-amino-3-hydroxy-4-hydroxymethyl-7-methyl-4²-furo[2,3-*c*]pyridine hydrobromide.

Introduction

The reaction of pyridoxal with cyanide in slightly alkaline solution produces a highly fluorescent compound and this reaction is commonly used in the quantitative analysis of pyridoxal or pyridoxal phosphate. As the name of this analytical procedure, 'cyanohydrin method' indicates, the product of this reaction was considered to be cyanohydrin (Bonavita & Scardi, 1959; Bonavita, 1960). Recently, Oishi & Fukui (1966*a,b*) have pointed out that the final reaction product which is strongly fluorescent is not cyanohydrin but 4-pyridoxolactone. We isolated a new compound in this reaction, which is considered to be an intermediate compound. The determination of the structure of this compound seemed to be of great help in elucidating the mechanism of the reaction.

Experimental

Pyridoxal (PAL) reacts with KCN in a phosphate buffer solution adjusted at pH 7.4. The intermediate product, 2-amino-3-hydroxy-4-hydroxymethyl-7-methyl-4²-furo[2,3-*c*]pyridine (PAL-CN) phosphate,



m.p. $159^\circ C$ (decomposition), (C: 40.13, H: 5.50, N: 10.29), was obtained under anaerobic conditions; it changes immediately to pyridoxolactone and cyanide anion in alkaline solution containing gaseous oxygen. PAL-CN hydrobromide was recrystallized from aqueous methanol solution in the form of pale yellow prisms with well developed {110} faces and elongated along the c axis. The elemental analysis indicated C: 36.79, H: 4.46, N: 9.73, which agrees well with the values calculated on the basis of $C_9H_{10}O_3N_2 \cdot HBr \cdot H_2O$.

The cell dimensions and space group were determined from precession photographs taken with Cu $K\alpha$ radiation ($\lambda=1.5418$ Å).

Crystal data

$C_9H_{10}O_3N_2 \cdot HBr \cdot H_2O$, M.W. 293.1, m.p. $157^\circ C$ (decomp.)

Monoclinic,

$$a = 10.15 \pm 0.015 \text{ \AA}$$

$$b = 17.70 \pm 0.02$$

$$c = 6.83 \pm 0.01$$

$$\beta = 110.8 \pm 0.1^\circ$$

$$U = 1137.3 \text{ \AA}^3$$

$$D_x = 1.712 \text{ g.cm}^{-3}$$

Linear absorption coefficient for Cu $K\alpha$ radiation,

$$\mu(\text{Cu } K\alpha) = 32.3 \text{ cm}^{-1}$$

$$F(000) = 592$$

Absent reflexions: $h0l$ when h is odd,
 $0k0$ when k is odd.

Space group: $P2_1/a$.

The three-dimensional intensity data were collected from equi-inclination Weissenberg photographs of layers ($h0l$)–($h8l$) and ($hk0$)–($hk3$) taken about the b and c axes. The multiple-film technique was employed and the intensities were measured by visual comparison with standard scales prepared for each axis. The specimens used were small enough for the absorption correction to be unnecessary and it was not applied. All the intensity data were then corrected for Lorentz and polarization factors. The resulting values were put on a single scale by correlating the structure factors on various layers and a total of 1306 structure factors were evaluated. A Wilson plot was then made, which gave an approximate scale factor and an overall temperature factor B of 2.33 \AA^2 .

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Determination of the structure

The position of the bromine atom was determined on the three-dimensional sharpened Patterson map. The first three-dimensional Fourier synthesis utilizing the phase angles given by the contributions of the bromine atoms showed eight light atoms as well defined peaks. Repeated cycles of structure factor and Fourier calculations revealed an unexpected aminofuran type structure (Fig. 1). Up to this point of the structure determination, a structure related to cyanohydrin had been assumed.

