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X-Ray Analysis of an Aminoglycoside Antibiotic P-2563 (P) Monohydrobromide

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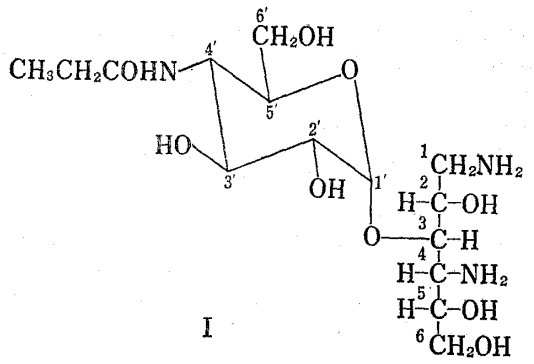
The structure of an aminoglycoside antibiotic P-2563 (P) has been confirmed to be 3-O-(4-deoxyl-4-propionamido-α-D-glucopyranosyl)-1,4-diamino-1,4-dideoxyhexytol by the X-ray analysis of its monohydrobromide dihydrate crystals. The pyranose ring is expectedly in a chair form and its substituents are equatorial except that at C(1′) which is axial. In the aminopolyol moiety, the arrangements around all of the C-C bonds are in staggered form. The carbon-carbon linkages from C(2) to C(6) are in *s-trans* zig-zag chain, and the five carbon atoms are almost coplanar. While the C(1) carbon atom is displaced from the plane resulting in the formation of an intramolecular hydrogen bond between the amino groups attached to C(1) and C(4).

Keywords——aminoglycoside antibiotic; P-2563 (P); crystal structure; conformation; X-ray analysis

P-2563 (P),[2] $C_{15}H_{31}N_3O_9$, is an aminoglycoside antibiotic isolated from the culture broth of *Pseudomonas fluorescence* P-2563, and is active against gram-positive and gram-nagetive bacteria. The structure shown in I has been assigned to the compound by chemical degradation studies and physicochemical properties.[5] We have undertaken the X-ray analysis for the confirmation of the structure and the establishment of the conformation of the molecule in the crystal.



I

Chart 1

Experimental

Single crystals of P-2563 (P) monohydrobromide were obtained as colorless prisms by recrystallization from a mixture of ethanol and water. Thermal and elemental analyses showed that the amount of water of crystallization varied considerably depending on the atmospheric humidity. For instance, when crystals were air-dried under the relative humidities of about 10, 50, and 90%, the amounts of water of crystallization were approximately 1, 2 and 4 molecules per one molecule of P-2563 (P), respectively. The crystals having 2 moles water of crystallization obtained by air-drying under the humidity of about 50% were seemed to be most promisong for the X-ray analysis. Specimens prepared under this condition were, therefore, coated with collodium in order to prevent moisture absorption or loss of the water of crystallization during the diffraction measurements.

Data for determination of the space group and cell dimensions were obtained using a Hilger & Watt's linear diffractometer (MoKα; $\lambda=0.7107$ Å) and photographic methods (CuKα; $\lambda=1.5418$ Å). Crystal system

1) Location: *Jusohonmachi, Yodogawa-ku, Osaka 532, Japan.*
2) P-2563 (P) has been identified with Sorbistin A_1,[3] isolated from the cultutre broth of *Pseudomonas sorbicinii sp. nov.*[4]
3) M. Konishi, S. Kamata, T. Tsuno, K. Numata, H. Tsukiura, T. Naito, and H. Kawaguchi, *J. Antibiot.* (Tokyo), *Ser. A*, **29**, 1152 (1976).
4) K. Tomita, Y. Hoshino, Y. Uenoyama, K. Fujisawa, H. Tsukiura, and H. Kawaguchi, *J. Antibiot.* (Tokyo), *Ser.* A, **29**, 1147 (1976).
5) K. Nara, Y. Sumino, K. Katamoto, S. Akiyama, and M. Asai, *Chem. Letters*, **1977**, 33; K. Nara, K. Katamoto, S. Suzuki, S. Akiyama, and E. Mizuta, *ibid.*, **1977**, 229.

