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Note

Crystal structure, conformation, and absolute configuration of kanamycin A

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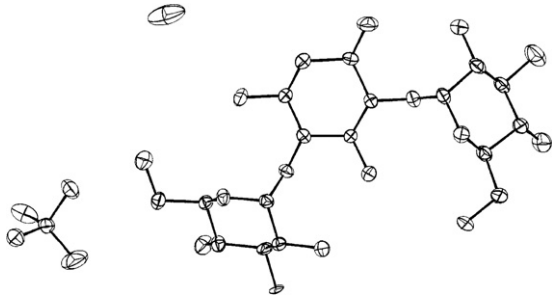
Abstract—Kanamycin, an antibiotic complex produced by *Streptomyces kanamycetius* isolated from Japanese soil, was described by Okami and Umezawa as early as 1957 and consists of three components: Kanamycin A (the major component), B, and C. The disulfate salt of kanamycin A [4-*O*-(6-amino-6-deoxy-α-D-glucopyranosyl)-6-*O*-(3-amino-3-deoxy-α-D-glucopyranosyl)-2-deoxystreptamine] is a broad-spectrum antibiotic that is used to treat gonorrhea, salmonella, tuberculosis, and many other diseases. Crystals of kanamycin A monosulfate monohydrate obtained from water are triclinic, space group $P1$, with $a = 7.2294(14)$, $b = 12.4922(15)$, $c = 7.1168(9)$, $\alpha = 94.74(1)$, $\beta = 89.16(1)$, $\gamma = 91.59(1)$, $V = 640.2(2)$ Å^3, $\mu(\mathrm{Cu\,K\alpha}) = 18.4\ \mathrm{cm}^{-1}$, FW 600.6, $D_{\mathrm{calc}} = 1.558\ \mathrm{g/cm}^3$, CAD-4 diffractometric data (2693 reflections, $2554 \geqslant 3\sigma(I)$), structure by SHELX-86 and refined by full-matrix least squares to a final R value of 0.038. The wrong conformer had an R value of 0.043. Both of the D-glucose moieties are attached to the deoxystreptamine by α linkages. This absolute configuration agrees with the earlier determination by both chemical and X-ray methods with photographic data. The (ϕ, ψ) values for the glycosidic linkages are 101.6°, −121.1°, 106.3°, and −140.4°, respectively. Kanamycin interacts with the ribosomal S12 protein to stabilize the codon–anticodon binding between mRNA and the aminoacyl tRNA and inhibits the elongation of peptide chains through a series of reactions resulting in the prevention of ribosomes from moving along mRNA.



Keywords: Kanamycin A; Crystal structure; Stereochemistry; Absolute configuration

The kanamycins are a family of three antibiotics, kanamycin A, B, and C, which were first reported in 1957. The disulfate salt of kanamycin A [4-*O*-(6-amino-6-deoxy-α-D-glucopyranosyl)-6-*O*-(3-amino-3-deoxy-α-D-glucopyranosyl)-2-deoxystreptamine] is the most widely used and studied of the three antibiotics. It is a broad-spectrum antibacterial agent that is used to treat gonorrhea, salmonella, tuberculosis, and many other diseases.[1] Several chemical methods have been employed to determine the absolute configuration of the streptamine ring of kanamycin A.[2–4] In addition, an early X-ray diffraction study of kanamycin monosulfate was undertaken,[5] but its precision was limited by the photographic technique available at that time. With the use of the current modern diffractometry, we have undertaken

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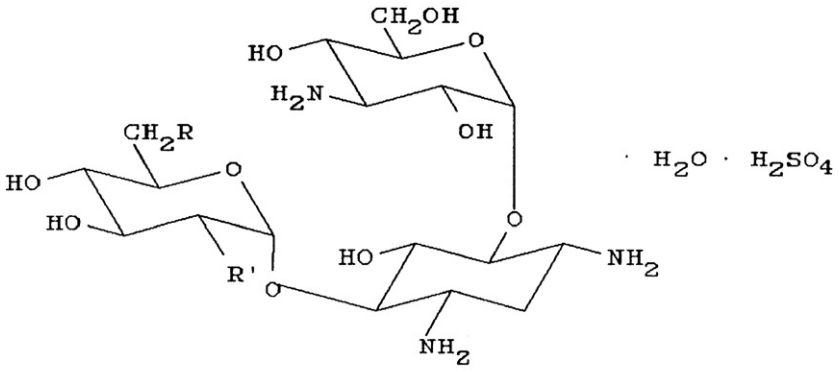
Kanamycin Antibiotics (sulfates)



