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Crystal Structure of Amikacin

Robert Bau* and Irina Tsyba

Department of Chemistry, University of Southern California, Los Angeles, CA 90089, U.S.A.

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Abstract

Amikacin is one of the important aminoglycoside antibiotics used against gram-negative bacteria. Here we report the crystal structure of amikacin that has been crystallized by vapor diffusion against polyethylene glycol. The molecule exists in a long, extended conformation, with all three six-membered rings in chair conformations and connected together by α-glycosidic linkages. The orientation between the A, B and C rings of the molecule is maintained by intramolecular hydrogen bonds involving the O5 hydroxyl group and the amide NH group.

Keywords: Antibiotics; Amino sugars; Glycosides; X-ray crystal structure

Introduction

The aminoglycoside antibiotics, which include gentamicin (Garamycin), tobramycin (Nebcin), amikacin (Amikin), and neomycin, are often used as the first defense against serious infections caused by various gram-negative bacteria.[1] Amikacin (**I**), a semisynthetic derivative of kanamycin A,[2] exhibits an extremely broad spectrum of antimicrobial activity and is particularly useful against strains resistant to other antibiotics.[1-3] Like many of the other aminoglycoside antibiotics, it is believed to act by binding to the 30S ribosomal subunit of bacteria, thereby interfering with normal protein biosynthesis.[4]

*e-mail: bau@chem1.usc.edu

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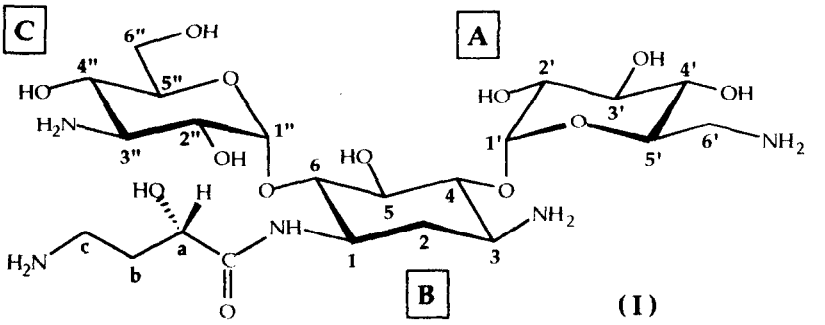
The structure of amikacin and its derivatives have been investigated by several groups using NMR[5,6] and other[7] techniques, which have established the basic chemical structure of the molecule as I. More recently, Cox, Serpersu and co-workers reported a detailed NMR investigation of amikacin while complexed to a bacterial enzyme,[8,9] a study which has shed some light on the mechanism of bacterial inactivation of aminoglycoside antibiotics. In this paper we describe the X-ray crystal structure analysis of this antibiotic, which establishes the spatial relationship between the various portions of the molecule.



Results and discussion

Amikacin[10] was successfully crystallized from an aqueous solution by the hanging-drop vapor-diffusion method.[11] Long needle-shaped crystals appeared within 2–4 days using 60-80% polyethylene glycol 400 as precipitant, with either 10% ethylene glycol or 10% octanol as additives. The crystals belong to the orthorhombic space group $P2_12_12_1$ with cell dimensions a = 6.687(2), b = 14.943(3), c = 29.575(8) Å. X–ray diffraction data were collected at low temperature (–150°C) with Cu Kα radiation using a Siemens X-1000 area detector, and processed using standard techniques [12]. The

structure was solved by direct methods[13] and refined to a final agreement factor of R(F)=6.1% for 1996 reflections.[14]

A molecular plot is shown in Figure 1 and interatomic distanes and angles are given in Table 1. The general structure of amikacin is very similar to that of the parent molecule kanamycin A,[15] with all three rings in chair configurations and the molecule as a whole in a long, extended conformation (Fig. 1). All the substitutents on the three rings are in equatorial positions, except the two glycosidic oxygens of the glucosamine rings (in other words, C_1'–O_a and C_1''–O_b are the only two axial bonds in the molecule). Thus, both glucosamine rings (A and C) are attached to the central deoxystreptamine ring (B) by α-linkages.