TABLE I. Crystal Data

Formula	$C_{15}H_{32}N_3O_9Br \cdot 2H_2O$
Crystal system	Orthorhombic
Space group	$P2_12_12_1$
Cell dimensions	$a=15.35$ Å
	$b=21.73$
	$c=8.48$
	$V=2828.6$ Å^3
Number of molecules in the unit cell	$z=4$
Calculated density	$\rho=1.21$ g cm^{-3}

and lattice constants are summarized in Table I. Reflection intensities were measured on the diffractometer about the *c*-axis, up to the 7th layer and the Weissenberg photographs about the *a*-axis, up to the 5th layer. Absolute values of the observed structure factors were deduced by the ordinary procedure, and 2306 independent data were derived.

Structure Determination

The position of Br atom was determined without ambiguity from a three-dimensional Patterson synthesis. Starting with this atom, the positions of the remaining 27 nonhydrogen atoms which constituteP-2563 (P) molecule were easily determined by the heavy atom method. After the refinement of the positional and thermal parameters by the least-squares method, 6 peaks were picked up from the difference Fourier map, phased by the 28 atoms, as candidates for the water molecules. For each of these positions, the occupancy factor of the water oxygen atom was estimated applying the least-squares method in which an occupancy factor of each position was treated as variable while holding the positional and thermal parameters unchanged. Temperature factors of the six positions were all