Kanamycin A: R = NH_2; R'= OH
Kanamycin B: R = R'= NH_2
Kanamycin C: R = OH; R'=NH_2

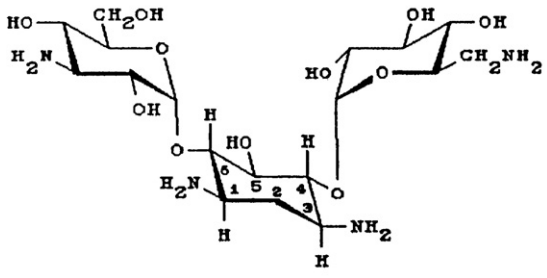
the task of determining the structure of kanamycin A with much greater precision and hence establish its absolute configuration unequivocally.[6]

We have undertaken the structural study of kanamycin A for the following three reasons: (i) Kanamycin A is a member of the series of three antibiotics, Kanamycin A, B, and C, which possess varying degrees of antibacterial activity, depending on the substituents. Consequently, a detailed investigation of the stereochemistry of kanamycin A will be a forerunner to the study of the other antibiotics,[6] which might facilitate the understanding of their biological activity. (ii) A detailed study of the structures of kanamycin antibiotics and their metal complexes in the solid state would provide the necessary database to investigate the specificity of the interaction of the drug with metal atoms. (iii) The structure of kanamycin A would provide accurate information of the glucopyranosyl and streptamine moieties that could be used in model-building studies of polysaccharides containing this as the monomeric unit. In this paper, we report the structure, stereochemistry, and absolute configuration of kanamycin A sulfate as deduced from single-crystal X-ray diffraction studies.

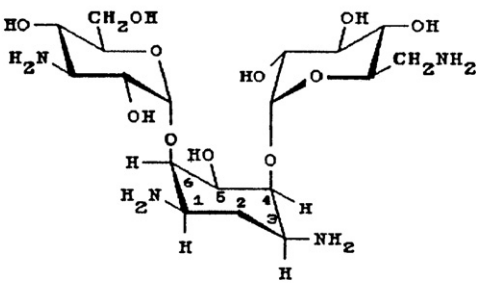
Table 1. Crystal data and structure refinement for kanamycin sulfate monohydrate

Empirical formula	$C_{18}H_{36}N_4O_{11}\cdot H_2O\cdot H_2SO_4$
CCDC number	610,593
Formula weight	600.6
Temperature	293(2) K
Wavelength (Cu Kα)	1.5418 Å
Crystal system	Triclinic
Space group	*P*1
Unit cell dimensions	$a = 7.229(1)$ Å $\alpha = 94.74(1)°$
	$b = 12.492(2)$ Å $\beta = 89.16(1)°$
	$c = 7.1168(9)$ Å $\gamma = 91.59(1)°$
Volume	640.2(2) $Å^3$
Z	1
Density (calculated)	1.558 Mg/m^3
Absorption coefficient	18.4 cm^{-1}
F(000)	320.2
Crystal size	$0.75 \times 0.3 \times 0.2$ mm^3
θ Range for data collection	0–150°
Reflections collected	2693, 2554 [$I \geqslant 3\sigma$]
Absorption correction	Multi-scan
Refinement method	Full-matrix least squares on F^2
Goodness-of-fit on F^2	1.043
Final *R* indices [$I > 4$sigma(I)]	$R1 = 0.0381$
Largest diff. peak and hole	0.347 and −0.219 e $Å^{-3}$

