The Crystal and Molecular Structure of the Phenethylammonium Salt of Fosfomycin
[Phenethylammonium (−)-(1*R*,2*S*)-Epoxypropylphosphonate Monohydrate: $C_8H_{12}N^+ . C_3H_6OPO_3^- . H_2O$]

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The structure of the phenethylammonium salt of fosfomycin has been determined. This salt crystallizes in the monoclinic system, space group $P2_1$, with two molecules in a unit cell of dimensions $a = 11.5397$ (17), $b = 6.1542$ (4), $c = 10.2053$ (13) Å and $\beta = 102.46$ (1)°. The structure was solved by the Patterson method and refined to an R of 3.3 for 1088 observed reflexions. The structure contains a molecule of water per asymmetric unit, hydrogen bonded to the phosphate group, the epoxy ring being in the *cis* conformation.

Introduction

Fosfomycin is a new antibiotic (Hendlin *et al.*, 1969; Glamkowski, Gal, Purick, Davidson & Sletzinger, 1970) produced by *Streptomyces fradiae*, grown in a variety of media, and is active against both Gram-positive and Gram-negative bacteria. The antibiotic is highly polar, optically active and has a low molecular weight. It inhibits a very early step in the peptido-glycan synthesis, binding irreversibly to the enzyme enol-pyruvate-uridine-diphospho-*N*-acetylglucosamine transferase. This enzyme is intracellular and the antibiotic gains access to it *via* a transport system whose normal substrate is glycerol phosphate. Fosfomycin exhibits a direct P–C linkage which is rare in nature. The formula of the phenethylammonium salt used for the X-ray analysis and the numbering system adopted throughout this paper are shown in Fig. 1. A stereoscopic view of the molecule in the asymmetric unit is given in Fig. 2.
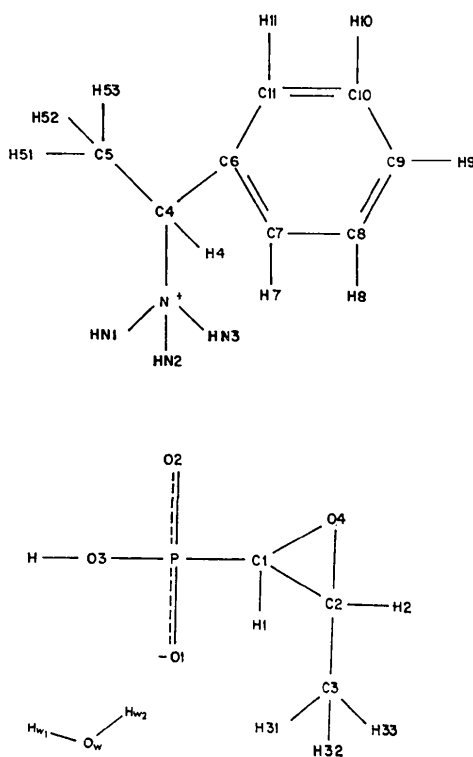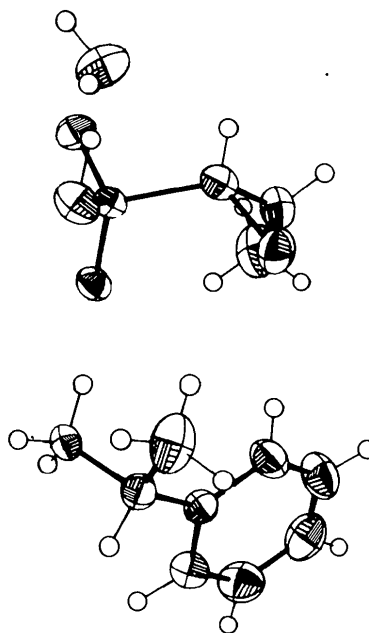

Fig. 1. Chemical formula and numbering scheme.

Fig. 2. A perspective view of the salt. The atoms are represented by 50% probability thermal ellipsoids.

Experimental

Suitable crystals of fosfomycin were kindly provided by Compañia Española de Penicilina y Antibioticos SA, Madrid. Precession and Weissenberg photographs showed $2/m$ reciprocal-lattice symmetry, and the systematic absence $0k0$ for k odd suggested the space group $P2_1$ or $P2_1/m$. Because of the optical activity of the compound, $P2_1$ was presumed to be correct and the structure determination and refinement were successfully carried out in this space group. Crystal data are given in Table 1.

