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Conformations of Fortimicins and Three-Dimensional Structure-Activity Relationship in the Aminoglycoside Antibiotics

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SUMMARY

The fortimicins are pseudodisaccharide antibiotics consisting of purpurosamine and fortamine moieties which are connected through an α-linkage. NMR spectra indicate that the fortamine ring in fortimicin A takes a chair conformation which corresponds to an inverted one in the conformation of fortimicin B free base. Fortimicin A is unstable in solution, and we were unable to obtain any crystals of it. To elucidate the structure of fortimicin A and to clarify the stereochemistry of fortimicins, we have undertaken to calculate their empirical force-fields starting from the X-ray structures of fortamines and fortimicin B free base. Nuclear Overhauser enhancement data were also employed to confirm the calculated relative orientation of the two rings around the glycosidic linkage. The 3-dimensional structure-activity relationship of aminoglycoside antibiotics is discussed on the basis of these results.

INTRODUCTION

Fortimicins are potent broad-spectrum antibiotics (1). The structures of two of their major members, fortimicin A and fortimicin B, have been determined by ^{1}H- and ^{13}C-NMR, mass spectra, and CD, combined with chemical degradations (2). Fortimicins are pseudodisaccharides consisting of purpurosamine and fortamine moieties which are connected through an α-glycosidic linkage. Studies of ^{1}H-NMR coupling constants (2) suggested that the fortamine rings in fortimicin A and in fortimicin B disulfate take a conformation which corresponds to an inverted one in the conformation of fortimicin B free base. In this paper the fortamine ring conformation of fortimicin A and fortimicin B disulfate is described as an "A"-type conformation and that of fortimicin B free base as a "B"-type conformation (Fig. 1). X-ray analysis has confirmed that the fortamine ring in fortimicin B free base is in the $^4C_1$ conformation (3). Removal of the glycine part from fortimicin A leads to a drastic structural change and reduces the antibiotic activity. For full explanation, the detailed structure of fortimicin A must be established. Unfortunately, suitable crystals of fortimicin A free base have not been obtained thus far. Accordingly we have tried to apply the molecular mechanics calculation, which has proved to be useful in modeling unknown structures based on the X-ray structures of fortamines (4–6) and fortimicin B free base (3). Stereochemical aspects of fortimicins should also be disclosed through this approach. In the present paper we report the calculated conformation of fortimicin A, its confirmation by ^{1}H-NMR and the nuclear Overhauser effect, and the 3-dimensional structure-activity relationship of aminoglycoside antibiotics.

CONFORMATIONAL ENERGY CALCULATIONS

The program used was MOLBD$_3$, by Boyd (7). In energy minimization all degrees of freedom were allowed for; no internal coordinate was kept fixed. Giglio's potentials (8) were used in the calculation of the nonbonded interactions and Boyd's potentials for others. To include the effect of the intramolecular hydrogen bond, we assumed a weak bond between the hydrogen atom and the hydrogen acceptor. The force constants, 1.0 kcal/Å^2 and 0.26 kcal/degree^2 for bond length and bond angle, respectively, were suitable for our calculation. To estimate the stabilization energy of hydrogen bond formation, the empirical inverse third-power expression $V_{hb} = -55.0/R^3$ (9) was employed, which was used to calculate the hydrogen-bond energies of the —OH $\cdots$ O system; R was originally the distance between oxygen atoms. To take the effect of the electrostatic interaction into account, the charges of +⅓ and +½ e.s.u. were assumed for the amino groups which contain three and two hydrogen atoms, respectively. The shielding 0.5 constant of Momany *et al.* (10) was introduced, which was originally proposed for intermolecular interactions. In this calculation we did not assume the planarity of the amide group. Therefore, appropriate correction should be made for the pertinent conformational energies. The energy barrier of rotation around the amide C—N bond in formamide has been measured by NMR methods to be 17.8 kcal/mole (11). We used this value as a force con-

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stant (k) for the equation

$$E_1 = \tfrac{1}{2}k[1 - \cos(2\phi)],$$

to estimate the labilizing energy values (E_1) corresponding to the relevant torsional angles (ϕ).

