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THE CRYSTAL STRUCTURE OF KANAMYCIN

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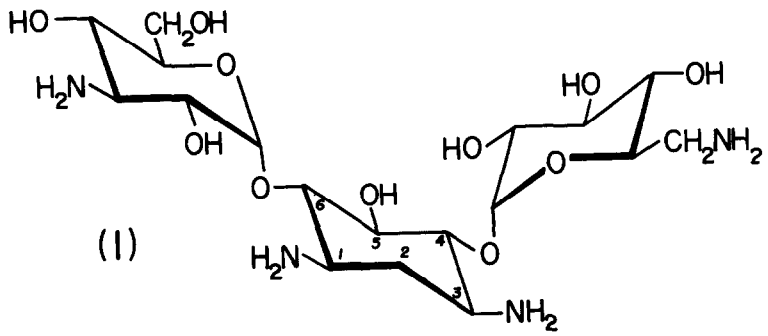
The structure (1) of kanamycin has been proposed by the chemical studies [1,2,3]. In the present paper, the authors report the crystal structure determination of kanamycin sulfate which absolutely confirms the previously proposed chemical structure.

Crystals of kanamycin monosulfate monohydrate (KMS) and kanamycin monoselenate monohydrate (KMSE) are prepared for the analysis. As shown in Table 1, crystals of KMS and KMSE are found to be isomorphous. The analysis was first carried out two-dimensionally by two methods;



(I)

4-O-(6-amino-6-deoxy-α-D-glucopyranosyl)-6-O-(3-amino-3-deoxy-α-D-glucopyranosyl)-2-deoxystreptamine

the one is the isomorphous replacement method using both KMS and KMSE, and the other is the anomalous dispersion method using KMSE by MoK_α radiation. Three-dimensional structure analysis of KMS was made by the consideration of three-dimensional Patterson function and two-dimensional result solved by the two methods.

	KMS	KMSE
Formula;	$C_{18}H_{36}N_4O_{11}\cdot H_2O\cdot H_2SO_4$	$C_{18}H_{36}N_4O_{11}\cdot H_2O\cdot H_2SeO_4$
Mol. weight;	600.6 (609.5 by X-ray)	647.5 (656.3 by X-ray)
Density;	1.589 gr/cm^3 (obs.)	1.682 gr/cm^3 (obs.)
	1.566 gr/cm^3 (calcd.)	1.659 gr/cm^3 (calcd.)
	a = 7.21Å α = 94.8°	a = 7.27Å α = 94.6°
	b = 12.47Å β = 89.7°	b = 12.51Å β = 88.6°
	c = 7.11Å γ = 91.5°	c = 7.15Å γ = 91.6°
	V = 636.8Å^3	V = 647.8Å^3
	Z = 1	Z = 1
Space group;	P1	P1

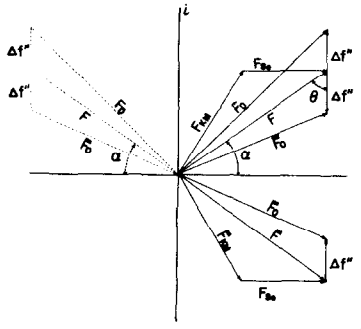
TABLE 1.

Three-dimensional intensity data of KMS were collected from the equi-inclination Weissenberg photographs. The layers hk0~hk3 were taken by CuK_α (λ=1.5418Å) radiation using multiple film technique. The intensities were estimated visually using a calibrated intensity scale. Two-dimensional intensity data (hk0), ($\bar{h}$k0), (h$\bar{k}$0) and ($\bar{h}\bar{k}$0) were collected for KMSE by MoK_α (λ=0.7107Å) radiation on a linear diffractometer (Hilger Co.).

Two-dimensional analysis:

The structure factors of KMSE (F) and their phase angles α(hk0) were calculated according to the method proposed by Ramachandran and Raman [4] using the equations (1) and (2) in Fig. 1. This leaves umbiguity in the phase angle determination about the imaginary axis in the Argand diagram.

Phase Angle Determination by the use of Anomalous Dispersion



$$F_0^2 = F^2 + \Delta f''^2 + 2 \cdot F \cdot \Delta f'' \cdot \cos\theta$$

$$\bar{F}_0^2 = F^2 + \Delta f''^2 - 2 \cdot F \cdot \Delta f'' \cdot \cos\theta$$

$$F_0^2 + \bar{F}_0^2 = 2(F^2 + \Delta f''^2)$$

$$F_0^2 - \bar{F}_0^2 = 4 \cdot F \cdot \Delta f'' \cos\theta$$

$$F = \sqrt{\frac{1}{2}(F_0^2 + \bar{F}_0^2) - \Delta f''^2} \qquad \cdots\cdots(1)$$

$$\cos\theta = \frac{F_0^2 - \bar{F}_0^2}{4 \cdot F \cdot \Delta f''} \qquad \cdots\cdots(2)$$

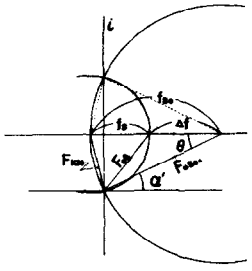
$$\sin\alpha = \cos\theta \qquad (\because\ \alpha = 90^\circ - \theta)$$

$$\cos\alpha = \pm\sqrt{1 - \cos^2\theta}$$

Fig. 1

The phase angles $\alpha'(hk0)$ of the observed structure factors $Fo_{Se}(hk0)$ can also be calculated by the isomorphous replacement method using a pair of reflections $Fo_{Se}(hk0)$ and $Fo_S(hk0)$ for each (hk0) plane by the equation (3) in Fig. 2.