Refinement of the structure was carried out using the block-matrix least-squares program of Okaya & Ashida (1957). After five cycles of refinement in which the individual anisotropic temperature factors were allowed for, the R value dropped to 0.095. The weighting system used for the least-squares calculations was: $w=1.0$, when $F_o \leq 15.0$ and $w=15.0/F_o$, when $F_o > 15.0$. The final atomic parameters and their estimated standard deviations are listed in Table 1. The list of observed and calculated structure factors is given in Table 2. The averages of the standard deviations of the positional parameters are estimated to be: for the bromine atom $\sigma(r)=0.002 \text{ \AA}$; for the oxygen atoms $\sigma(r)=0.011 \text{ \AA}$; for the nitrogen atoms $\sigma(r)=0.013 \text{ \AA}$ and for the carbon atoms $\sigma(r)=0.015 \text{ \AA}$. The atomic scattering factors used for the present structure determination were: for bromine, those of Thomas & Umeda (1957) and for oxygen, nitrogen and carbon, those of Berghuis, Haanappel, Potters, Loopstra, MacGillavry & Veenendaal (1955).

Discussion of the structure

The molecular structure

Before the present structure determination was carried out, interpretation of the infrared absorption spectra had been the subject of many discussions. The compound shows a 1650 cm^{-1} absorption band but has no absorption band assignable to the cyanide group and, therefore the spectra could not be interpreted in terms of the cyanohydrin type structures which had been assumed for the present compound. It was also not possible to arrive at a correct structure from the spectral data only. However, the main feature of the infrared absorption bands has now been well accounted for by the present structure. The 1650 cm^{-1} band is assigned to the $C(2)-N(8)$ stretching vibration. The result of the present structure determination strongly indicates that pyridoxal cyanohydrin, a labile primary product formed by the addition of cyanide ion to pyridoxal, would change immediately to give the present compound as a result of an intramolecular reaction (Takanashi, Tamura, Yoshino & Iitaka, 1968*). A schematic diagram of the reaction is shown in Fig. 2.

* The chemical formula proposed for the present compound was incorrectly cited in this paper.