Initial structure. Conformational energies of the fortimicin system were calculated for three types of fortimicins: fortimicin B free base, protonated fortimicin B, and fortimicin A. As initial structures of fortimicin B we adopted three structures. The first one was the structure obtained by X-ray analysis, where the intramolecular —OH ··· N hydrogen bond was maintained. The second structure was the same as the first, but the constraint by the hydrogen bond was removed. The third structure was a hypothetical fortimicin B with fortamine in Conformation A; the structure, with the exception of the fortamine moiety, was taken from the X-ray results for fortimicin B free base, and the X-ray results for fortamine dihydrochloride were used for the A-type fortamine moiety. These two component structures were matched by sharing the plane determined by the C-1, C-5, and C-6 atoms as a common plane. Here we call the three initial structures FB[2], FB(−HB), and FB(A), respectively. The following initial structures for the protonated fortimicin B were also built and examined in a similar manner. They were the protonated fortimicin B with the A-type fortamine [FB^+(A)] and that with the B-type fortamine [FB^+(B)]. The former was constructed in the same manner as that of FB(A) and the latter structure was based on the X-ray results of fortimicin B with replacement of protonated amino groups, where the C—N bond lengths of fortamine dihydrochloride were employed and the positions of the hydrogen atoms were calculated assuming sp^3 hybridization for each nitrogen atom with an N—H distance of 1.00 Å. The constraint of the intramolecular hydrogen bond was removed and the effects from the counter-anion were neglected. We constructed two initial structures of fortimicin A. One was fortimicin A with the A-type fortamine [FA(A)] and another was that with the B-type fortamine [FA(B)]. The molecular skeletons (except for the glycine part) were built in the same manner as above. The dimensions of the glycine part were taken from the X-ray results of γ-glycine (12). As the terminal amino group is *trans* to the imino group in glycylglycine (13), we joined the glycine part to the fortimicins to maintain this geometry. There is a freedom of rotation around the C(4)—N(4) bond. By looking at the Dreiding model, we took four rotamers around this bond as the initial structures.

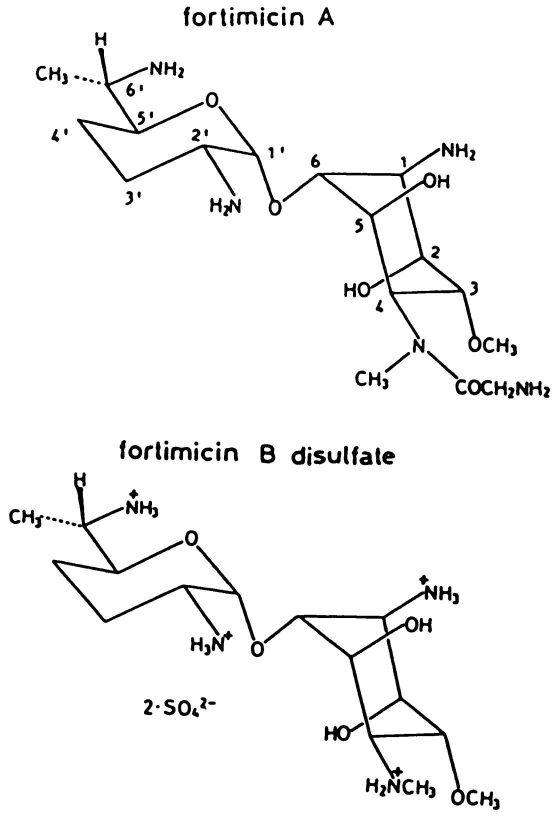
Energy minimization. The conformational energies of the initial structures were minimized by using $MOLBD_3$. The program $MOLDB_3$ was originally written for hydrocarbons; for the molecular geometrical quantities relevant to nitrogen and oxygen atoms, the X-ray results on fortamines and fortimicin B free base were employed. We examined in advance whether this process could be successfully applied to the present molecular systems. Preliminary computations using the X-ray results of (+)-isomethyl *p*-bromophenylcarbamate (14) were undertaken. The $MOLBD_3$ gave reasonable and almost identical energies for the three crystallographically independent molecules. This proved that the program would be satisfactory for fortimicins. When the conformational energies of the structures exceeded 1000 kcal/mole, the structures were considered improbable and further refinement was not attempted.

NMR experiments. NMR experiments were performed on a JEOL PS-100 NMR spectrometer operating at a probe temperature of 25° and in the external lock mode. NOE of signal intensity was measured from the difference between the integrated signal areas (at least three times) before and after saturation of the signal of interest.