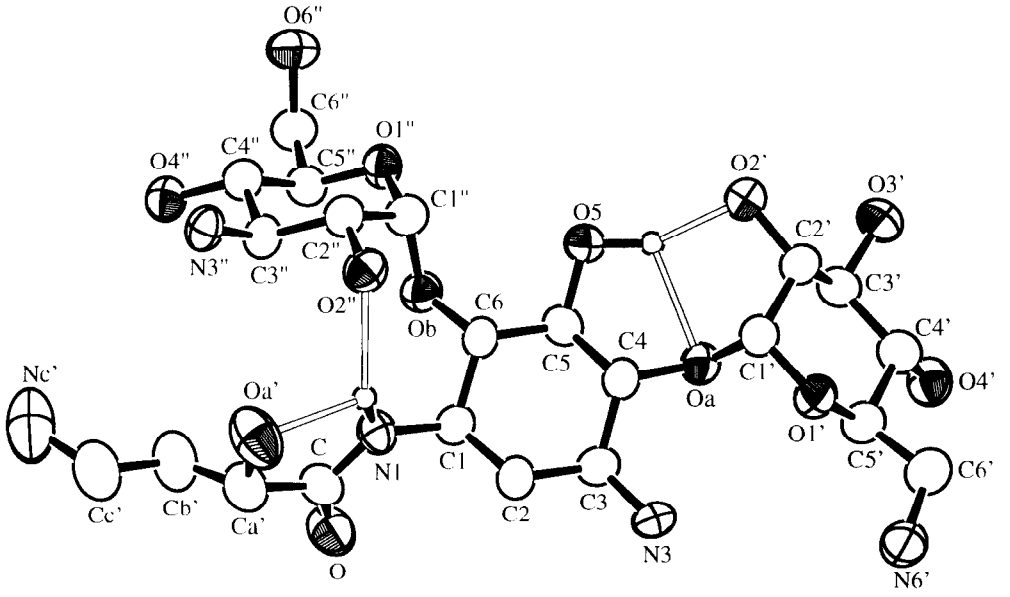


Figure 1. Crystal structure of amikacin, with key H bonds shown as hollow lines.

Key torsion angles in the molecule, around atoms O_a, O_b and N_1, are listed in Table 2. The conformation around the C_1'–O_a–C_4 bridge is maintained by a bifurcated hydrogen bond involving the O_5 hydroxyl group (Figure 1; H...O_2' = 1.77Å, H...O_a = 2.18Å), while the conformation around the C_1''–O_b–C_6 linkage is influenced by a similar but weaker bifurcated hydrogen bond involving the amide NH group (Figure 1; H...O_2'' = 2.24Å, H...O_a' = 2.12Å).

All distances and angles in the molecule are normal (Table 1). The C-O-C angles in the A and C rings (average 115.3°) and in the glycosidic bridges (average 116.2°) both have values which are significantly larger than the normal sp^3-hybridized value of 109.5°. This phenomenon has been noticed before in other carbohydrate structures,[16] and could suggest a certain amount of sp^2 character for these bridging oxygen atoms. There is also a slight but perceptible shortening of the single-bonded C–O distances associated with C_1' and C_1'', the carbons bonded to two oxygen atoms. These four distances (C_1'–O_1'; C_1'–O_a; C_1''–O_1''; C_1''–O_b) average to 1.414Å, whereas all other C–O single bonds in the molecule have a mean value of 1.438Å. This type of bond shortening has been attributed[16] to a slight amount of double-bond character for the C–O bonds in these O-C-O linkages.

As mentioned earlier, the amikacin molecule exists in a rather long, extended conformation. The 3'-hydroxy group, the site of modification by bacterial enzymes,[8] is close to one end of this extended structure, while the hydroxyaminobutyryl chain (atoms N_1 to N_c') is located at the other extremity of the molecule. The structure we find in the solid state is in remarkably close agreement to the one predicted by energy minimization calculations, which was used in the NMR studies of amikacin/enzyme interactions in solution.[9] However, it is very different from the results of an earlier NMR report,[6] which proposed the existence of certain intramolecular H bonds (between O_2' and N_3'', and between N_3 and O_a') that are not found in the present study.