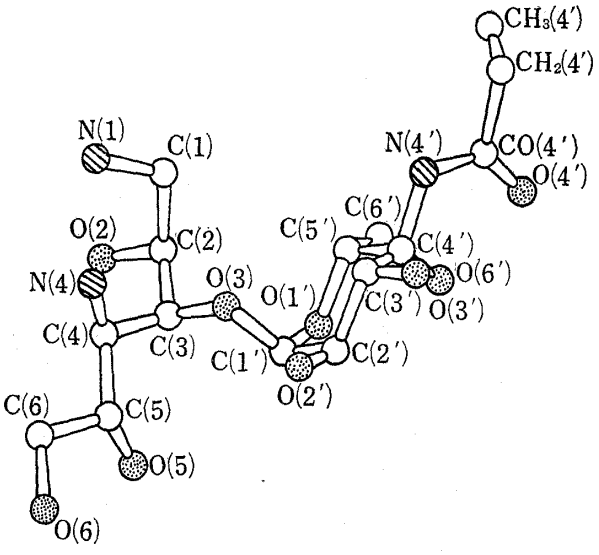


Fig. 1. Perspective View of P-2563 (P) Molecule

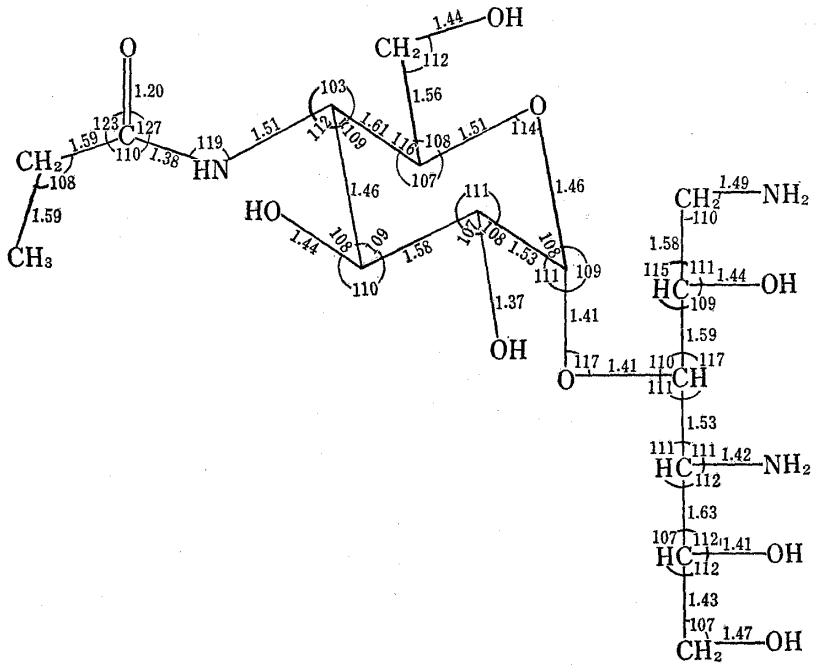


Fig. 2. Bond Lengths and Angles

assigned to 6.5. The occupancy factor of each position (k_j) contributes to the structure factor as follows.

$$F(hkl) = \sum_j k_j f_j \exp(-B_j \sin\theta/\lambda) \exp 2\pi i(hx_j + ky_j + lz_j)$$

The occupancy factor of the 28 atoms became almost 1.0 (0.8—1.2), while those of the six positions were converged to the values shown in Table II. The ratios of the chance in which the positions were occupied by water molecules were determined from these values as estimated moles of water in Table II. Finally, atomic coordinates and isotropic temperature factors were refined by the least-squares method to an R value of 0.156. The atomic parameters are listed in Table III. A perspective view of the molecule, bond distances and angles can be seen in Figs. 1 and 2, respectively. The packing of the molecule in the unit cell and the hydrogen bonding are shown in Fig. 3.

The absolute configuration of the molecule was determined by the anomalous dispersion method. Intensities of Friedel pairs were compared visually on a-axis Weissenberg photographs (CuK_α) and structure factors of these pairs were calculated using the atomic parameters of Table III. The result given in Table IV indicates that the co-ordinates of Table III represent the mirror image of the correct absolute configuration when drown by right-handed set of axes. All figures of this report were drown to give the absolute configuration correctly.