RESULTS AND DISCUSSION

The calculated minimal energies for fortimicins are listed in Table 1.

'A' TYPE CONFORMATION


fortimicin A

fortimicin B disulfate

'B' TYPE CONFORMATION

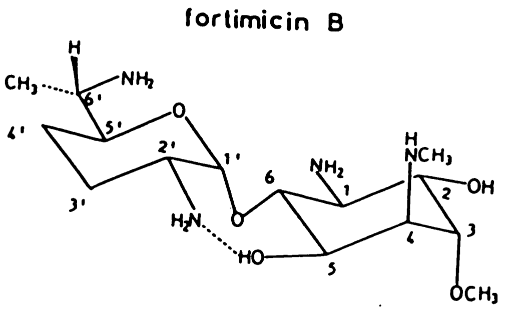

fortimicin B

FIG. 1. *Structure of fortimicins*

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[2] The abbreviations used are: FB, fortimicin B; FA, fortimicin A; NOE, nuclear Overhauser enhancement.

TABLE 1

Conformational energies of fortimicins

Fortimicin	Conformational energy
	kcal/mole
FB	19.5
FB(A)	20.9
FB(−HB)	20.4
FB^+(A)	23.6
FB^+(B)	28.2
FA	22.1
FA(B)	25.4

Structure of fortimicin B free base. The calculated structure of FB is in good agreement with that determined by X-ray analysis. The distance between H-1′ and H-6 (2.32 Å) in the calculated structure is still shorter than the sum of the van der Waals radii. The corresponding value in the crystal is 2.14 Å. This highly repulsive nonbonded interaction seems to be compensated by the strong intramolecular hydrogen bond in the crystal. The stabilization energy due to the hydrogen bond formation was 2.64 kcal/mole. In the calculated molecular structure of FB(−HB), without the intramolecular hydrogen bond, this H-1′ ··· H-6 repulsion is apparently released by the rotation of fortamine and purpurosamine around the glycosidic bond, but the conformational energy is higher than that of FB. These results indicate that fortimicin B free base would adopt the FB conformation *in vacuo* just as in the crystal, and that the intramolecular hydrogen bond plays a decisive role.

Structure of fortimicin B under acidic conditions. The energy difference between the two structures is large, as shown in Table 1, and this shows that the A-type conformation of fortamine is indispensable to the structure of protonated fortimicin B.

It has been suspected that the ring inversion in fortimicin B disulfate may be due to the nature of fortamine itself. However, in a series of X-ray analyses of fortamines (4–6), we have disclosed that the rings in fortamine itself (4), in its dihydrochloride (5), and in its sulfate (6) take the B-, A-, and B-type conformations, respectively. On the other hand, in solution the NMR spectra indicated that all of them take essentially the same (B-type) conformation (6). These facts mean that, although the protonation at the amino groups may be *sine qua non* for the ring inversion from B to A, the A-type conformation is rather sensitive to environmental effects. This argument is supported by energy calculation, which shows that the energy difference between the A-type and B-type conformations is remarkably small in the acidic forms, as shown in Table 2. To understand the mechanism of the ring inversion in fortimicin B disulfate and fortimicin A, we must also consider the intramolecular effect of the purpurosamine moiety on the fortamine moiety. Electrostatic perturbation from the purpurosamine moiety is enough for this. About 82% of the electrostatic energy which destabilizes FB^+(B) is associated with the amino group at position 2′. Therefore, it may be concluded that under acidic conditions the ring inversion in fortimicin B is energetically favored mainly as a result of removal of the electrostatic repulsion of the protonated amino groups in both rings.

