The Gentamicin Antibiotics. Part I.† Structure and Absolute Stereochemistry of Methyl Garosaminide

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Methanolysis of the gentamicin C complex gave the anomeric methyl glycosides of a monosaccharide (named garosamine) shown to be methyl 3-deoxy-4-*C*-methyl-3-methylamino-L-arabinopyranoside (III).

GENTAMICIN C, a broad-spectrum antibiotic complex,‡ has been isolated [1] from submerged fermentations of *Micromonospora*. The complex has been shown [2-4] to contain three closely related, non-reducing pseudotrisaccharides named gentamicins C_1, C_2, and C_{1a}.

† Preliminary communication, D. J. Cooper, and M. D. Yudis, *Chem. Comm.*, 1967, 821.

‡ Garamycin[R].

[1] M. J. Weinstein, G. H. Luedemann, E. M. Oden, and G. H. Wagman, 'Antibacterial Agents and Chemotherapy,' American Society for Microbiology, 1963, p. 1.

[2] M. J. Weinstein, G. H. Luedemann, E. M. Oden, G. H. Wagman, J. P. Rosselet, J. A. Marquez, C. T. Coniglio, W. Charney, H. L. Herzog, and J. Black, *J. Medicin. Chem.*, 1963, **6**, 463.

[3] G. H. Wagman, J. A. Marquez, and M. J. Weinstein, *J. Chromatog.*, 1968, **34**, 210.

[4] D. J. Cooper, H. M. Marigliano, M. D. Yudis, and T. Traubel, *J. Infectious Diseases*, 1969, **114**, 342.

Methanolysis of gentamicin C in refluxing methanol saturated with hydrogen chloride gave the methyl glycosides of an amino-monosaccharide (named garosamine) and three pseudodisaccharides named gentamines C_1, C_2, and C_{1a}, corresponding to the parent gentamicins C_1, C_2, and C_{1a}, respectively. The methyl garosaminides were isolated by conversion of the hydrochloride mixture initially obtained into the free bases followed by chromatography on a silica gel column. An oily, hygroscopic product was shown to be an anomeric mixture of pyranosides by the ^1H n.m.r. spectrum ($CDCl_3$; 60 MHz), which showed the anomeric proton signals as doublets (ratio 1 : 2) at δ 4·75 (J 3·6 Hz) and 4·18 (J 6·5 Hz). Integration was consistent with the presence of seventeen protons, three of which were exchangeable. The mass spectrum showed the molecular ion at m/e 191 ($C_8H_{17}NO_4$); the base peak at m/e 160 corresponds to M — OMe.

Acetylation of the anomeric mixture with acetic anhydride in methanol gave crystalline methyl N-acetylgarosaminide, which was fractionally crystallized from absolute ethanol to give the pure β-anomer. The ^1H n.m.r. spectrum [$(CD_3)_2SO$; 60 MHz] of this compound at 35° was unexpectedly complex (Figure 1), and was consistent with a mixture (*ca.* 2 : 1) of isomers arising from restricted rotation about the >N–CO· bond.[5] As expected the spectrum of a solution at 120° was much simpler. The N-acetyl derivative was readily deacetylated by refluxing with hydrazine hydrate.[6] The resulting methyl garosaminide was purified by column chromatography and obtained as a colourless syrup.

Oxidation of methyl garosaminide with periodate resulted in the consumption of 1·8 mol. *of oxidant*; no additional uptake was noted in the ensuing 48 hr. Under the same conditions methyl N-acetylgarosaminide consumed no periodate. The ready cleavage of garosamine from the parent antibiotic by methanolysis precludes the presence of an amino-function at C-2,[7] thus C-2 must carry a hydroxy-group. In light of this, the periodate oxidation results indicated methyl garosaminide to be a 3-amino-pyranoside.