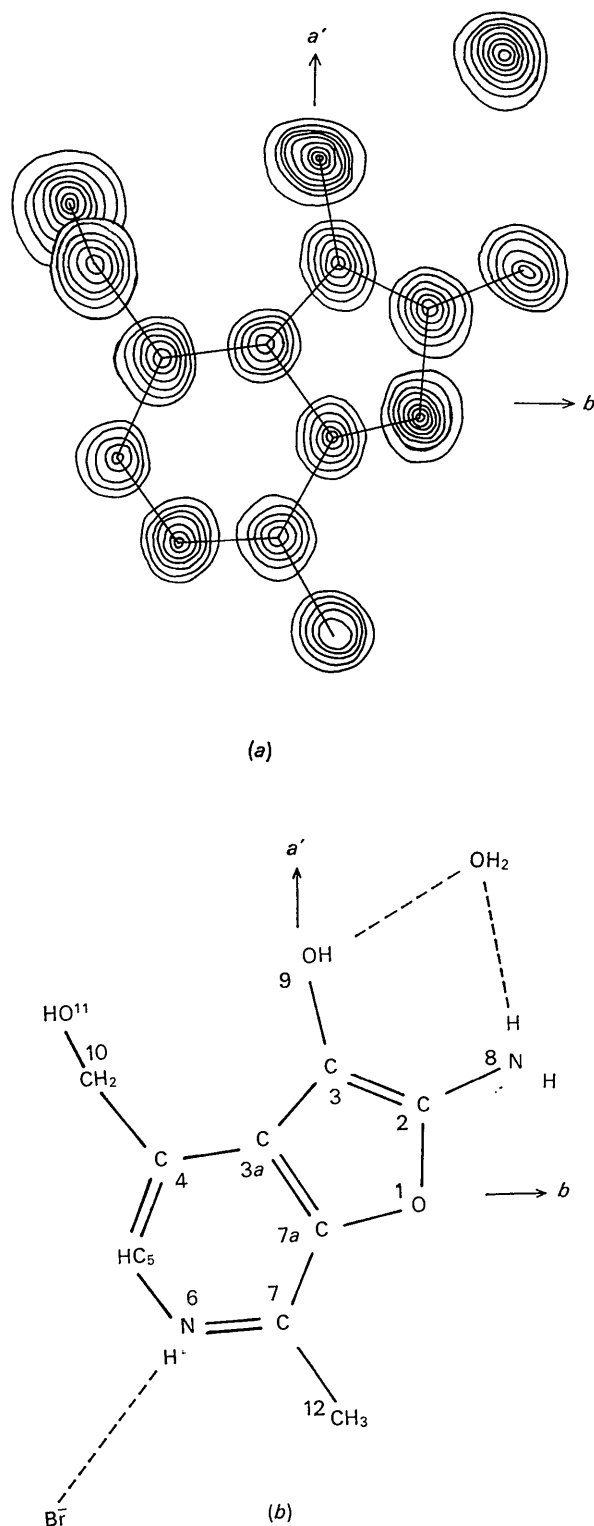


Fig. 1. (a) Composite electron density map of the structure viewed along the c axis. Contours are drawn at 1, 2, 3, ... $e \cdot \text{\AA}^{-3}$. Those for bromine atom are omitted. (b) Molecular structure. Broken lines indicate hydrogen bonds to water molecules and the bromine atom.

The bond lengths and angles, calculated from the coordinates given in Table 1, are shown in Figs. 3 and 4.

The mean standard deviation of the bond lengths is 0.01 Å and that of the bond angles estimated for the conjugated C=C-C bonds is 0.8°. The bond lengths involved in the pyridine and furan rings indicate a conjugate system extending over these two rings. The bond lengths in the pyridine ring vary from 1.35 to 1.43 Å. The average values for C-C and C-N bonds are 1.39 and 1.36 Å respectively. These values are consistent with those found in gaseous pyridine, determined by the method of electron diffraction (Almenningen, Bastiansen & Hansen, 1955) and microwave spectroscopy (Bak, Hansen & Rastrup-Andersen,

1954). The value obtained by electron diffraction was 1.377 Å (mean value of C-C and C-N) and the values obtained by microwave spectroscopy were 1.390 and 1.400 Å for C-C and 1.340 Å for C-N. In the crystals of nicotinamide (Wright & King, 1954) and ethionamide hydrochloride (Colleter & Gadret, 1968), the bond lengths in the pyridine rings are found to be within the range 1.37-1.40 Å for C-C and 1.33-1.37 Å for C-N bonds. It is also interesting to compare the endocyclic bond angles in the pyridine rings. For the free molecules of pyridine in the gaseous state, the angles subtended at the nitrogen atom and the α , β and γ carbon atoms are 116.7, 124.0, 118.6 and 118.1° respectively (Bak, Hansen & Rastrup-Andersen, 1954).

Table 1. *The final atomic fractional coordinates, thermal parameters and their standard deviations*