Table 1. *Crystal data*

$C_3H_6PO_4^- \cdot C_8H_{12}N^+ \cdot H_2O$, $M_r = 277 \cdot 243$
Monoclinic, space group $P2_1$
$a = 11 \cdot 5397\ (17)$ Å $D_c = 1 \cdot 240\ \mathrm{g\ cm^{-3}}$
$b = 6 \cdot 1542\ (4)$ $F(000) = 232$
$c = 10 \cdot 2053\ (13)$ $\mu = 2 \cdot 038\ \mathrm{cm^{-1}}$
$\beta = 102 \cdot 46\ (1)^\circ$ Crystal size: $0 \cdot 5 \times 0 \cdot 35 \times 0 \cdot 2$ mm
$V = 706 \cdot 823$ Å^3 $\lambda(\mathrm{Mo}\ K\alpha) = 0 \cdot 71069$ Å
   $Z = 2$

Intensity data were collected on an automatic PW 1100 four-circle diffractometer with graphite-monochromatized Mo $K\alpha$ radiation using the $\omega/2\theta$ scan mode. 1665 independent reflexions were scanned of which 1235 were above the assigned threshold value of $2\sigma(I)$ [where $\sigma(I)$ is from counting statistics]; two standard reflexions were monitored during data collection at intervals of 37 reflexions and showed no significant change in intensity. The intensities were corrected for geometrical factors but not for absorption.

Structure determination and refinement

The structure was solved by the heavy-atom method. The position of the P atom was obtained from the Harker section $(u,\frac{1}{2},v)$ of the three-dimensional Patterson map; the P atom x and z coordinates were obtained from this section, the coordinate y was fixed at $\frac{1}{4}$. A Fourier electron density calculation based on the P position alone showed the phosphate group. Two successive Fourier syntheses were sufficient to establish the coordinates of all the atoms other than H. After three cycles of isotropic and three more of anisotropic refinement with unit weights, the R value was $7 \cdot 2$. All H atoms except $H(w2)$ were located in $(F_o - F_c)$ maps. The difference Fourier synthesis gave no clue to the position of this H atom and it was located by geometrical considerations, $H(w1)$ having been positioned in the previous difference synthesis. The H atoms were included in the model with isotropic temperature

Table 2. *Final atomic parameters for non-hydrogen atoms*

E.s.d.'s are given in parentheses.

	x	y	z
P	0·1194 (1)	0·2500 (0)	0·2740 (1)
O(1)	0·0654 (2)	0·1303 (5)	0·1479 (2)
O(2)	0·1001 (2)	0·1560 (4)	0·4021 (2)
O(3)	0·0740 (2)	0·4928 (5)	0·2655 (3)
O(4)	0·3587 (2)	0·3229 (5)	0·3982 (3)
O(w)	0·1141 (2)	0·7220 (5)	0·0717 (2)
N	0·0697 (2)	0·3238 (5)	0·6410 (3)
C(1)	0·2755 (3)	0·2676 (8)	0·2737 (3)
C(2)	0·3677 (3)	0·1194 (9)	0·3322 (4)
C(3)	0·3482 (5)	−0·0835 (8)	0·4017 (6)
C(4)	0·1748 (3)	0·4318 (6)	0·7318 (4)
C(5)	0·2156 (4)	0·6140 (8)	0·6519 (5)
C(6)	0·2670 (3)	0·2621 (8)	0·7892 (3)
C(7)	0·3650 (3)	0·2229 (8)	0·7347 (4)
C(8)	0·4479 (4)	0·0693 (9)	0·7909 (5)
C(9)	0·4363 (4)	−0·0458 (8)	0·9017 (5)
C(10)	0·3394 (4)	−0·0118 (9)	0·9551 (4)
C(11)	0·2564 (4)	0·1433 (9)	0·9011 (4)

Table 3. *Final atomic parameters for hydrogen atoms, and* C—H, N—H *and* O—H *bonds*

E.s.d.'s are given in parentheses.