Stereochemistry of Kanamycin A



Two equatorial linkages



Two axial linkages

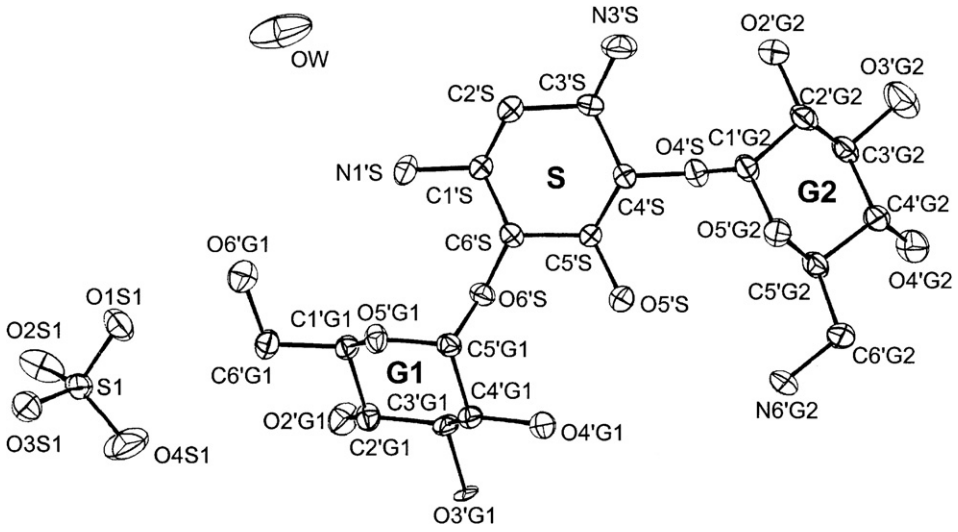


Figure 1. An ORTEP diagram showing the molecular structure of kanamycin sulfate and the atomic numbering scheme. Displacement ellipsoids are drawn at 50% probability level. The water molecule, chloride ion, and hydrogen atoms are omitted for clarity.

1. Experimental

Samples of kanamycin A were purchased from Sigma–Aldrich Chemical Company. Excellent crystals of the compound were obtained by slow evaporation of an aqueous solution at room temperature. Crystals of kanamycin sulfate monohydrate ($C_{18}H_{36}N_4O_{11}\cdot H_2SO_4\cdot H_2O$) are triclinic, space group $P1$, with cell dimensions $a = 7.2294(14)$, $b = 12.4922(15)$, $c = 7.1168(9)$, $\alpha = 94.74(1)$, $\beta = 89.16(1)$, $\gamma = 91.59(1)$, $V = 640.2(2)$ Å^3. A crystal of approximate dimensions $0.12 \times 0.18 \times 0.25$ mm^3 was chosen for data collection. Diffraction data were collected at 298 K using an Enraf–Nonius CAD-4[7] diffractometer with graphite monochromated Cu Kα radiation ($\lambda = 1.5418$ Å). Table 1 gives the crystal data and the parameters used in the refinement of the structure. The data were analyzed using a structure determination package (SDP)[7] from the Enraf–Nonius company on a microvax computing system. A total of 2695 reflections had their intensities integrated and scaled, of which 2554 were considered significant. The structure was solved by direct methods and refined by full-matrix least squares on F^2 with anisotropic displacement parameters for the nonhydrogen atoms using Bruker, SHELXT[8] version 6.10. Hydrogen atoms were located from a difference Fourier map and were refined isotropically. The structure refined to a goodness of fit (GOF)[†] of 1.043 and final residuals[‡] of $R_1 = 0.0381\%$ ($I > 3\sigma(I)$) for the correct conformer. The other conformer had an R value of 0.043. A total of 2693 reflections were employed for 513 parameter determinations, with three restraining parameters. The flack parameter was −0.05(2).[9] The final fractional coordinates, equivalent isotropic displacement parameters [U(eq)] of the atoms in the structure, bond lengths and angles are deposited with the manuscript (see Supplementary data).

The structure of kanamycin has been reported from chemical studies.[2–4] Later, the absolute configuration of kanamycin was determined by using the anomalous effect of the selenium atom using photographic data.[5] In this paper, we report the determination of the absolute configuration of kanamycin sulfate monohydrate using single-crystal diffractometric data. The sulfur atoms scatter anomalously for Cu Kα radiation. The R values for $R(+)$ and $R(-)$ are 0.0381 and 0.0434, and the ratio is 1.176. The absolute configuration, as found from X-ray investigation, is in good agreement with those deduced from chemical studies and earlier studies with photographic data. All the three six-membered rings exist in the 'chair' conformation in which all the substituents except the two glycosidic oxygens of the two glucosamine moieties occupy the most stable equatorial positions. Both the 3-amino-3-deoxy-D-glucose and the 6-amino-6-deoxy-D-glucose moieties are attached to the deoxystreptamine ring by α linkages. In general, the determination of the absolute configuration of the compounds by chemical methods is rather difficult in the whole chemical structural studies. Our present study on the absolute configuration of kanamycin has not only confirmed the chemical structure, but also gives very valuable justification to the chemical studies about the positions of the C-4 and C-6 of the deoxystreptamine ring by the copper complex methodology.

An ORTEP[10] diagram of the molecular structure of kanamycin sulfate monohydrate and atomic numbering scheme is shown in Figure 1. Displacement ellipsoids are

[†] $\mathrm{GOF} = \left[\sum[w(F_o^2 - F_c^2)^2]/(n-p)\right]^{1/2}$, where n and p denote the number of data and parameters.

