Chemistry of the Gentamicins. II. Stereochemistry and Synthesis of Gentosamine. Total Structure of Gentamicin A

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Abstract: The anomeric mixture of methyl gentosaminides obtained upon methanolysis of gentamicin A was resolved, and the components were identified as methyl 3-methylamino-3-deoxy-D-xylopyranosides. Methyl 3-methylamino-3-deoxy-β-D-xylopyranoside and 3-methylamino-3-deoxy-D-xylose hydrochloride were synthesized from D-arabinose and identified as methyl β-gentosaminide and gentosamine hydrochloride, respectively. Gentosamine is linked α-glycosidically to 2-deoxystreptamine. The remaining stereochemical uncertainties in gentamicin A are thus clarified, and its structure can be regarded as O-2-amino-2-deoxy-α-D-glucopyranosyl-(1→4)-O-[3-methylamino-3-deoxy-α-D-xylopyranosyl-(1→6)]-1,3-diamino-1,2,3-trideoxyscylloinositol.

Gentamicin A is one of the numerous antibiotics which comprise the gentamicin complex. Resolution of the complex and chromatographic characterization of the gentamicin antibiotics were described previously.[2,3] Gentamicin A[4] as well as gentamicins C_1, C_2, and D[5,6,7,8] together with the recently synthesized kanamycins A,[9] B,[10] and C,[11] belong to the group of 4,6-disubstituted 2-deoxystreptamine antibiotics.[12] Gentamicins C_1, C_2, and D contain garosamine[13] and purpurosamines[6,7] rather than glucosamine and gentosamine which are constituents of gentamicin A.[4]

Gentamines, representing neamine analogs derived from gentamicins, constitute units consisting of a monosaccharide moiety linked to C_4 of 2-deoxystreptamine. Gentamicin A can be regarded as a derivative of a gentamine which is identical with paromamine, thus differing considerably from other members of the complex, but exhibiting a considerable similarity to the paromomycins and particularly to kanamycin C. This chemical relationship of metabolites demonstrates a strikingly similar biogenetic ability of the gentamicin producing *Micromonosporae* and the kanamycin- and paromomycin-producing *Streptomycetes*. Whether the gentamines of gentamicins C_1, C_2, or D are identical with O-purpurosaminyl-(1→4)-2-deoxystreptamine or O-garosaminyl-(1→4)-2-deoxystreptamine is not as yet certain, although biogenetic considerations based on the structural similarity of gentosamine and garosamine tend to favor the former alternative.[12]

Previous studies on gentamicin A led to a gross structure[4] with undetermined stereochemistry of the gentosamine moiety. In this report we wish to describe the anomeric methyl gentosaminides, the structure elucidation and synthesis of methyl β-gentosaminide and gentosamine, as well as the stereochemistry of the glycosidic linkage between paromamine and gentosamine, thus establishing the complete stereochemistry of gentamicin A.

Results and Discussion

Methanolysis of gentamicin A afforded a crystalline mixture of the anomeric methyl gentosaminides which was identified as methyl 3-methylamino-3-deoxypentapyranosides.[4] Factional crystallization afforded pure methyl β-gentosaminide **6** (Scheme I), mp 140°, as well as the α anomer, mp 122°.

The proton magnetic resonance spectrum of the glycoside with mp 140° is shown in Figure 1. The anomeric proton at δ 4.76 is coupled to H_2 by 7.5 Hz, clearly suggesting the diaxial configuration of these two hydrogen atoms. This coupling constant of 7.5 Hz, together with a spin–spin interaction of 9 Hz between H_2 and H_3, gives rise to a quartet at δ 3.72, representing the chemical shift of H_2. Thus, the hydrogen atom at C_3 is also present in axial position. This assumption is confirmed by a triplet with approximate, relative intensities of 1:2:1 at δ 2.92 which can be regarded as the chemical shift of H_3, the perturbance being generated by equal spin–spin interactions with the protons on C_2 and C_4. The coupling constant of 9 Hz between H_3 and H_4 is observed in the splitting pattern of H_4, centered at δ 4.10; this signal is further perturbed by couplings with the axial and equatorial protons on C_5. The coupling constant of 4.5 Hz between H_4 and the equatorial hydrogen on C_5 is in agreement with the corresponding dihedral angle of 60° and coupling to the axial proton on C_5 is revealed by a characteristic value of 9.5 Hz. The signals for axial and equatorial protons on C_5 appear as quartets with chemical shift values of δ 3.77 and 4.38, respectively.