	x	y	z	U (Å^2)	C—H (Å)
H(1)	0·283 (4)	0·366 (8)	0·193 (4)	1·9 (1·0)	1·01 (5)
H(2)	0·346 (4)	0·121 (10)	0·290 (5)	4·0 (1·4)	0·98 (3)
H(31)	0·283 (3)	−0·075 (5)	0·441 (3)	2·8 (0·7)	0·93 (3)
H(32)	0·419 (4)	−0·136 (9)	0·470 (4)	4·2 (1·3)	1·00 (4)
H(33)	0·329 (5)	−0·198 (10)	0·327 (6)	5·9 (1·8)	1·03 (6)
H(4)	0·137 (3)	0·478 (7)	0·802 (3)	0·6 (0·9)	0·96 (4)
H(51)	0·241 (6)	0·571 (14)	0·570 (7)	8·6 (2·3)	0·97 (8)
H(52)	0·150 (5)	0·729 (12)	0·624 (6)	5·9 (1·8)	1·03 (7)
H(53)	0·285 (5)	0·678 (9)	0·712 (6)	2·9 (1·7)	0·98 (5)
H(7)	0·366 (4)	0·301 (9)	0·645 (5)	5·2 (1·5)	1·03 (5)
H(8)	0·516 (5)	0·047 (9)	0·760 (5)	4·3 (1·5)	0·92 (6)
H(9)	0·494 (4)	−0·135 (8)	0·939 (4)	2·9 (1·1)	0·89 (4)
H(10)	0·329 (4)	−0·089 (8)	1·04 (4)	4·3 (1·2)	0·98 (5)
H(11)	0·182 (4)	0·162 (9)	0·937 (5)	3·2 (1·3)	1·01 (5)

					N—H (Å)
H(N1)	0·089 (3)	0·276 (9)	0·555 (4)	2·1 (1·0)	1·00 (4)
H(N2)	0·003 (3)	0·418 (6)	0·618 (3)	0·0 (0·7)	0·95 (3)
H(N3)	0·044 (3)	0·201 (6)	0·680 (3)	1·1 (0·8)	0·93 (4)

					O—H (Å)
H(O3)	0·089 (7)	0·556 (13)	0·173 (8)	6·6 (2·3)	1·07 (8)
H(w1)	0·055 (4)	0·701 (9)	−0·006 (5)	3·8 (1·5)	0·88 (5)
H(w2)	0·088 (6)	0·846 (13)	0·099 (7)	4·9 (2·2)	0·85 (8)

$H(w1)-O(w)-H(w2) = 106 \cdot 5\ (5 \cdot 7)^\circ$

factors which were not refined at this stage. For the last cycle of least-squares refinement the H atom positions and their B values were refined. The final weighted and unweighted R values were both $3 \cdot 3$ $[R_w = (\Sigma\ w\Delta^2/\Sigma\ w|F_o|^2)^{1/2}]$ with $\langle w\Delta^2 \rangle$ showing no trends.

The weighting scheme was $w = Kw_1 w_2$ with $w_1 = 1/\sigma_f^2$ and $w_2 = 1/\sigma_s^2$ where σ_f and σ_s are as follows:

$$\sigma_f = (1 \cdot 2462 - 0 \cdot 2377 |F_o| + 0 \cdot 0161 |F_o|^2)$$
$$\text{if} \qquad 0 < |F_o| < \ 8 \cdot 35$$
$$\sigma_f = (0 \cdot 1061 + 0 \cdot 0319 |F_o|)$$
$$\text{if} \quad 8 \cdot 35 < |F_o| < 12 \cdot 51$$
$$\sigma_f = (0 \cdot 2880 + 0 \cdot 0124 |F_o|)$$
$$\text{if} \quad 12 \cdot 51 < |F_o| < 66 \cdot 44$$
$$\sqrt{\sigma_s} = (4 \cdot 6959 - 17 \cdot 8403 s + 20 \cdot 2215 s^2)$$
$$\text{for any } s \text{ values.}$$

s is $\sin \theta / \lambda$ and $K = 1 \cdot 0152$. A final difference map showed no features greater than $0 \cdot 2$ e Å^{-3} or related to any part of the structure.

The atomic scattering factors for all the atoms and dispersion corrections for the P atom were those in *International Tables for X-ray Crystallography* (1974).*

The final atomic parameters and their standard deviations for atoms other than H are listed in Table 2. Those for the H atoms together with C—H bonds are given in Table 3. Table 4 gives the bond lengths and angles for non-hydrogen atoms.

Computer programs used were those of the XRAY system (1970) on a Univac 1108 computer.

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* Lists of structure factors and anisotropic thermal parameters have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 32946 (7 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 13 White Friars, Chester CH1 1NZ, England.