The temperature factors are expressed in the form

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

	<i>x</i>	<i>y</i>	<i>z</i>	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
Br	0.4547 (1)	0.2044 (1)	0.0385 (2)	0.0100 (1)	0.0027 (0)	0.0312 (4)	-0.0020 (0)	0.0025 (2)	-0.0008 (1)
C(2)	0.1300 (7)	0.0906 (4)	0.2995 (15)	0.0066 (7)	0.0015 (2)	0.0190 (25)	-0.0002 (3)	0.0016 (11)	0.0007 (8)
C(3)	0.1850 (7)	0.0218 (4)	0.3816 (14)	0.0056 (7)	0.0016 (2)	0.0170 (23)	0.0000 (3)	0.0014 (11)	0.0012 (8)
C(3a)	0.0764 (7)	-0.0302 (4)	0.3189 (14)	0.0056 (7)	0.0014 (2)	0.0175 (23)	0.0003 (3)	0.0031 (10)	-0.0007 (7)
C(4)	0.0591 (8)	-0.1088 (4)	0.3534 (15)	0.0067 (7)	0.0018 (2)	0.0187 (24)	0.0005 (3)	0.0040 (11)	0.0001 (8)
C(5)	-0.0722 (8)	-0.1375 (5)	0.2648 (16)	0.0079 (8)	0.0018 (2)	0.0216 (27)	-0.0001 (3)	0.0045 (13)	-0.0008 (8)
C(7)	-0.1770 (7)	-0.0184 (4)	0.1240 (13)	0.0056 (7)	0.0021 (2)	0.0142 (21)	0.0003 (3)	0.0028 (10)	0.0009 (8)
C(7a)	-0.0455 (7)	0.0112 (4)	0.2045 (14)	0.0066 (7)	0.0014 (2)	0.0178 (24)	-0.0007 (3)	0.0018 (11)	-0.0009 (7)
C(10)	0.1795 (9)	-0.1605 (5)	0.4666 (18)	0.0085 (8)	0.0018 (2)	0.0306 (34)	0.0004 (4)	0.0062 (14)	0.0026 (8)
C(12)	-0.3053 (8)	0.0252 (6)	0.0047 (18)	0.0047 (7)	0.0032 (3)	0.0293 (32)	0.0004 (4)	0.0018 (13)	0.0012 (10)
N(6)	-0.1853 (6)	-0.0934 (4)	0.1571 (13)	0.0053 (6)	0.0023 (2)	0.0232 (23)	-0.0007 (3)	0.0020 (10)	-0.0006 (8)
N(8)	0.1842 (7)	0.1600 (4)	0.3044 (15)	0.0086 (8)	0.0015 (2)	0.0306 (29)	-0.0003 (3)	0.0019 (12)	0.0013 (7)
O(1)	-0.0127 (5)	0.0865 (3)	0.1946 (10)	0.0065 (5)	0.0016 (2)	0.0168 (16)	0.0000 (2)	0.0007 (8)	0.0011 (5)
O(9)	0.3234 (5)	0.0060 (3)	0.4995 (11)	0.0050 (5)	0.0025 (2)	0.0259 (20)	-0.0002 (3)	0.0001 (8)	0.0004 (6)
O(11)	0.2584 (6)	-0.1799 (4)	0.3366 (13)	0.0081 (6)	0.0030 (2)	0.0318 (25)	0.0010 (3)	0.0057 (10)	-0.0025 (7)
O(W)	0.4682 (6)	0.1379 (4)	0.6042 (14)	0.0070 (6)	0.0031 (2)	0.0378 (26)	-0.0006 (3)	0.0049 (10)	0.0009 (7)

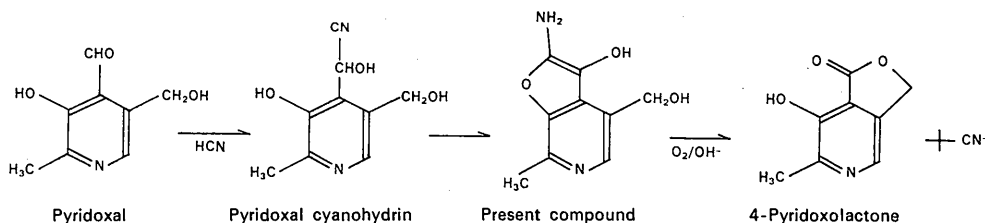


Fig. 2. Proposed reaction mechanisms of pyridoxal with cyanide.

$$-0.158X + 0.205Y + 0.966Z = 1.621$$

and

$$-0.175X + 0.185Y + 0.967Z = 1.622,$$

where X , Y and Z are measured in Å and taken along the crystallographic axes, a^* , b and c respectively. The deviations of the atoms from the least-squares planes

are shown in Table 3. It is clearly seen that the pyridine and furan rings are almost coplanar and all the atoms except the terminal oxygen atom O(11) of the hydroxy-methyl group are nearly on the plane within the maximum deviation of 0.027 Å. The C(10)–O(11) bond is turned away from the plane forming hydrogen bonds to the bromine atom and the water molecule.