(1) Author to whom correspondence should be addressed at: Hoffmann-La Roche Inc., Nutley, N. J. 07110.
(2) H. Maehr and C. P. Schaffner, *J. Chromatogr.*, 30, 572 (1967).
(3) G. H. Wagman, J. A. Marquez, and M. J. Weinstein, *ibid.*, 34, 210 (1968).
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(5) Gentamicin D was previously designated C_{1a}.
(6) D. J. Cooper, H. M. Marigliano, T. Traubel, and M. D. Yudis, 5th International Symposium on the Chemistry of Natural Products, London, July 8–13, 1968, p 435.
(7) D. J. Cooper, H. M. Marigliano, M. D. Yudis, and T. Traubel, *J. Infec. Dis.*, **119**, 342 (1969).
(8) Chemical investigations concerned with the determination of the amino sugar linkages to 2-deoxysteptamine previously reported for gentamicin A[4] were repeated in this laboratory with gentamicins C_1, C_2, and D. The almost quantitative liberation of 2-deoxystreptamine after periodate oxidation and hydrolysis confirmed the presence of 4,6-disubstituted 2-deoxystreptamine moieties in gentamicins C_1, C_2, and D and hence substantiates the speculative assignment by Cooper and coworkers.[7]
(9) (a) M. Nakajima, A. Hasegawa, N. Kurihara, H. Shibata, T. Ueno, and D. Nishimura, *Tetrahedron Lett.*, 623 (1968); (b) S. Umezawa, K. Tatsuta, and S. Koto, *Bull. Chem. Soc. Jap.*, 42, 533 (1969).
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(12) K. L. Rinehart, Jr., *J. Infec. Dis.*, **119**, 345 (1969).
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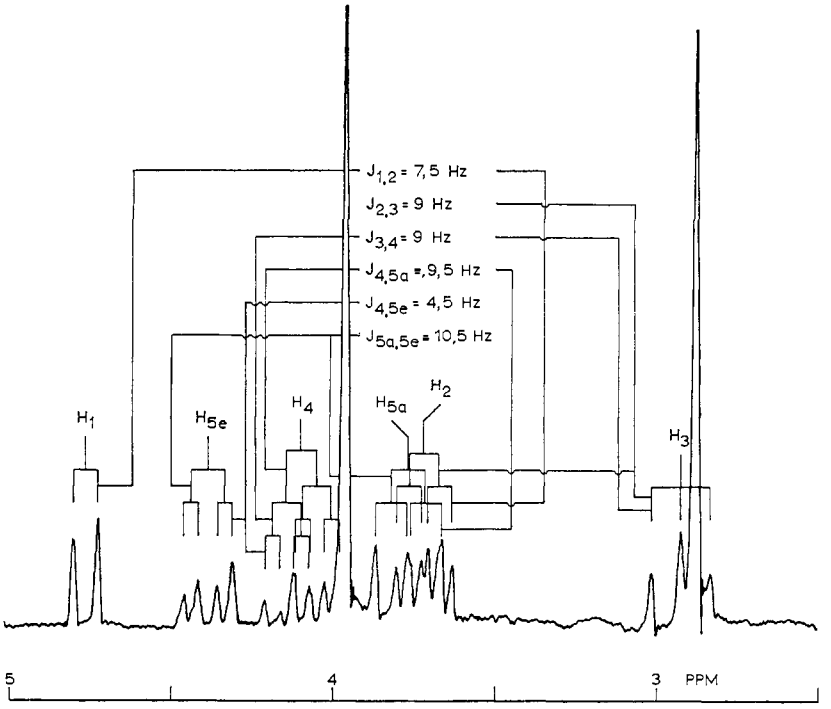
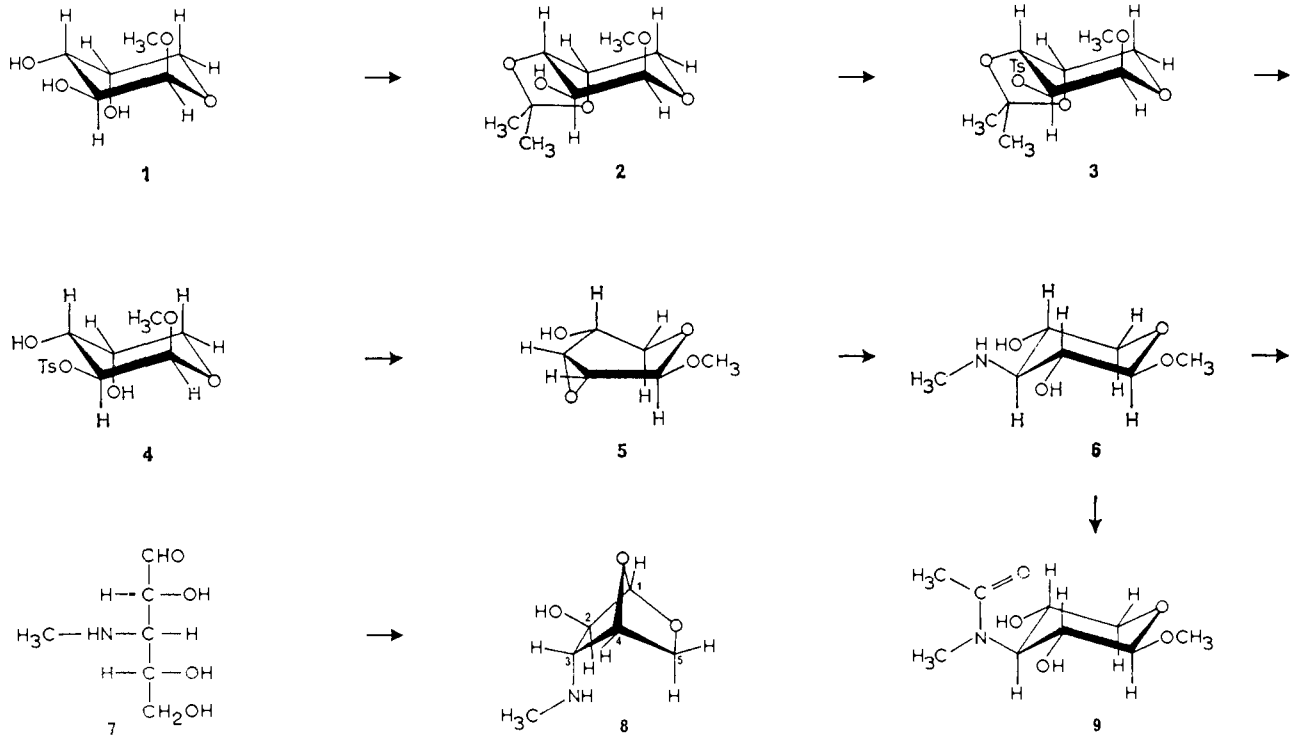