Results and discussion

The phosphate group

The phosphate group has a peculiarity which is unique in antibiotics, that is, the direct linkage P—C, which normally takes place through an oxygen bridge P—O—C. The P—C length is $1 \cdot 809$ Å which is identical to the P—C bond length of $1 \cdot 807$ Å in β-ciliatine (Okaya, 1966), the first example of a P—C linkage in biological material. A similar P—C value is found in nitrilotrimethylenetriphosphonic acid (Daly & Wheatley, 1967). The P—O distances in fosfomycin appear to depend on the precise state of the O atom. The longest, $1 \cdot 578$ Å, corresponds to the P—OH bond and is slightly longer than the average in organic phosphates (monoester monoanions), but still shorter than the P—O single-bond length of $1 \cdot 71$ Å calculated by the Schomaker & Stevenson (1941) rule. The shortest P—O distances, P—O(1) $1 \cdot 499$ Å and P—O(2) $1 \cdot 490$ Å, can be described as PO⁻ bonds, each O atom bearing a fractional negative charge; that is to say in mono-anionic monophosphates the negative charge on the phosphate group is mainly delocalized among the phosphate P—O(1) and P—O(2) bonds but there is a tendency for P—O⁻ to be slightly longer than P=O. The O—P—O valence angles in fosfomycin are 108–$117°$, the largest angle involving the unprotonated atoms O(1) and O(2). The angle between planes O(2)—P—O(1) and O(3)—P—C(1) is $87°$.

The epoxy ring

The epoxy rings are not frequently described but they are very interesting since they readily open and can react very easily. It is generally accepted that in the electronic structures of systems such as oxiranes their bent bonds create regions of greater electron density outside the ring. However, the final difference Fourier map of fosfomycin did not show any region of greater electron density around the oxirane ring. On the other hand, the strain makes the opening of the ring easier by the action of both nucleophilic and electrophilic reagents. It is interesting to consider the geometry of this epoxy ring and its surroundings. The Csp^3–Csp^3 single bond C(1)—C(2) is significantly short, $1 \cdot 47$ Å, as is the adjacent bond C(2)—C(3), $1 \cdot 48$ Å. The C—O bonds are slightly different from each other, C(1)—O(4) being longer than C(2)—O(4), and the valence angles have an average value of $60°$.

The oxirane ring shows a *cis* conformation, the hydrogen atoms H(1) and H(2) being displaced $0 \cdot 86$ and $0 \cdot 88$ Å respectively from the plane of the epoxy ring. The dihedral angle between the oxirane ring and the plane P—O(2)—C(1) is $83°$; the rotation angle around P—C(1) is $154°$. This conformation probably results from the *cis* orientation of the epoxy ring.

Table 4. *Bond lengths and angles for non-hydrogen atoms*

E.s.d.'s are given in parentheses.