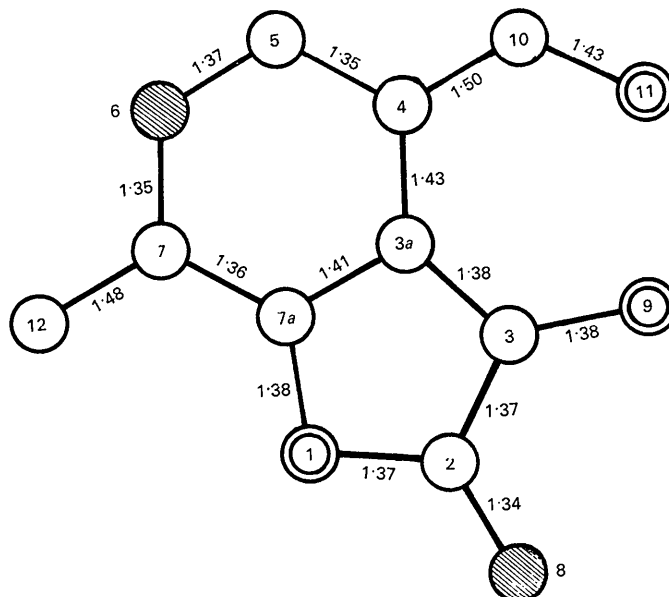


Fig. 3. Bond lengths (Å). Open circles indicate carbon, double circles indicate oxygen and shaded circles indicate nitrogen atoms.

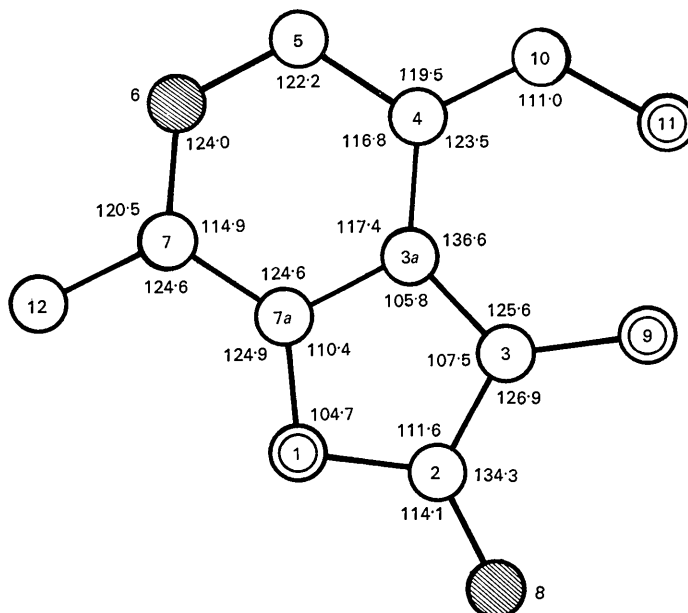


Fig. 4. Bond angles (°).

The crystal structure

The projections of the crystal structure along the a and the c axes are shown in Figs. 5 and 6 respectively. The numbers denoting the molecules indicate that the

molecules are: I at (x, y, z) , II at $(\frac{1}{2} + x, \frac{1}{2} - y, z)$, III at $(-x, -y, 1 - z)$ and IV at $(\frac{1}{2} - x, \frac{1}{2} + y, 1 - z)$, with x , y and z coordinates given in Table 1. The subscript attached to the molecular number indicates transla-