Figure 1. Proton magnetic resonance spectrum of methyl β-gentosaminide.

First-order analysis of the nmr spectrum of the methyl gentosaminide of mp 140°, therefore, established xylo configuration and the structure can be regarded as methyl 3-methylamino-3-deoxy-β-D-xylopyranoside **6** or its mirror image.

Scheme I



The D configuration of the methyl gentosaminides is confirmed by comparison of the molecular rotations of the known methyl D-xylopyranosides, M_D^{α} +252 and M_D^{β} −106,[14] with the corresponding values of +246 and −106 of the α- and β-methyl gentosaminides, respectively.

To provide definite structure proof, **6**, **7**, and **9** were synthesized as outlined in Scheme I which illustrates a significant conformational structure for each compound.

Methyl β-D-arabinopyranoside **1** was prepared from D-arabinose by a modification of the procedure described for the enantiomer.[15] Treating **1** with acetone and phosphorus pentoxide[16] yielded methyl 3,4-O-

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(15) C. S. Hudson, *J. Amer. Chem. Soc.*, 47, 265 (1925).

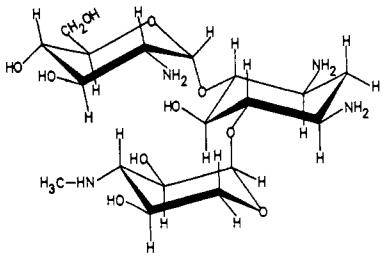

Figure 2. The structure of gentamicin A.

isopropylidene-β-D-arabinopyranoside **2**[17] in 83% yield (bp 90°, 0.5 mm). Tosylation of **2** in pyridine was carried out for 48 hr as described.[17] After recrystallization from aqueous ethanol, **3** was obtained in 95% yield (mp 134°; $[\alpha]^{25}D$ −179, *c* 1.0 chloroform). The isopropylidene group of the resulting methyl 2-O-tosyl-3,4-O-isopropylidene-β-D-arabinopyranoside **3** was selectively removed in refluxing 1 *M* acetic acid[18] to give methyl-2-O-tosyl-β-D-arabinopyranoside **4**. Displacing the tosyl group intramolecularly in crude **4** according to the method described for the enantiomer[18] afforded methyl 2,3-anhydro-β-D-ribopyranoside **5**[19] in 67% yield (bp 115°, 0.1 mm). Opening of the epoxide ring in **5** with methylamine yielded **6** almost exclusively. Treatment of **6** with acetic anhydride in methanol afforded **9** which exhibited negative ninhydrin and Pan–Dutcher reactions and was completely inert toward sodium metaperiodate, thus confirming structures **6** and **9** as methyl 3-methylamino-3-deoxy-β-D-xylopyranoside and methyl 3-(N-methylacetamido)-3-deoxy-β-D-xylopyranoside, respectively.

Methyl β-gentosaminide obtained from gentamicin A and the synthetic material **6** were found to be identical. They exhibited identical mobilities on paper chromatograms, virtually superimposable ir and nmr spectra, similar optical rotations, and gave mmp 139°. The absolute stereochemistry of both methyl gentosaminides is thus established.

Treating methyl-β-gentosaminide in 1.5 *N* hydrochloric acid at 100° for 20 hr yielded predominantly gentosamine **7**, isolated as the hydrochloride, with traces of a hitherto unidentified nonreducing compound, presumably the anhydrogentosamine, 1,4-anhydro-3-methylamino-3-deoxy-α-D-xylopyranoside **8**.[4] In addition, the hydrolyzate still contained traces of unreacted starting material. Increasing acid concentrations resulted in the formation of increasing amounts of **8**.

Gentosamine hydrochloride derived from gentamicin A and the synthetic material were found to be identical by mixture melting point, mobility on paper strips, and optical rotation.

The stereochemistry of the glycosidic O-gentosaminyl-(1→6)-2-deoxystreptamine linkage in gentamicin A can be assigned on the basis of the nmr spectrum of tetra-N-acetylgentamicin A.[4] There are two doublets centered at positions 29 and 44 Hz, respectively, downfield from the HOD signal with coupling constants of 3.5 Hz each. These signals represent the anomeric hydrogen atoms of the two amino sugar moieties. The hydrogen atoms on the C_2 positions in both glucosamine and gentosamine are axially orientated, thus *cis* relationships must be involved between the hydrogen atoms on C_1 and C_2 in both amino sugar moieties. This then confirms the α-glucosidic linkage in paromamine[4] and establishes a second α-glycosidic linkage between gentosamine and paromamine. The structure of gentamicin A is thus established in all stereochemical details and is shown in Figure 2.

Experimental Section

Melting points were obtained on a Reichert Thermopan hot stage and are uncorrected. Nmr spectra of methyl gentosaminide samples were recorded in deuterium oxide on a 100-MHz spectrometer using the δ scale with tetramethylsilane as reference. Tetra-N-acetylgentamicin A was studied on a 60-MHz instrument with water as internal standard at a probe temperature of 31°. Optical rotations were measured with a Perkin-Elmer photoelectric polarimeter. Infrared spectra of a 1-mg sample per 100 mg of potassium bromide each were recorded on a Perkin-Elmer Model 21 double-beam recording spectrometer. Methanol was dried over magnesium methylate; hydrogen chloride was dried with calcium chloride. Paper chromatography was performed descendingly on Whatman No. 1 with 1-butanol, pyridine, water, and acetic acid, 6:4:3:1, v/v, the spots were detected with ninhydrin.