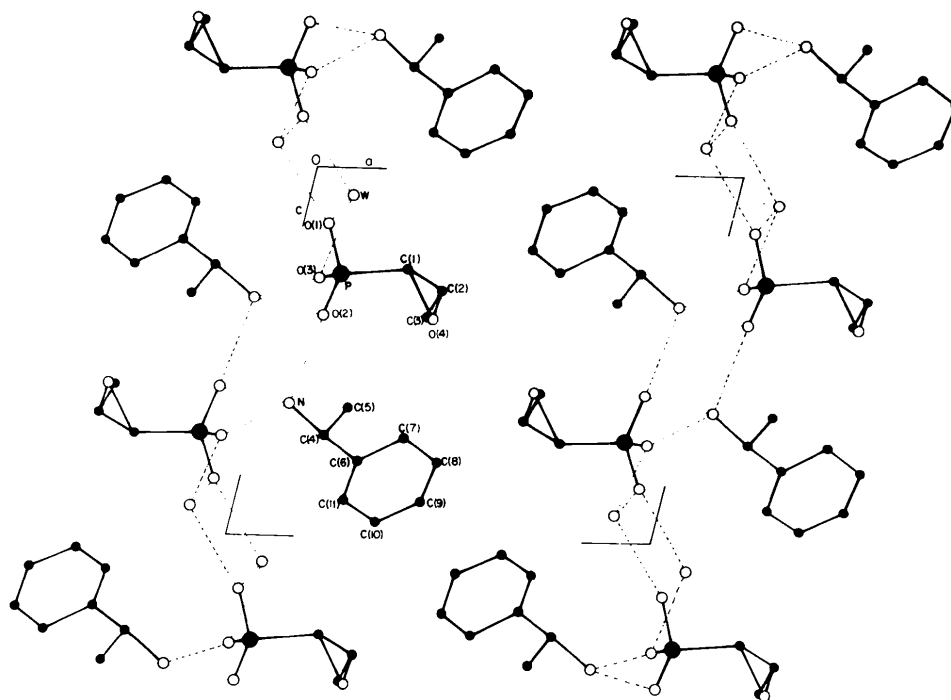|			
P—O(1)	1·497 (3) Å	O(1)—P—O(2)	116·7 (2)°
P—O(2)	1·490 (3)	O(1)—P—O(3)	110·3 (1)
P—O(3)	1·580 (3)	O(1)—P—C(1)	105·6 (2)
P—C(1)	1·809 (4)	O(2)—P—O(3)	107·7 (2)
O(4)—C(1)	1·447 (4)	O(2)—P—C(1)	112·0 (2)
O(4)—C(2)	1·436 (6)	O(3)—P—C(1)	103·5 (2)
C(1)—C(2)	1·467 (6)	P—C(1)—O(4)	119·2 (3)
C(2)—C(3)	1·476 (7)	P—C(1)—C(2)	125·0 (3)
N—C(4)	1·512 (4)	O(4)—C(1)—C(2)	59·1 (3)
C(4)—C(5)	1·519 (7)	C(1)—O(4)—C(2)	61·2 (3)
C(4)—C(6)	1·515 (5)	O(4)—C(2)—C(1)	59·8 (3)
C(6)—C(7)	1·384 (5)	O(4)—C(2)—C(3)	118·6 (4)
C(7)—C(8)	1·380 (6)	C(1)—C(2)—C(3)	125·6 (4)
C(8)—C(9)	1·365 (7)	N—C(4)—C(5)	107·2 (3)
C(9)—C(10)	1·362 (7)	N—C(4)—C(6)	109·7 (3)
C(10)—C(11)	1·379 (7)	C(5)—C(4)—C(6)	116·3 (3)
C(11)—C(6)	1·384 (6)	C(4)—C(6)—C(7)	122·0 (4)
		C(4)—C(6)—C(11)	120·2 (3)
		C(7)—C(6)—C(11)	117·7 (4)
		C(6)—C(7)—C(8)	120·4 (4)
		C(7)—C(8)—C(9)	121·4 (4)
		C(8)—C(9)—C(10)	119·2 (4)
		C(9)—C(10)—C(11)	120·4 (5)
		C(10)—C(11)—C(6)	121·2 (4)

Fig. 3. Hydrogen bonding.

The phenethylammonium cation

The geometry of the cation is fairly regular. The benzene ring is in a plane, the average bond length being 1·376 Å and the average bond angle, 120·05°. The C—N bond, 1·513 Å, is longer by 5σ than the average value, 1·479 Å, quoted in *Interatomic Distances and Configuration in Molecules and Ions* (1965) for the bond linking a C atom to a four-covalent N atom. In terms of the standard deviations of this bond length, such a large difference is significant. The angle between the benzene ring and the N—C(4)—C(6) plane

is 82°. The N atom is involved as a proton donor in three N—H···O bonds.

Hydrogen bonding

The packing of the molecules is characterized by a three-dimensional network of hydrogen bonds, where the molecules run parallel to the c axis and in which all the O atoms from the phosphate group are involved; the water molecule serves both as donor to the two longer bonds and as an acceptor of the shorter bond. The details of the hydrogen bonding appear in Table 5 and in Fig. 3.

Table 5. *The geometry of the hydrogen bonds*

E.s.d.'s are given in parentheses.

Symmetry code for acceptor

(i)	$-x, y - \frac{1}{2}, -z$	(iv)	$-x, y - \frac{1}{2}, -z + 1$
(ii)	$x, y - 1, z$	(v)	$-x, y + \frac{1}{2}, -z + 1$
(iii)	x, y, z		

D—H···A*	D···A	H···A	$\angle D$—H···A
O(w)—H(w1)···O(1^i)	2·697 (3) Å	1·83 (5) Å	168·8 (5·5)°
O(w)—H(w2)···O(1^{ii})	2·695 (4)	1·85 (8)	173·2 (8·0)
O(3)—H(O3)···O(w^{iii})	2·514 (4)	1·49 (8)	158·2 (7·6)
N—H(N1)···O(2^{iii})	2·740 (4)	1·75 (4)	162·2 (4·1)
N—H(N2)···O(2^{iv})	2·800 (4)	1·87 (3)	165·1 (2·3)
N H(N3)···O(3^v)	2·913 (4)	2·04 (4)	156·7 (2·8)

* D is the donor and A is the acceptor atom.