Phase Angle Determination by the Isomorphous Replacement Method



$$F_{oS}^2 = F_{oSe}^2 + \Delta f^2 - 2 \cdot F_{oSe} \cdot \Delta f \cdot \cos\theta$$

$$\cos\alpha' = \cos\theta = \frac{F_{oSe}^2 - F_{oS}^2 + \Delta f^2}{2 \cdot F_{oSe} \cdot \Delta f} \qquad \cdots\cdots(3)$$

$$(\Delta f = f_{Se} - f_S)$$

Fig. 2

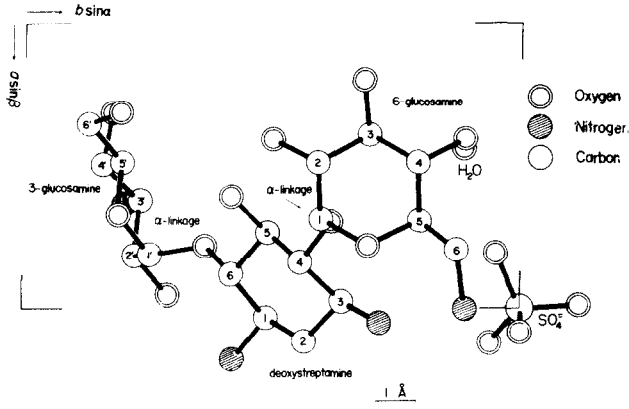
This method, however, leaves umbiguity in the phase angle determination about the real axis. Both umbiguities in the anomalous dispersion and isomorphous replacement methods can be solved by comparing the phase angles $\alpha(hk0)$ and $\alpha'(hk0)$. Two kinds of electron density projections, $\rho_{an.}(x,y)$ and $\rho_{iso.}(x,y)$ were then computed using the phases thus determined. Resulting ρ maps are very similar or essentially identical. Several trial Fourier and difference Fourier syntheses were then calculated by $Fo_S(hk0)$ of KMS. The reliability factor dropped down to 23.7%

when one sulfur and 34 light atoms were included. Many trials to find out the projected image of the molecules on the Fourier map, constructing various stereo-models of the known chemical structure, lead to a reasonable interpretation of the map. A subsequent electron density projection calculated with all atoms revealed the whole kanamycin molecule together with a molecule of crystal water. This structure was refined by the least squares method (R=15.9%).

Three-dimensional analysis of KMS:

Three-dimensional Patterson synthesis was first calculated. It was then tried to obtain the Z-coordinates of each atom in kanamycin by considering the Patterson function and the relative configuration of the molecule using the scale model which was derived by the last two-dimensional analysis. As a result, twenty-seven Z-coordinates for light atoms of 15 oxygens, 4 nitrogens and 6 carbons could be obtained which account for the Patterson function and the stereo-model very well. The Fourier and difference Fourier synthesized with a sulfur and 27 light atoms (R=41.3%) revealed the other all light atoms but one oxygen of crystal water. Three cycles of the least squares refinement were then performed and the reliability factor dropped to 22.6%. In the subsequent Fourier and difference Fourier syntheses (R=20.8%), one of the four nitrogen atoms and one oxygen of the crystal water were excluded because the former was found to have too large temperature factor (Ca. 13.8Å^2) and the latter could not be recognized so definitely as yet. The resulting Fourier and difference Fourier map, however, showed these last two light atoms in the reasonable positions. Three more cycles of least squares calculation gave the R factor of 10.6% for 1154 reflections.

The molecular structure of kanamycin sulfate is shown in Fig. 3.



Kanamycin:
Absolute Structure (along c-axis)

Fig.3

The absolute configuration was determined by the use of the anomalous dispersion effect due to the selenium atom when MoK_α was used ($\Delta f'=-0.1$, $\Delta f''=2.4$). Fig. 3 shows the absolute configuration of the molecule. All three six-membered rings exist in the "chair" form in which all the substituents but the two glycoside oxygens of two glucosamine moieties occupy the more stable equatorial positions. Accordingly, both 3-amino-3-deoxy-D-glucose and 6-amino-6-deoxy-D-glucose moieties in kanamycin are attached to deoxystreptamine by α-linkages. Generally, the determination of the absolute configuration of the compounds like kanamycin by the chemical methods is the most difficult part in the whole structural studies. The present study on the crystal structure of kanamycin has not only confirmed the structure already known, but also justified the configurational chemical studies about the positions C-4 and C-6 of the deoxystreptamine moiety by a copper complex method [1,2]. The structure in Fig. 3 here presented conforms with the chemical observations, that is, isolation of 6-amino-6-deoxy-D-glucose and 3-amino-3-deoxy-D-glucose by acid hydrolysis of kanamycin [3] and isolation of optically inactive 5-O-methyl-2-deoxystreptamine by hydrolysis of exhaustively methylated N-acetylkanamycin [5].

The bond distances found in the molecule are very normal i.e. the average values are 1.49Å for S-O, 1.55Å for C-C, 1.48Å for C-N and 1.45Å for C-O. The bond angles calculated around each atoms are also normal.

The calculations of the present study were performed on the HITAC 5020E computor at the University of Tokyo and CDC 3600 computor at the JAIF Control Data Center, Tokyo.

Crystallographical data in detail will be reported.

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