TABLE 2

Conformational energies of fortamine

Form	Conformational energy
	kcal/mole
Fortamine	6.09
Fortamine (A-type)	6.91
Protonated fortamine (B-type)	7.37
Protonated fortamine (A-type)	7.69

Structure of fortimicin A. The conformation of FA(B) with the intramolecular —NH ··· O hydrogen bond is more stable than that without it by approximately 1.2 kcal/mole. We started from four rotamers around the glycine part of FA(A). One of them is sufficiently stable (by approximately 10 kcal/mole) as compared with the second stable rotamer. As shown in Table 1, the FA(A) conformation is more stable by 3.3 kcal/mole than that of the FA(B) conformer. The main effect which destabilizes the B-type conformation is nonbonded interaction between the axial substituents and the glycine part of the fortamine moiety. The stereoscopic drawing of the molecular structure of FA(A) which corresponds to the minimal energy is shown in Fig. 2. In this structure the amide plane is nearly parallel to the C(4)—H bond, and the distance between the carbonyl oxygen atom and the H-4 atom is 2.74 Å. This arrangement seems to favor the interpretation that the anomeric effect of the amide carbonyl group causes the remarkably low field shift (δ = 5.36) of the H-4 proton (2).

Inversion of the fortamine ring from the B- to the A-type conformation is expected to produce the relatively strong 1,3-diaxial interaction between the amino group and the hydroxyl group. In the FA(A) structure, however, the distance between the oxygen atom and the nitrogen atom is 3.02 Å, which is unstrained. The empirical force-field calculation indicates that strain energy cannot be confined to pertinent parts of the molecule but will be shared by almost all of the atoms in the molecule to relieve the local strain. Therefore, we must compare the stability of some structures after self-relaxation of their intramolecular strain energies has been attained.

Structure of fortimicins in solution. Information about molecular structures in solution is essential for discussion of the structure-activity relationship of antibiotics. All of the calculated torsion angles in the rings corresponding

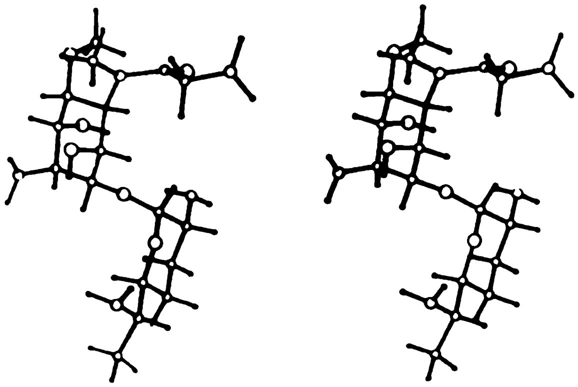

FIG. 2. *Minimal energy structure of fortimicin A*

This structure was drawn by TSD:XTAL, a program for the NOVA 3 computer (15).