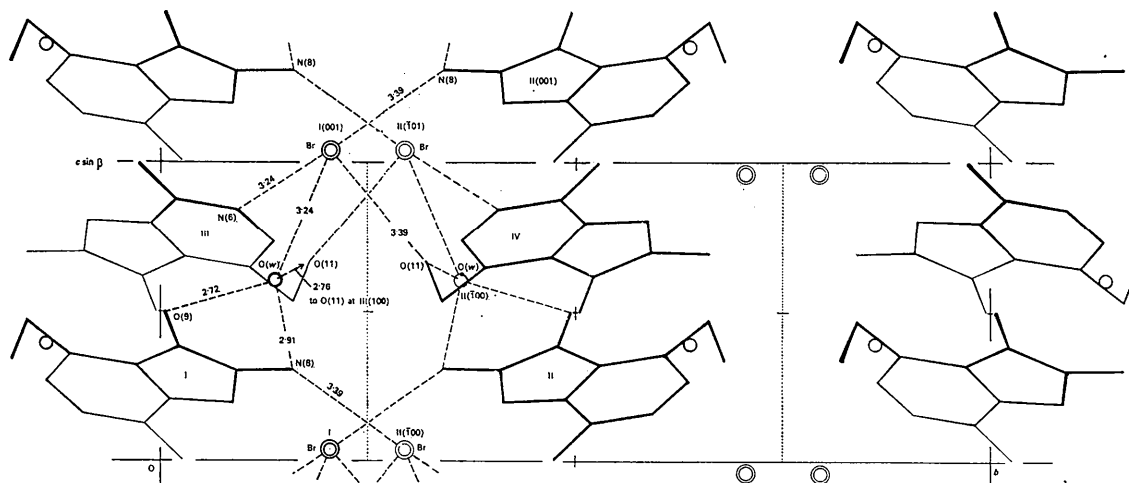


Fig. 5. Projection of the crystal structure along the a axis showing the stacking of the molecules and the hydrogen bonds (shown by broken lines).