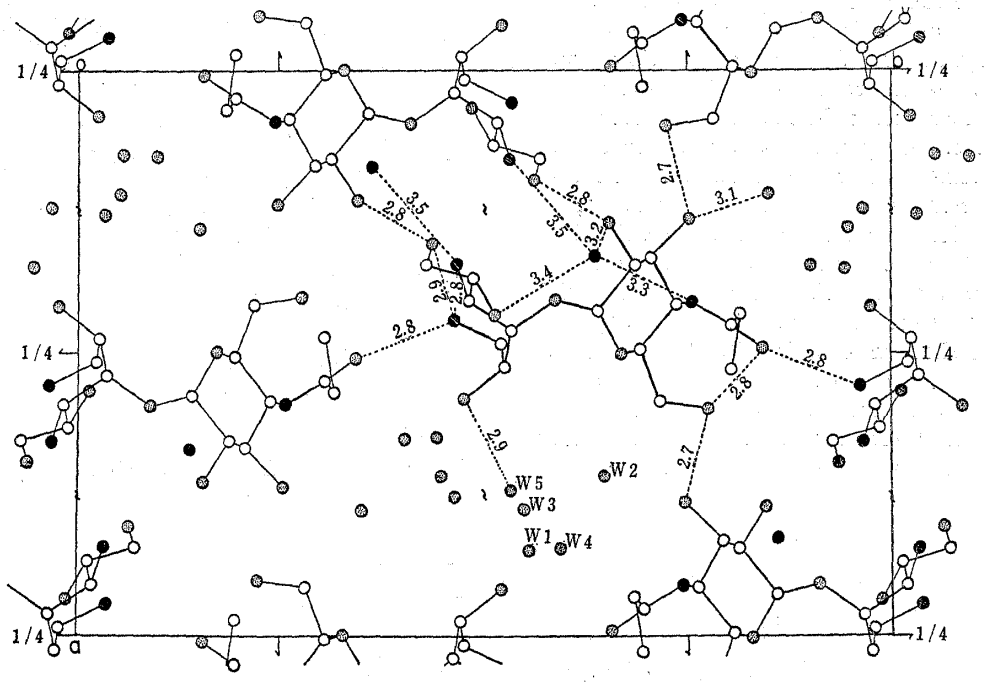


Fig. 3. Content of the Unit-Cell Seen along the c-Axis

Broken lines show possible hydrogen bonds and numbers are distances between hydrogen donors and acceptors.
●: Br, ◎: O, ◍: N, ○: C.

TABLE II. Estimation of Water of Crystallization by Least-squares Method

No.	k_j	Estimated mol
1	0.241	1/4
2	0.716	3/4
3	0.305	1/3
4	0.430	1/3
5	0.303	1/3
6	0.142	0

Table III. Atomic Co-ordinates and Temperature Factors with Their Standard Deviations in Parentheses

Atom		x/a	y/b	z/c	B
Br	Br	0.1757(02)	0.3623(02)	0.4506(04)	3.9(0.1)
C (1)	C	0.4816(18)	0.5239(12)	0.7123(32)	1.9(0.5)
N (1)	N	0.4419(17)	0.4624(16)	0.7323(27)	2.6(0.5)
C (2)	C	0.5263(18)	0.5280(12)	0.5471(38)	2.3(0.5)
O (2)	O	0.5801(14)	0.4754(09)	0.5178(26)	3.8(0.5)
C (3)	C	0.4616(19)	0.5369(13)	0.4044(32)	2.2(0.6)
O (3)	O	0.4075(11)	0.5875(08)	0.4330(22)	1.9(0.3)
C (4)	C	0.4095(20)	0.4810(13)	0.3530(35)	2.4(0.6)
N (4)	N	0.3429(15)	0.4679(10)	0.4614(32)	3.0(0.5)
C (5)	C	0.3727(18)	0.4896(12)	0.1753(33)	1.8(0.5)
O (5)	O	0.4356(14)	0.5146(09)	0.0748(27)	3.5(0.5)
C (6)	C	0.3446(21)	0.4306(14)	0.1236(38)	3.1(0.7)
O (6)	O	0.3079(13)	0.4390(09)	−0.0331(26)	3.3(0.4)
C (1′)	C	0.4241(18)	0.6407(14)	0.3459(31)	2.2(0.5)
O (1′)	O	0.5017(12)	0.6700(08)	0.4081(22)	2.3(0.4)
C (2′)	C	0.3493(17)	0.6858(11)	0.3574(29)	1.4(0.5)
O (2′)	O	0.2752(12)	0.6555(08)	0.3151(22)	2.1(0.4)
C (3′)	C	0.3362(18)	0.7082(11)	0.5319(33)	2.2(0.5)
O (3′)	O	0.2678(14)	0.7524(10)	0.5382(28)	3.6(0.4)
C (4′)	C	0.4150(19)	0.7381(12)	0.5846(33)	2.3(0.6)
N (4′)	N	0.4115(16)	0.7541(11)	0.7564(30)	2.7(0.5)
CO(4′)	C	0.4531(22)	0.8059(15)	0.8081(40)	3.5(0.7)
CH_2(4′)	C	0.4355(24)	0.8154(17)	0.9896(44)	4.6(0.8)
CH_3(4′)	C	0.5232(39)	0.8028(26)	1.0817(77)	10.3(1.7)
O (4′)	O	0.4929(14)	0.8418(09)	0.7313(26)	3.4(0.5)
C (5′)	C	0.4942(20)	0.6906(13)	0.5759(37)	3.0(0.6)
C (6′)	C	0.5852(22)	0.7179(15)	0.6117(40)	3.3(0.7)
O (6′)	O	0.6001(15)	0.7736(10)	0.5255(31)	4.6(0.5)
W1	1/4O	0.8513(94)	0.5590(64)	0.5169(186)	8.0(4.3)
W2	3/4O	0.2810(33)	0.1519(25)	0.2849(65)	8.6(1.5)
W3	1/3O	0.2212(75)	0.0503(52)	0.9062(146)	8.2(3.4)
W4	1/3O	0.6560(60)	0.4030(41)	0.1302(108)	6.9(2.3)
W5	1/3O	0.7433(54)	0.5377(38)	0.4485(123)	6.6(2.0)