[‡] $R_1 = (\sum||F_o| - |F_c||)/\sum|F_o|$; $wR_2 = [\sum[w(F_o^2 - F_c^2)^2]/\sum[w(F_o^2)^2]]^{1/2}$ where $w = 1/[\sigma^2(F_o^2) + (a\cdot P)^2 + b\cdot P]$ and $P = [(Max;0,F_o^2) + 2\cdot F_c^2]/3$.

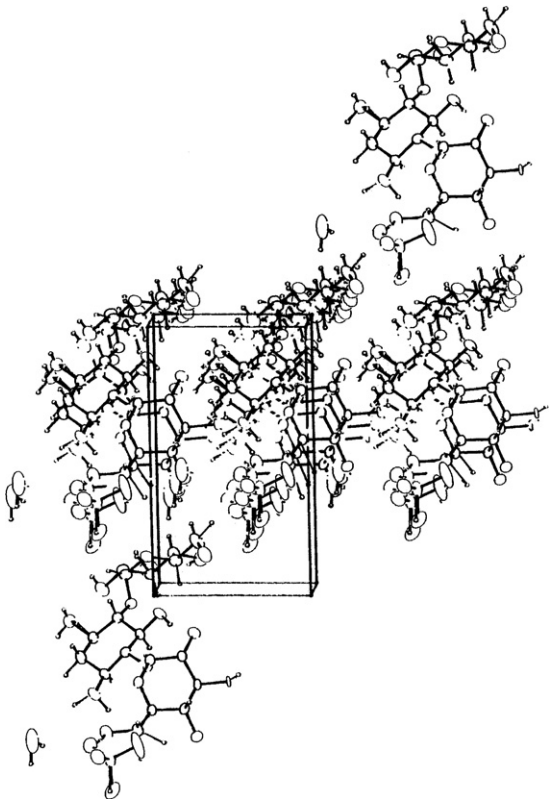


Figure 2. Hydrogen-bonding interactions shown by dashed lines in the crystal structure of kanamycin sulfate monohydrate.

drawn at 50% probability level. The water molecule, chloride ion, and hydrogen atoms are omitted for clarity. The molecule of kanamycin A exists in a long extended conformation with all the three rings in the chair conformation. The conformation of amikacin,[11] a semi-synthetic derivative of kanamycin A, is very similar to that of kanamycin reported in this paper. The conformation of the A/B rings is essentially unchanged between amikacin and kanamycin A, whereas the B/C ring junctions are significantly different. Figure 2 gives the hydrogen-bonding scheme in the crystal structure. The hydrogen bond involving the O5′ of the central B ring is common to both amikacin and kanamycin A, suggesting the essential role it plays in maintaining the relative orientation of the A/B ring systems. The orientation of B/C is different between these two compounds due to the change of the NH_2 group at the N1 position of kanamycin by the long hydroxyaminobutyryl side chain in amikacin. A table of bond distances and angles has been deposited. All distances and angles in the molecule are essentially normal. There are two variations, which are worthy of mention here. The C–O–C angle in the A and C rings (115.8°) and in the bridging oxygens (117.4°) is larger than the normal sp^3-hybridized value of 109.5°. This phenomenon has been observed in other carbohydrate structures and is well documented in the literature.[12] This could suggest a certain amount of sp^2 character for these bridged oxygen atoms. There is also a slight shortening of the C–O distances of carbon atoms attached to two oxygen atoms due to the contribution of double-bond character for the C–O bonds in the carbohydrate structures.[12] Table 2 gives the important torsion angles in the structure, and Table 3 gives the various hydrogen-bond distances and angles in the crystal packing. The (ϕ,ψ) values for the glycosidic linkages are 101.6°, −121.1°, 106.3°, and −140.4°, respectively.

Table 3 lists the hydrogen-bond distances and angles in the structure. The crystal structure is stabilized by an extensive network of very strong N–H···O and O–H···O hydrogen bonds involving all the hydroxyl groups in the molecule and the sulfate ion. The water molecule donates two hydrogen bonds to the sulfate oxygens and accepts one from the sulfate oxygen. Figure 2 shows the network of hydrogen bonding in the structure.