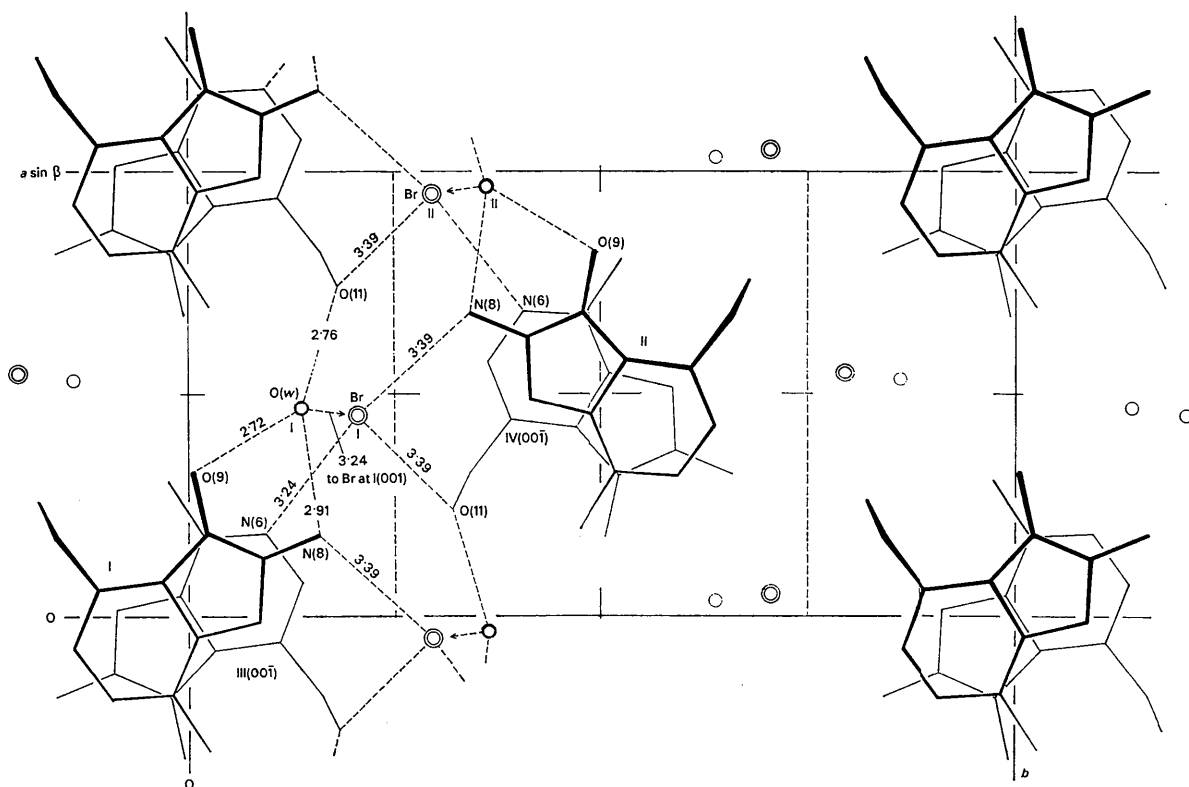


Fig. 6. Projection of the crystal structure along the c axis.

tions along the three edges of the unit cell. It is seen that the structure consists of infinite columns of molecules stacked along the *c* axis. Within the column the molecular planes are arranged parallel to each other but their normals are inclined at an angle of $14^{\circ}55'$ to the *c* axis. As shown in Fig. 5, there are two different modes of molecular overlap, one being across the centre