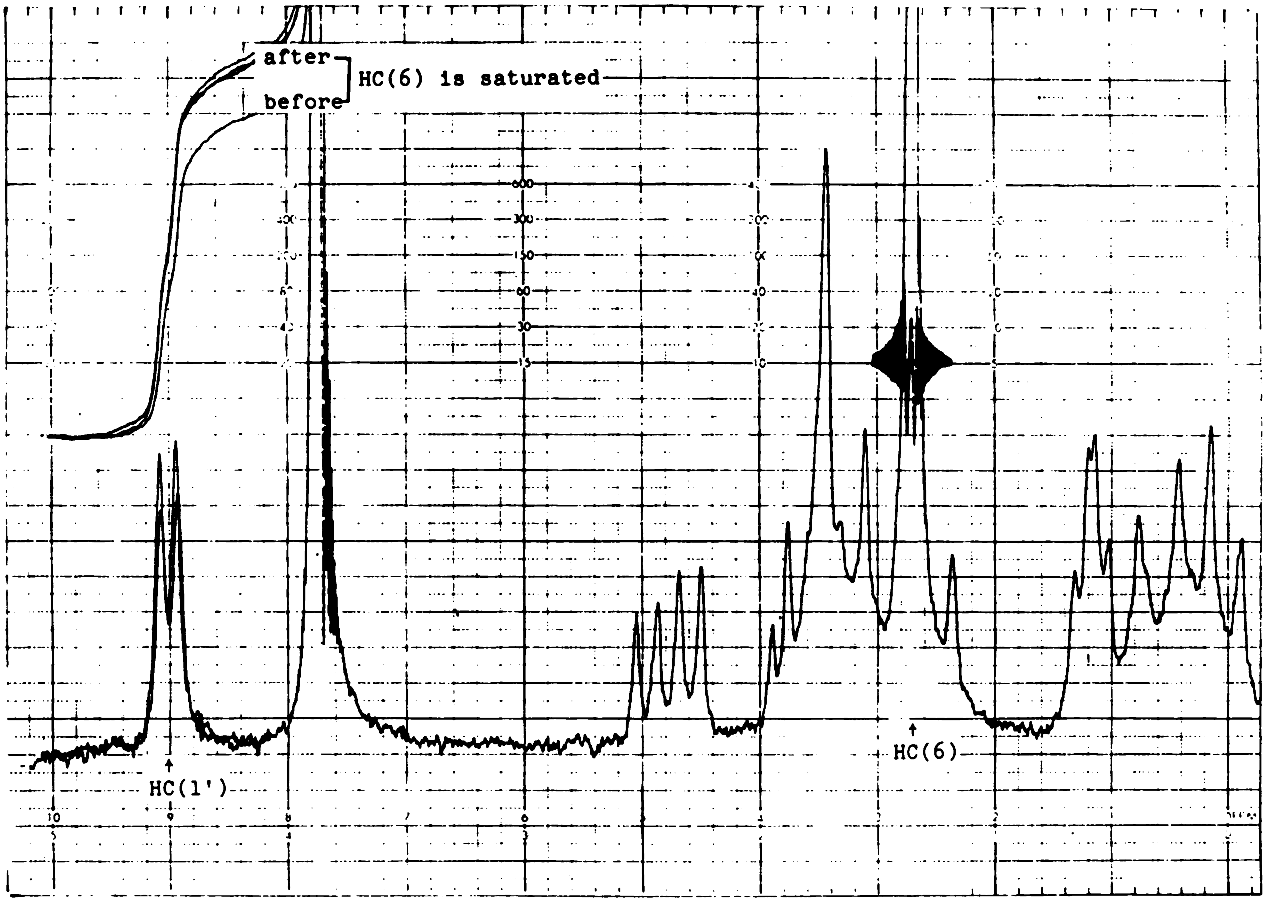

FIG. 3. *Nuclear Overhauser enhancement of the intensity of the H-1′ proton with irradiation of the H-6 proton in fortimicin B free base*

to the minimal energies explain qualitatively the coupling constants of ^{1}H-NMR obtained by Egan *et al.* (2).

The rotation around the glycosidic bond, on the other hand, is possible to some extent in solution, furnishing freedom in the relative arrangement of the functional groups. Given a distance between the H-1′ and H-6 atoms, the possible conformation can be limited to a certain range. To determine the internuclear distance, we made use of NOE in NMR, which occurs for pairs of protons in close spatial proximity. In the ^{1}H-NMR spectrum of fortimicin A, all of the protons on the fortamine moiety have been assigned unequivocally, and the H-1′ proton is sufficiently separated from other signals (16). The spectrum for fortimicin B free base in D_2O is shown in Fig. 3, where the signal intensity of the H-1′ proton is increased when the H-6 proton is saturated. The percentages of enhancement for fortimicin B free base, fortimicin B tetrahydrochloride, and fortimicin A tetrahydrochloride are listed in Table 3. The observed NOE value of 16.9% in the solution of fortimicin B free base corresponds to an internuclear distance of 2.64 Å according to the equation of Bell and Saunders (17), although the conformational energy calculations showed that the distance between the pertinent proton atoms in FB and FB(−HB) are 2.32 and 2.89 Å, respectively. For these flexible molecules, especially around the glycosidic bond, the equation overestimates the internuclear distance, as is known in the case of peptide molecules (18). This means that the fortimicin B free base molecule in solution should be nearly equal to the structure of FB and, accordingly, the intramolecular hydrogen bond should be conserved. The observed NOE value of 4.5% for fortimicin A can explain qualitatively the internuclear distance in the FA(A) structure (2.89 Å). Therefore, it may be concluded that the conformation of fortimicin A free base in solution will be quite similar to that calculated by conformational energy calculations. The internuclear distance in the FB^+(A) calculated by molecular mechanics seems to be somewhat shorter than the corresponding distance deduced from the observed NOE percentage value.

TABLE 3

Nuclear Overhauser enhancement

Fortimicin	NOE
	%
Fortimicin B free base (*pD* = 11.4)	16.9
Fortimicin B tetrahydrochloride (*pD* = 1.8)	3.5
Fortimicin A tetrahydrochloride (*pD* = 0.7)	4.5

Three-dimensional structure-activity relationship in the aminoglycoside antibiotics. For aminoglycoside antibiotics with similar chemical structures and similar biological behavior, many studies have been attempted, mainly from a synthetic chemical point of view, to determine the structure-activity relationship. The number of X-ray analyses of aminoglycoside antibiotics is quite small, perhaps owing to the difficulty in obtaining suitable crystals. Consequently, little attention has been paid to the 3-dimensional structure-activity relationship of aminoglycoside antibiotics.