Table IV. Comparison of the Observed and Calculated Intensity Differences Used for the Establishment of the Absolute Configuration

h	k	l	$\lvert F_c(hkl)\rvert/\lvert F_c(\bar{h}\bar{k}\bar{l})\rvert$	$I_o(hkl)/I_o(\bar{h}\bar{k}\bar{l})$
1	12	1	0.85	>1
1	14	1	0.80	>1
1	12	6	0.84	>1
2	11	2	1.11	<1
2	1	3	1.24	<1
2	1	7	0.85	>1
3	13	1	1.12	<1
3	21	1	1.13	<1
3	10	2	0.96	>1
3	1	3	0.89	>1
3	1	6	0.86	<1
4	1	4	0.88	>1
4	2	4	0.89	>1
4	3	4	0.85	>1
5	10	1	1.17	<1
5	17	1	1.24	>1*)
5	3	2	0.72	>1

* The observation marked with an asterisk is not in accord with other data.

Results and Discussion

The structure of P-2563 (P) obtained from the present analysis was in complete accord with the previously assigned structure (I).[5]

The molecule of P-2563 (P) is composed of an aminosugar moiety and an α-glycosidically linked aminopolyol part. The projection of the molecule on the least-squares plane of the 6-membered ring of the aminosugar is given in Fig. 4 in which the displacements of the atoms from the plane are also shown. It is clearly seen from the figure that the pyranose ring is in a chair form and its substituents are equatorial except that at C(1′) which is axial. The carbonyl bond of the propionamido and the C(6′)–O(6′) bond are nearly perpendicular to the plane and an intramolecular hydrogen bond is formed between these two functional groups.

Torsion angles around the carbon atoms constituting the backbone of the aminopolyol moiety are shown in Fig. 5. All of these arrangements are in the staggered conformation and the carbon-carbon linkages from C(2) to C(6) are in *s-trans* zig-zag chain conformation. As the result, the five carbon atoms C(2), C(3), C(4), C(5) and C(6), are almost coplanar. The projection diagram of the molecule on the least-sequares plane calculated from these five atoms is presented in Fig. 6. The hydroxyl oxygen at C(6) also lie on the plane, while the

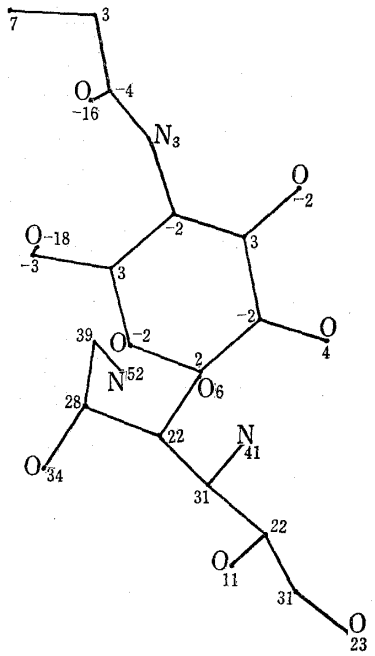


Fig. 4. Projection of the Molecule on the Least-squares Plane of Pyranose Ring

Displacements of atoms from the plane are shown in Å units but multiplied by 10.