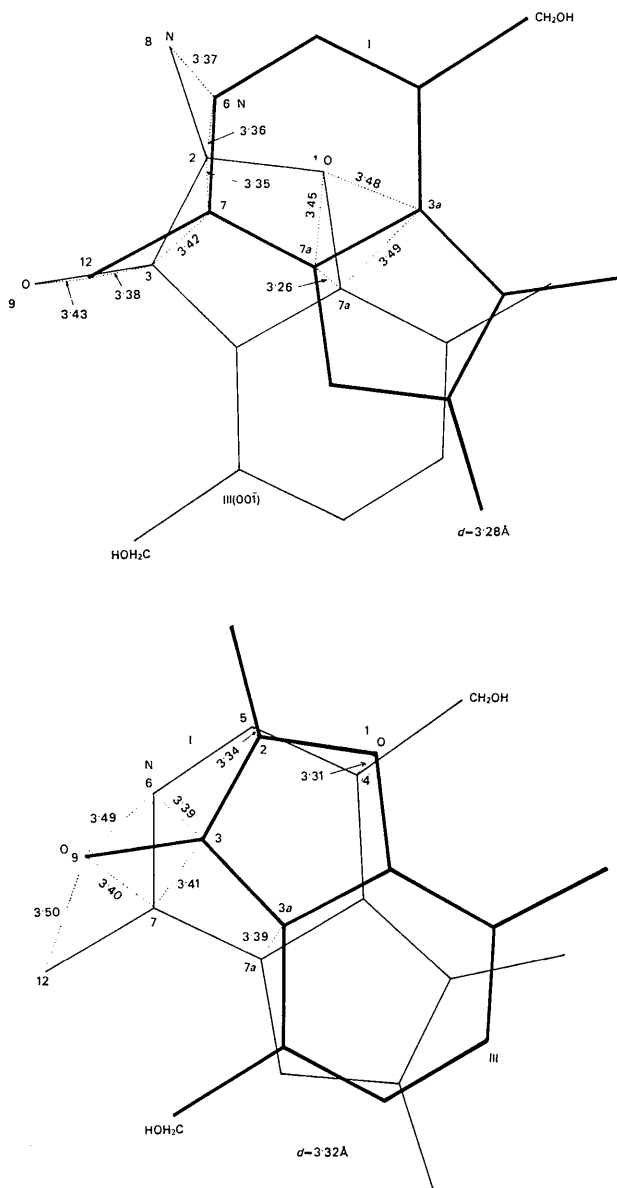


Fig. 7. Overlapping of the molecules within the molecular stack viewed along the normals of the molecular planes. The shortest intermolecular contacts of atoms less than 3.5 Å are shown by dotted lines. It should be noted that in each pair of molecules, the molecules are related by a centre of symmetry. *d* denotes the interplanar distances between the molecular planes. (a) The molecular overlap across the centre of symmetry at (0,0,0). (b) The molecular overlap across the centre of symmetry at (0,0,1/2).

Table 3. Deviations of atoms from the least-squares planes

The atoms marked by * are not included in the least-squares calculations.

Least-squares plane through the pyridine ring
 $-0.158X + 0.205Y + 0.966Z = 1.621$

Deviations	
C(3a)	-0.009 Å
C(4)	0.019
C(5)	-0.014
C(7)	0.011
C(7a)	-0.006
N(6)	-0.001
C(3)*	0.052
C(10)*	-0.021
C(12)*	0.022
O(1)*	0.039

Least-squares plane through the furan ring
 $-0.175X + 0.185Y + 0.967Z = 1.622$

Deviations	
C(2)	-0.017 Å
C(3)	0.016
C(3a)	-0.009
C(7a)	-0.001
O(1)	0.011
C(4)*	0.051
C(7)*	0.047
N(8)*	-0.037
O(9)*	0.032

Least-squares plane through the pyridine and furan rings
 $-0.165X + 0.197Y + 0.966Z = 1.639$

Deviations	
C(2)	-0.005 Å
C(3)	0.019
C(3a)	-0.027
C(4)	0.014
C(5)	-0.007
C(7)	0.009
C(7a)	-0.021
N(6)	0.008
O(1)	0.010
C(10)*	-0.027
C(12)*	0.022
N(8)*	-0.004
O(9)*	0.043

of symmetry at (0,0,0) and the other across the symmetry centre at (0,0,1/2). The spacings between the mean planes of the molecules and the shortest interatomic distances between the molecules of the pair are shown in Fig. 7. The intermolecular spacings, 3.28 and 3.32 Å, found within the molecular stack are close to those found in pyrimidines and purines. The spaces between the columns of the molecules are filled up by the water molecules and bromide ions and several kinds of hydrogen bonds, shown in Table 4, are formed between them. As shown in Fig. 6 and Table 4, each molecule accepts six hydrogen bonds: three from the water molecules and three from the bromide anions. The assumed positions of the hydrogen atoms are also shown in Table 4. The nitrogen atom of the pyridine ring is protonated and forms a fairly strong hydrogen bond to the bromide ion.

Table 4. *Hydrogen bond lengths and angles*

Hydrogen bonds attached to each molecule			
Hydrogen bonds	Lengths	Angles	
N(6)—H···Br ⁻	3·24 Å	C(5)—N(6)···Br ⁻	107·5°
		C(7)—N(6)···Br ⁻	128·2
N(8) / H···O(W)H ₂	2·91	C(2)—N(8)···O(W)	100·9
\ H···Br ⁻	3·39	C(2)—N(8)···Br ⁻	116·0
O(9)—H···O(W)H ₂	2·72	C(3)—O(9)···O(W)	110·6
O(11) / H—O(W)—H	2·76	C(10)—O(11)···O(W)	111·6
\ H···Br ⁻	3·39	C(10)—O(11)···Br ⁻	110·1

Hydrogen bond between the water molecule and the bromide ion



Hydrogen bonds around the water molecule

O(11)···O(W)···Br ⁻	148·6°
O(11)···O(W)···O(9)	110·4
O(11)···O(W)···N(8)	87·8
O(9)···O(W)···N(8)	67·8
O(9)···O(W)···Br ⁻	111·0
N(8)···O(W)···Br ⁻	104·0

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