Here we compare the relative position of the amino

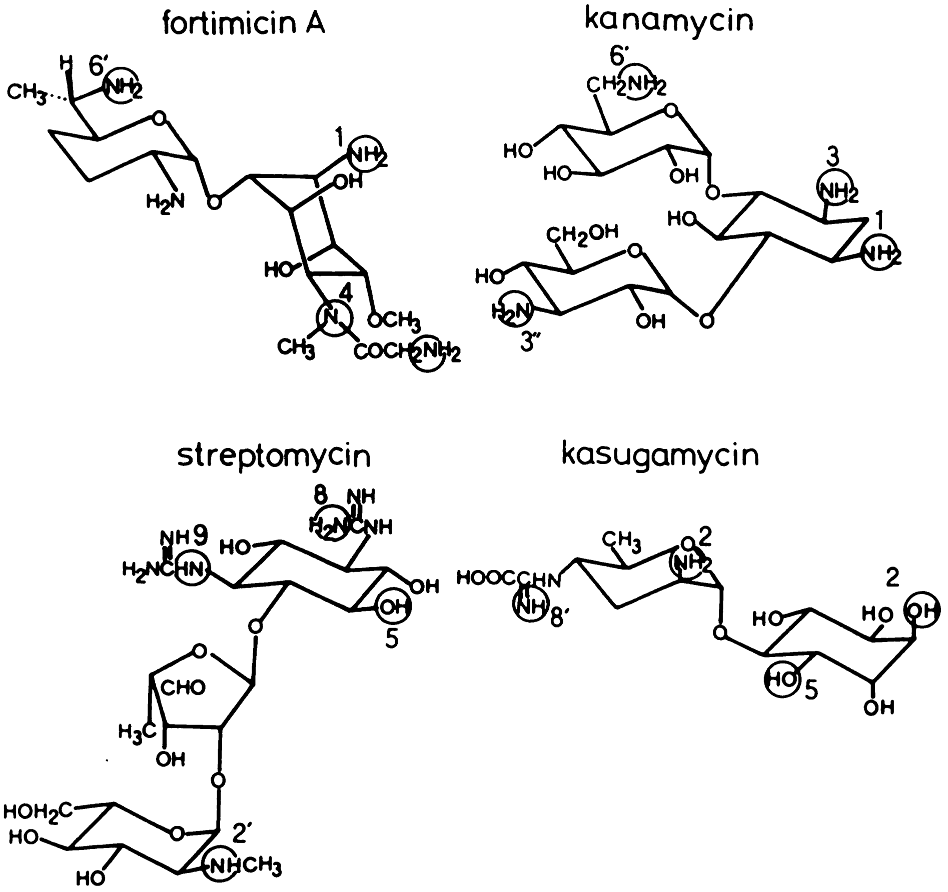

FIG. 4. *Chemical structures of fortimicin A, kanamycin, kasugamycin, and streptomycin*

groups and hydroxyl groups of kanamycin (19), kasugamycin (20), streptomycin (21), and fortimicin A, whose chemical structures are given in Fig. 4. Distribution of the amino and hydroxyl groups is apparently quite different among them. However, minimal rotation around the glycosidic linkage and peripheral bonds brings about essentially the same disposition of these functional groups as shown in Fig. 5, where the average distances between the pertinent functional groups and their estimated standard deviations are given. Fortimicin A in Fig. 5 is obtained by rotation around the O—C(6) and C(4)—N(4) bonds by −35° and −40°, respectively. In the case of kanamycin the rotations around the O—C(1′), C(1″)—O, and O—C(4) bonds are 35°, −30°, and 30°, and in streptomycin the rotations around the O—C(2′) and N(7)—C(1) bonds are −40° and 90°. In the case of kasugamycin the rotations around the O—C(1′) and N(7′)—C(4′) bonds are 10° and 30°. These rotations are free of the nonbonded repulsion in the molecules, and the intramolecular hydrogen bond in these structures can be maintained. Therefore, these conformers are energet-