Table 1. Molecular Parameters in Amikacin.

(a) Bond Distances (Å)

O(1')–C(1')	1.410(14)	O(2')–C(2')	1.442(13)
O(3')–C(3')	1.438(13)	O(4')–C(4')	1.433(13)
O(1')–C(5')	1.440(13)	O(5)–C(5)	1.434(12)
O a–C(1')	1.411(15)	O a–C(4)	1.443(12)
O–C	1.246(14)	O a'–C a'	1.430(16)
O b–C(1'')	1.434(13)	O b–C(6)	1.451(13)
O(1'')–C(1'')	1.401(14)	O(2'')–C(2'')	1.446(14)
O(4'')–C(4'')	1.438(15)	O(6'')–C(6'')	1.431(14)
O(1'')–C(5'')	1.432(15)	N(1)–C	1.345(15)
N(1)–C(1)	1.472(13)	N(3)–C(3)	1.479(15)
N(6')–C(6')	1.473(16)	N(3'')–C(3'')	1.471(12)
C(1)–C(2)	1.510(16)	C(1)–C(6)	1.510(15)
C(2)–C(3)	1.512(15)	C(3)–C(4)	1.517(15)
C(4)–C(5)	1.522(16)	C(5)–C(6)	1.518(14)
C(1')–C(2')	1.492(15)	C(2')–C(3')	1.515(15)
C(3')–C(4')	1.502(17)	C(4')–C(5')	1.537(15)
C(5')–C(6')	1.496(18)	Nc'–Cc'	1.497(25)
C(1'')–C(2'')	1.538(14)	C(2'')–C(3'')	1.517(18)
C(3'')–C(4'')	1.517(16)	C(4'')–C(5'')	1.526(14)
C(5'')–C(6'')	1.504(17)	C–Ca'	1.525(16)
Ca'–Cb'	1.478(22)	Cb'–Cc'	1.522(19)

(b) Bond Angles

C(1')–O(1')–C(5')	115.0(9)	C(1')–Oa–C(4)	118.5(8)
C(1'')–Ob–C(6)	113.8(8)	C(1'')–O(1'')–C(5'')	115.6(8)
C–N(1)–C(1)	123.6(9)	N(1)–C(1)–C(6)	108.1(8)
N(1)–C(1)–C(2)	111.7(9)	C(6)–C(1)–C(2)	110.9(9)
C(1)–C(2)–C(3)	112.5(9)	N(3)–C(3)–C(2)	109.5(9)
N(3)–C(3)–C(4)	113.6(10)	C(2)–C(3)–C(4)	108.6(8)
Oa–C(4)–C(3)	111.4(8)	Oa–C(4)–C(5)	108.9(8)
C(3)–C(4)–C(5)	110.3(10)	O(5)–C(5)–C(6)	108.6(8)
O(5)–C(5)–C(4)	110.1(9)	C(6)–C(5)–C(4)	109.2(8)
O b–C(6)–C(1)	106.6(9)	O b–C(6)–C(5)	111.7(8)
C(1)–C(6)–C(5)	111.0(8)	O(1')–C(1')–O a	111.4(9)
O(1')–C(1')–C(2')	110.3(9)	O a–C(1')–C(2')	109.4(10)
O(2')–C(2')–C(1')	110.7(8)	O(2')–C(2')–C(3')	113.1(10)
C(1')–C(2')–C(3')	109.4(9)	O(3')–C(3')–C(4')	108.9(9)
O(3')–C(3')–C(2')	110.6(9)	C(4')–C(3')–C(2')	108.0(10)
O(4')–C(4')–C(3')	109.1(10)	O(4')–C(4')–C(5')	109.1(8)
C(3')–C(4')–C(5')	112.6(9)	O(1')–C(5')–C(6')	104.4(10)
O(1')–C(5')–C(4')	113.0(8)	C(6')–C(5')–C(4')	111.1(10)