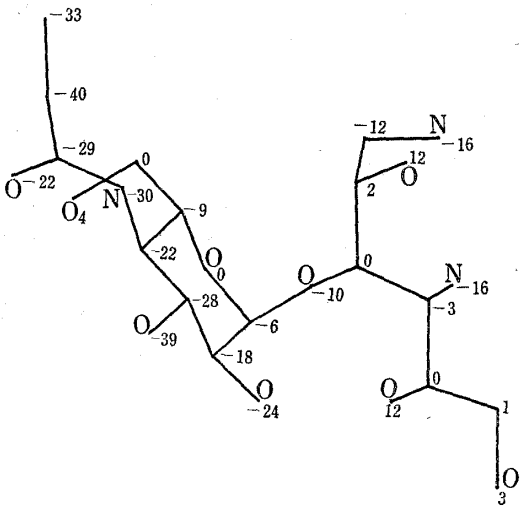


Fig. 6. Projection of the Molecule on the Least-squares Plane of C(2), C(3), C(4), C(5) and C(6) Atoms

Displacements of atoms from the plane are shown in Å units but multiplied by 10.

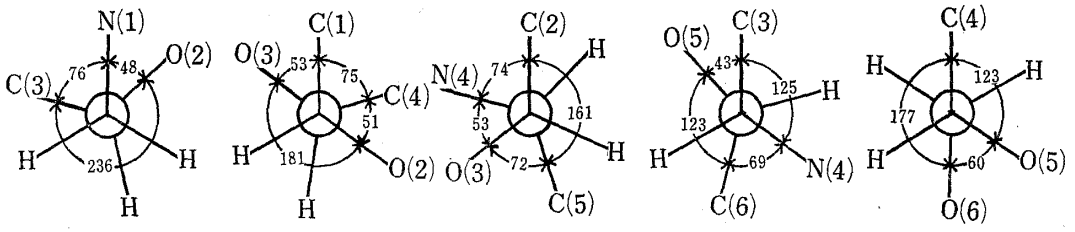


Fig. 5. Torsion Angles around Backbone Carbon of Aminopolyol

Estimated hydrogen atoms are also shown.

C(1) carbon and the amino nitrogens at C(1) and C(4) are displaced from the plane and an intramolecular hydrogen bond is formed between these amino groups.

Since hydrogen atoms were not located even in the difference Fourier map at the final stage, some of the hydrogen bonds remain uncertain. Intermolecular hydrogen bonds shown in Fig. 3 are deduced from atomic distances between possible hydrogen donors and acceptors. The amino group at C(1) seems to form intermolecular hydrogen bonds with the hydroxyl at C(6) of the adjacent molecule translated by one unit along the *c*-axis and with the carbonyl oxygen in the propionamido moiety of the molecule related by the twofold screw axis symmetry along the *b*-axis. The hydroxyls at C(6′) and C(2′) of the aminosugar form hydrogen bonds with the C(3′) hydroxyl and the C(6) hydroxyl of the two different neighbors, respectively. The bromine ion has four coordinated atoms at distances between 3.2 and 3.5 Å. Two of them are nitrogen atoms of the C(4′) amido group and the C(4) amino in different molecules which are related by the 2_1 screw axis along the *c*-axis. Remaining two are the hydroxyl oxygens at C(2′) and C(5) of the molecule generated by one unit translation in the *c* direction of the molecule to which the above amido group belongs.

As already stated in the structure determination part, two molecules of water of crystallization per asymmetric unit are distributed over the five positions (W1—W5) with positional disorder from cell to cell. Unexpectedly, only one hydrogen bond is probable between the P-2563 (P) molecule and one of these water molecules, O(2′)···W(5). No hydrogen bond is found between the water molecules. Therefore, these water molecules do not play an important role for the molecular linking in the crystal but merely fill the large spaces formed by the three dimensional network of the P-2563 (P) molecules. Because of the large tolerance of the gap, the uptake or the removal of water molecules may easily happen depending on the change in the atmospheric humidity.

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