Methyl α- and β-Gentosaminides. The mixture of the anomeric methyl gentosaminide bases as derived from gentamicin A was dissolved in a minimum amount of hot methanol. The solution was diluted with 3 vol of absolute ethanol and with acetone. The resulting crystalline product was isolated after 24 hr and four recrystallizations from methanol–ethanol–acetone mixtures afforded methyl β-gentosaminide **6**, mp 140°, $[\alpha]^{23}D$ −60 (*c* 0.5, water), R_f 0.54. *Anal.* Calcd for $C_7H_{15}NO_4$: C, 47.45; H, 8.53; N, 7.90. Found: C, 47.53; H, 8.45; N, 7.82. The nmr spectrum of this compound is shown in Figure 1.

The mother liquor of the first crystallization was evaporated to dryness and the residue was redissolved in absolute ethanol. Addition of benzene afforded a crystalline material and four subsequent recrystallizations from ethanol–benzene mixtures yielded methyl α-gentosaminide, mp 122°, $[\alpha]^{23}D$ +139° (*c* 0.3, water), R_f 0.54. *Anal.* Found: C, 47.65; H, 8.52; N, 7.83.

Methyl β-D-Arabinopyranoside (1). A solution of 99.3 g of D-arabinose in 3 l. of 0.67 *M* methanolic hydrogen chloride was refluxed for 18 hr and the reaction mixture was neutralized with Amberlite IRA-400, carbonate form, previously dehydrated with methanol. The resin was filtered off, washed extensively with methanol, the filtrate and washings were evaporated to a volume of approximately 400 ml, and crude **1** was allowed to crystallize at 4° overnight. The azeotropically dehydrated mother liquor was again refluxed with 1.2 l. of methanolic hydrogen chloride, and crude **1** was isolated as above. Repeating the reequilibration and isolation process twice more afforded 92 g of crude **1**. Recrystallization from a mixture of 300 ml of methanol and 950 ml of absolute ethanol at 4° gave 81 g of pure **1** (75% yield), mp 169°, $[\alpha]^{23}D$ −245.7 (*c* 0.9, water), after washing with ethyl acetate and ether and drying at 60° for 3 hr at reduced pressure. Bishop and Cooper reported a value of $[\alpha]D$ −242°.[20]

Methyl β-Gentosaminide. (Methyl 3-Methylamino-3-deoxy-β-D-xylopyranoside) (6). A solution of 3.20 g of **5** in 50 ml of 40% aqueous methylamine was heated at 105° under 15 atm N_2 pressure

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(18) B. R. Baker and R. E. Schaub, *J. Org. Chem.*, **19**, 646 (1954).
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(20) C. T. Bishop and F. P. Cooper, *Can. J. Chem.*, **41**, 2743 (1963).

for 24 hr. The slightly tan solution was evaporated to dryness and the crystalline residue was redissolved in hot absolute ethanol. The solution was diluted with one-fourth volume of benzene and allowed to crystallize at 4°. After three recrystallizations, 1.70 g of colorless, rhombohedral crystals of **6** was obtained (44% yield): R_f = 0.54, mp 139°, $[\alpha]^{25}D$ −59° (*c* 0.5, water).

Anal. Calcd for $C_7H_{15}NO_4$: C, 47.44; H, 8.53; N, 7.91. Found: C, 47.41; H, 8.69; N, 7.75.