Kanamycin and several other aminoglycosides have been extensively studied in the solution state by NMR methods.[13] Based on proton assignments and NOESY experiments, a stacking arrangement between the xylose ring and the 2,6-amino-2,6-dideoxyglucose rings has been suggested for butirosin, whereas the 2-deoxystreptamaine ring in kanamycin was found to be in an

Table 2. Torsion angles (°) for kanamycin sulfate monohydrate

Angle	Glucopyranose ring G1	Glucopyranose ring G2	Angle	Streptamine ring S
C1′–C2′–C3′–C4′	−58.2(4)	−56.9(4)	C1′–C2′–C3′–C4′	−54.1(4)
C2′–C3′–C4′–C5′	53.1(4)	58.4(4)	C2′–C3′–C4′C5′	49.8(4)
C3′–C4′–C5′–O5′	−50.4(4)	−59.3(4)	C3′–C4′C5′–C6′	−52.7(4)
C4′–C5′–O5′–C1′	55.1(4)	60.9(4)	C4′–C5′–C6′–C1′	60.4(4)
C5′–O5′–C1′–C2′	−59.5(4)	−58.0(4)	C5′–C6′–C1′–C2′	−64.9(4)
O5′–C1′–C2′–C3′	60.1(4)	54.9(4)	C6′–C1′–C2′–C3′	61.6(4)
Other linkage torsion angles				
C5′S–C6′S–O6′S–C5′G1 (ϕ)	118.8(3)		C5′S–C4′S–O4′S–C1′G2	−131.2(3)
C1′S–C6′S–O6′S–C5′G1	−121.1(3)		C3′S–C4′S–O4′S–C1′G2 (ϕ)	106.3(4)
C6′S–O6′S–C5′G1–C4′G1	−137.0(3)		C4′S–O4′S–C1′G2–C2′G2 (ψ)	−140.4(3)
C6′S–O6′S–C5′G1–O5′G1 (ψ)	101.6(3)		C4′S–O4′S–C1′G2–O5′G2	97.5(4)

Table 3. Hydrogen-bond distances and angles in kanamycin sulfate

Donor D	Hydrogen H	Acceptor A	*d*(D–H) in Å	*d*(H···A) in Å	*d*(D···A) in Å	∠DHA (°)	Reference
N1′S	H1N1′S	O2S1	0.88	2.21	3.224	150.2	$x, y, 1-z$
N1′S	H2N1′S	O4S1	0.82	2.12	3.418	144.9	$x, y, 1-z$
N3′S	H1N3′S	O4′G1	0.84	2.02	2.856	145.6	$1+x, y, z-1$
O5′S	HO5′S	O3′G2	1.11	2.19	3.056	145.9	$1+x, -y, z$
N3′G1	H1N3′G1	O6′G1	0.74	2.18	2.792	130.9	$x, y, z+1$
O6′G1	HO6′G1	O2S1	1.03	2.01	2.783	173.2	x, y, z
O3′G2	HO3′G2	N6′G2	1.03	2.12	2.806	172.0	$x, y, z-1$
O4′G2	HO4′G2	O2′G2	0.73	2.17	3.012	169.0	$x-1, y-1, z-1$
N6′G2	H1N6′G2	O2S1	0.56	2.41	3.415	165.0	$x-1, y-1, z-1$
N6′G2	H2N6′G2	O3S1	0.58	1.92	2.683	164.0	$x-1, y-1, z-1$
OW	H1OW	O4S1	0.89	2.14	2.748	172.0	$x+1, y, z-1$
OW	H2OW	O2S1	0.64	2.23	2.806	151.2	$x, y, 1-z$
O4S1	HO4S1	OW	1.13	1.94	2.748	172.5	$x-1, y, 1+z$
O2′G1	HO2′G1	O1S1	0.89	2.19	3.230	164.0	$x, y, 1+z$
C2′G1	HC2′G1	OW	1.02	2.42	3.385	164.0	$x-1, y, z$
C6′G2	HC6′G2	O2S1	1.04	2.09	3.100	161.4	$x-1, y-1, z-1$

extended conformation, departing from the stacked configuration. Kanamycin interacts with the ribosomal S12 protein to stabilize the codon–anticodon binding between mRNA and the aminoacyl tRNA and inhibits the elongation of peptide chains through a series of reactions resulting in the prevention of ribosomes from moving along mRNA.[14] It is not yet clear as to what role the absolute configuration of kanamycin plays apart in this codon–anticodon interaction. Several analogs of kanamycin A containing the 6-amino-6-deoxyglycofuranoses have been synthesized and tested for possible potential activity against resistant bacteria producing 6′-*N*-acetyltransferase.[15] Their structure and absolute configuration studies should yield valuable information on the conservation of the absolute configuration of kanamycin and its possible role in their potential activity.

Acknowledgements

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Supplementary data

Crystallographic data for this structure have been deposited with the Cambridge Crystallographic Data Centre as supplementary data no. 610593. Copies of the data may be obtained free of charge upon request from The Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk; Web: http://www.ccdc.cam.ac.uk). Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2006.09.008.

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