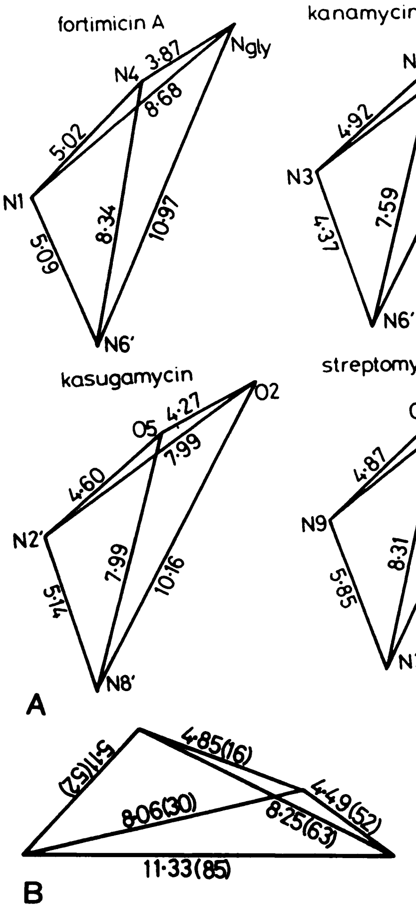

FIG. 5. *Relative dispositions of and distances between functional groups of some aminoglycoside antibiotics*

A. The relative dispositions (in angstroms) of functional groups in some aminoglycoside antibiotics. Values are based on X-ray analyses, but some rotations are made around glycosidic bonds and peripheral bonds.

B. The average distance (in angstroms) between the corresponding functional groups with their estimated standard deviations.

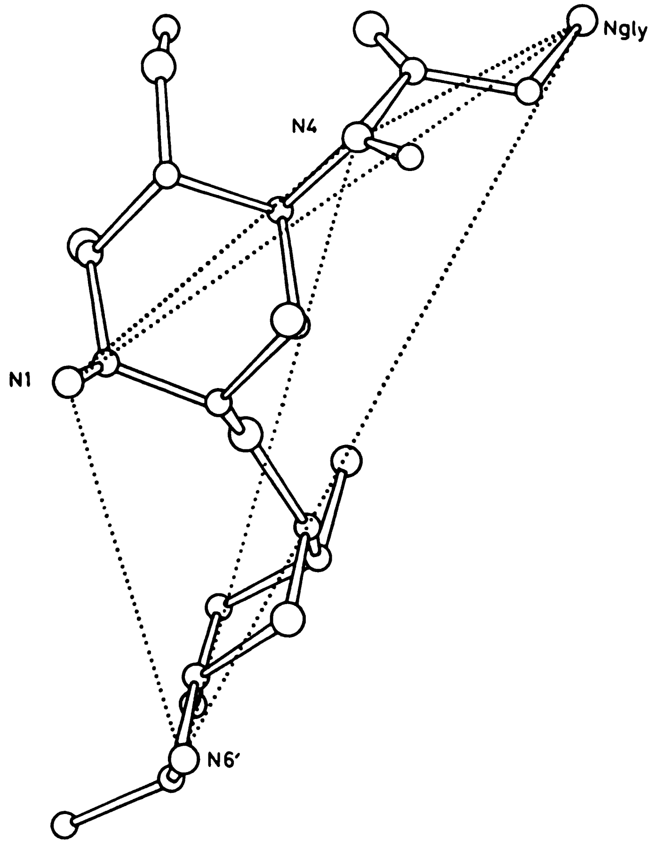

FIG. 6. *Perspective drawing of the supposed active form of fortimicin A*

ically allowed. A perspective drawing of the supposed form of active fortimicin A is shown in Fig. 6. It is noteworthy that all of the pertinent functional groups lie on the same side of the molecules and they are approximately on the surface of a right cylinder with a radius of approximately 5.5 Å.

It appears that such a common pattern of the arrangement of the functional groups may actually contribute to the binding in one of the pertinent receptor sites in certain biopolymers. Admittedly, the present observation is phenomenological and the facts presented here are oversimplified. But the approach through this line may be of use in designing better aminoglycoside antibiotics, because the receptor-complement feature will be essential to exploring *in vivo* drug interaction in greater detail.

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