Gentosamine Hydrochloride (3-Methylamino-3-deoxy-D-xylose Hydrochloride) (7). A solution of 0.530 g of 6 in 20 ml of 1.5 *M* hydrochloric acid was heated on a steam bath for 20 hr. The cooled stirred solution was adjusted to pH 5 by the portionwise addition of Dowex 1-X8 (carbonate form), and the resin is filtered off and washed with water. Filtrate and washings were concentrated and crystallization was allowed to proceed from methanol–ethanol–acetone mixture at 4°. One recrystallization from the same solvent system gave 0.304 g of gentosamine hydrochloride (51% yield), mp 173° dec, $[\alpha]^{25}D$ −4° (extrapolated to 0 time), with a constant rotation, $[\alpha]^{25}D$ +28°, after 40 min. *Anal.* Calcd for $C_6H_{14}NO_4Cl$: C, 36.10; H, 7.07; N, 7.02. Found: C, 36.46; H, 7.25; N, 6.91.

Methyl N-Acetyl-β-gentosaminide. [Methyl 3-(N-Methylacetamido)-3-deoxy-β-D-xylopyranoside] (9). A solution of 0.100 g of 6 in 1 ml of methanol and 0.15 ml of acetic anhydride was allowed to react at room temperature for 24 hr. Methanol was evaporated under reduced pressure and the syrup was diluted with 1 ml of absolute ethanol. The solution was kept at 4° for 24 hr after addition of 1.5 ml of ether. The resulting needles were recrystallized once from the same solvent mixture affording 85 mg of **9** as long needles (69% yield), mp 183°, $[\alpha]^{25}D$ −59.5 (*c* 0.8, water). *Anal.* Calcd for $C_9H_{15}NO_5$: C, 49.30; H, 7.82; N, 6.39. Found: C, 49.27; H, 7.89; N, 6.23.

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Total Synthesis of Indole and Dihydroindole Alkaloids. I.[1] Introduction and the Transannular Cyclization Approach

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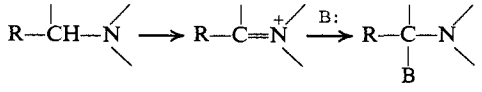
Abstract: An introduction to the transannular cyclization reaction and its application to indole and dihydroindole alkaloid syntheses is presented. The first successful laboratory realization of this process has been accomplished by converting 4β-dihydrocleavamine (I) into 7β-ethyl-5-desethylaspidospermidine (V). The partial synthesis of (+)-aspidospermidine (X) from (−)-quebrachamine (IX) illustrates an extension of this approach to the natural Aspidosperma series. The stereochemistry of this reaction has been established by X-ray analysis on the N_b-methiodide of the N_a-acetyl derivative VI. This study made available the first compound bearing the Aspidosperma skeleton for which an absolute configuration was available. Its importance in connection with the determination of absolute configuration in the natural Aspidosperma series is noted.

The vast domain of indole and dihydroindole alkaloids offers a formidable challenge to the synthetic chemist. Coupled with the recent interests in alkaloid biosynthesis, there is considerable stimulus for developing chemical reactions which not only provide laboratory syntheses of these natural products, but which may have some parallel in the natural pathways inherent in the living plant system. Clearly, reactions which are fundamentally simple, but general in their application to the elaboration of these alkaloid systems, would be highly desirable. We chose to study such a process, and the results presented in this and the succeeding publications have been obtained from such an investigation.

The reaction selected for this work is, in its simplest terms, straightforward and well known. It involves the creation of an electrophilic center (iminium salt) in



the original amine, as indicated, followed by reaction of the latter intermediate with a nucleophile (B:) to yield the desired product. The intervention of imines as possible intermediates in alkaloid biosynthesis has long been recognized,[2] and this functional group has also played a major role as an intermediate in some elegant alkaloid syntheses, particularly in the pyrrolidine, piperidine, and quinolizidine series.[2,3]

We felt that this reaction could be of general application to the indole and dihydroindole group where tremendous structural variety is available, particularly from the recent investigations in the Vinca, Iboga, and Aspidosperma families. Indeed, postulates on the possible biosynthetic pathways of these alkaloids have invoked these imine intermediates[4] and their use in the synthesis of some indole alkaloids had already been demonstrated.[5] Similarly, mechanistic interpretations on the interesting acid-catalyzed rearrangements of the alkaloid catharanthine have employed such intermediates although no direct experimental evidence was available for such postulates.[6,7]

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