Table 1 (continued)

N(6')–C(6')–C(5')	117.3(11)	O(1")–C(1")–O b	107.5(9)
O(1")–C(1")–C(2")	110.1(9)	O b–C(1")–C(2")	113.1(8)
O(2")–C(2")–C(3")	109.4(9)	O(2")–C(2")–C(1")	111.1(9)
C(3")–C(2")–C(1")	111.6(10)	N(3")–C(3")–C(2")	108.0(9)
N(3")–C(3")–C(4")	110.0(8)	C(2")–C(3")–C(4")	108.1(9)
O(4")–C(4")–C(3")	109.3(8)	O(4")–C(4")–C(5")	110.0(9)
C(3")–C(4")–C(5")	109.1(9)	O(1")–C(5")–C(6")	106.6(9)
O(1")–C(5")–C(4")	109.1(9)	C(6")–C(5")–C(4")	113.7(9)
O(6")–C(6")–C(5")	110.0(9)	O–C–N(1)	123.8(11)
O–C–C a'	120.2(11)	N(1)–C–C a'	116.0(11)
O a'–C a'–C b'	110.0(11)	O a'–C a'–C	108.3(11)
C b'–C a'–C	112.6(12)	C a'–C b'–C c'	112.8(14)
N c'–C c'–C b'	111.0(19)		

Table 2: Selected Torsion Angles in Amikacin

107.3	C4 – Oa – C1' – O1'	(ϕ1a) [a]
–130.5	C4 – Oa – C1' – C2'	(ϕ1b) [a]
120.4	C1' – Oa – C4 – C5	(ψ1a) [a]
–117.8	C1' – Oa – C4 – C3	(ψ1b) [a]
151.9	C6 – O b – C1" – O1"	(ϕ2a) [a]
–86.3	C6 – O b – C1" – C2"	(ϕ2b) [a]
146.0	C1" – O b – C6 – C1	(ψ2a) [a]
–92.6	C1" – O b – C6 – C5	(ψ2b) [a]
–0.9	C1 – N1 – C – O	
176.9	C1 – N1 – C – Ca'	
147.8	C – N1 – C1 – C6	
–89.8	C – N1 – C1 – C2	
–61.7	O1" – C5" – C6" – O6" [b]	
58.5	C4" – C5" – C6" – O6" [b]	
65.6	O1' – C5' – C6' – N6' [b]	
–172.3	C4' – C5' – C6' – N6' [b]	

footnotes for Table 2: (a) The torsion angles (ϕ_a, ϕ_b, ψ_a, ψ_b) in this table are related to the angles (ϕ_1, ϕ_1', ϕ_2, ϕ_2') respectively as defined by Sundaralingam[16] for the conformation of oligosaccharides.

(b) Torsion angles related to the exocyclic hydroxymethyl and aminomethyl groups. The $C_6"$–$O_6"$ bond is in the gg conformation (*gauche* to both $C_5"$–$O_1"$ and $C_5"$–$C_4"$), while the C_6'–N_6' bond is in the gt *conformation* (*gauche* to C_5'–O_1' and *trans* to C_5'–C_4')

In conclusion, our results suggest that the two bifurcated hydrogen bonds shown in Figure 1 are important factors in influencing the internal conformation of the amikacin molecule. In comparing the structure of amikacin with its parent, kanamycin A[15] (in which the entire amide side chain in the N_1 position is replaced by an NH_2 group), one finds that the A/B ring conformations are essentially unchanged, while the B/C ring junctions are significantly different. The torsion angles around the C_1'-O_a-C_4 bridge are virtually identical between amikacin and kanamycin A, suggesting that the H bonds involving the O_5 hydroxyl group, which is common to both molecules, are probably playing a pivotal role in controlling the A/B ring orientation. In contrast, the torsion angles around the C_6-O_b-C_1'' bridge are significantly different between the two molecules, which suggests that the replacement of the NH_2 group in the N_1 position of kanamycin A by the long hydoxyaminobutyryl side chain in amikacin has modified the conformational angles around the B/C ring junction in a substantial way.

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