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Structure Cristalline du Turanose [*O*-α-D-Glucosyl-(1 → 3)-β-D-fructose]

PAR ALAIN NEUMAN, DANIÈLE AVENEL ET HÉLÈNE GILLIER-PANDRAUD

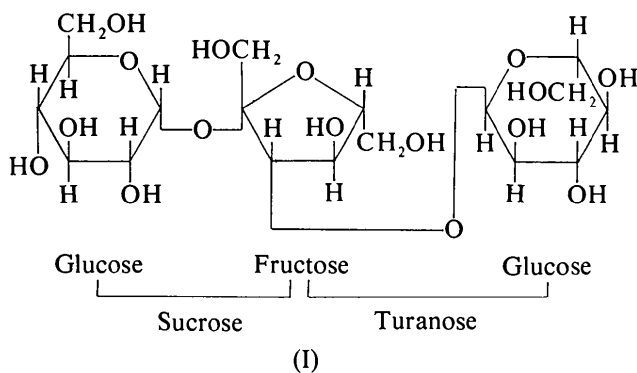*UER Expérimentale de Médecine et de Biologie Humaine (Université Paris XIII), 74 rue Marcel Cachin, 93000 Bobigny, France*

The crystal structure of turanose has been determined by direct methods and refined by least-squares calculations to a final R value of 0·039. The space group is $P2_12_12_1$. The lattice parameters are $a = 8·124$ (1), $b = 9·212$ (2), $c = 19·254$ (3) Å. The glucosyl unit is in the $^4C_1$ chair conformation. The fructose ring adopts a pyran form with a $^2C_5$ conformation as in β-D-fructopyranose. The conformation of the α(1 → 3) linkage is compared with the α(1 → 2) linkage in aldotriouronic acid. There exists an intramolecular hydrogen bond.
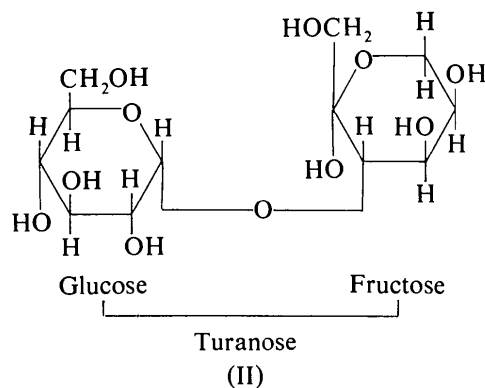
Introduction

Le turanose [*O*-α-D-glucosyl-(1 → 3)-β-D-fructose] est un produit d'hydrolyse du mélézitose (I) [*O*-α-D-glucopyrannosyl-(1 → 2)-*O*-β-D-fructofurannosyl-(3 → 1)-α-D-glucopyrannoside].

nouveau cycle. Ce but primitif a dû être abandonné car notre travail a montré que le résidu fructosyl, présent dans le turanose, n'existe pas sous forme furannique mais sous forme pyrannique (II).

(I)

(II)

A partir de la structure du mélézitose (Avenel, Neuman & Gillier-Pandraud, 1976*a*) et de trois trisaccharides déjà étudiés, raffinose pentahydraté (Berman, 1970), L-kestose (Jeffrey & Park, 1972) et plantéose dihydraté (Rohrer, 1972), une comparaison de la stéréochimie de la partie sucrose a été proposée (Avenel, Neuman & Gillier-Pandraud, 1976*b*). Il nous a semblé intéressant d'étendre cette étude à la partie turanose et de mettre en évidence les modifications conformationnelles provoquées par l'adjonction d'un

Nous avons alors étudié les rapports entre la partie fructose du turanose et le fructose libre qui se présente dans le cristal sous forme pyrannique (Kanters, Roelofsen, Alblas & Meinders, 1977). Par ailleurs, la liaison α(1 → 3) existant dans le turanose est l'analogue de la liaison α(1 → 2) reliant l'acide glucuronique et un des xyloses de l'acide aldotriouronique (Moran & Richards, 1973). Ces deux composés constituent les seuls exemples d'une telle liaison étudiée par diffraction des rayons X. Il était donc intéressant de comparer les résultats obtenus dans ces deux cas.