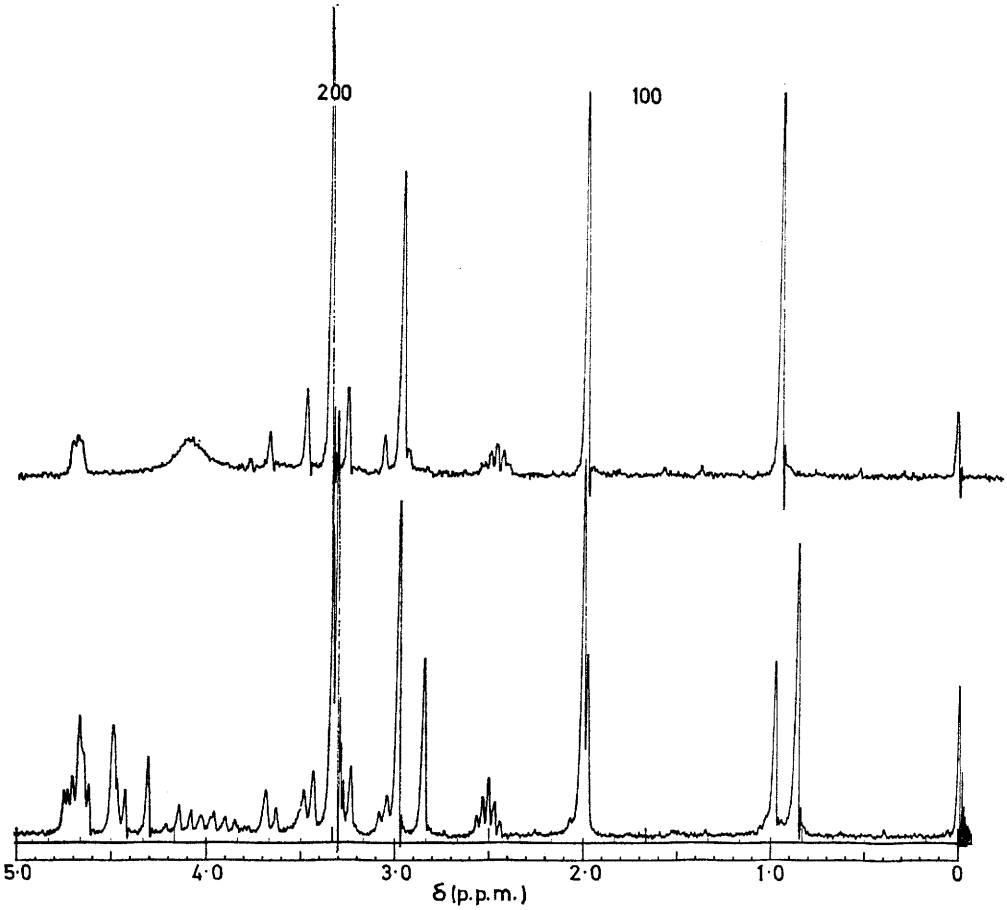


FIGURE 1 ^1H N.m.r. spectra [$(CD_3)_2SO$; 60 MHz] of methyl N-acetyl-3-deoxy-4-C-methyl-3-methylamino-β-L-arabinopyranoside at 35° (lower curve) and 120° (upper curve)

[5] W. A. Szarek, S. Wolfe, and J. K. N. Jones, *Tetrahedron Letters*, 1964, 2743.

[6] M. L. Wolfrom and B. O. Julaiano, *J. Amer. Chem. Soc.*, 1960 **82**, 2588.

[7] K. L. Rinehart, 'The Neomycins and Related Antibiotics,' E. R. Squibb Lectures on Chemistry of Microbial Products, Wiley, New York, 1964, p. 9.

The ^{1}H n.m.r. spectrum ([$^2H_6$]benzene; 60 MHz) of methyl garosaminide (pure β-anomer) (Figure 2) supports unequivocally structure (I) or its enantiomer. The splitting patterns and magnitudes of the coupling constants [8] establish the relative stereochemistry at C-1,

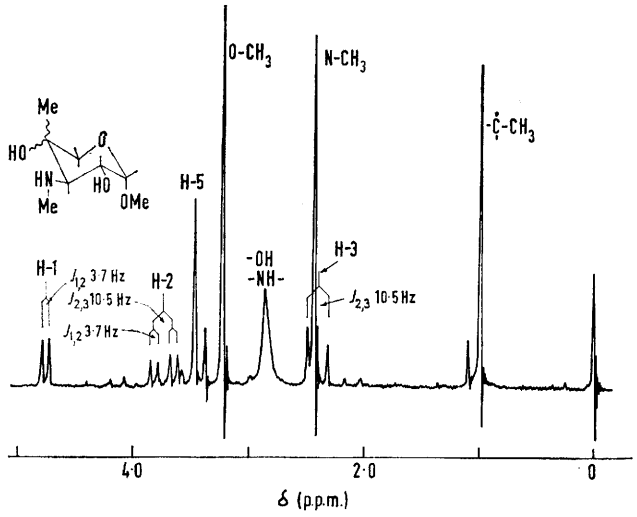
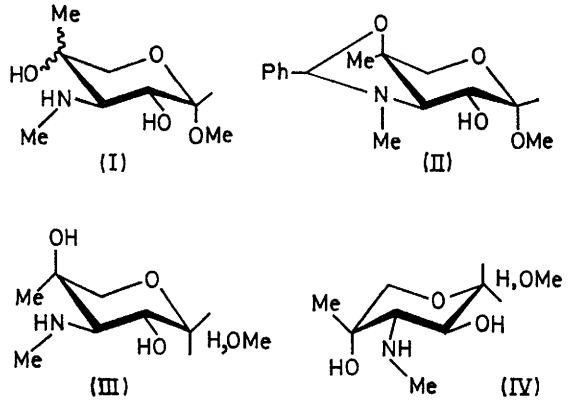


FIGURE 2 ^{1}H N.m.r. spectrum ([$^2H_6$]benzene; 60 MHz) of methyl 3-deoxy-4-*C*-methyl-3-methylamino-L-arabinopyranoside

C-2, and C-3. The large value of $J_{2,3}$ (10·5 Hz) requires H-2 and H-3 to be *trans*-diaxial. The doublet observed for H-3 shows that C-4 must be disubstituted, a fact confirmed by the uncoupled H-5 (two-proton) signal.



The reaction of methyl garosaminide with benzaldehyde proceeded exothermically and stereospecifically in ethanol to give a single crystalline oxazolidine [(II) or its enantiomer]. The ^{1}H n.m.r. spectrum ($CDCl_3$; 60 MHz) of this compound was first-order (see Experimental section) and indicated that the pyranose ring conformation was essentially the same as that of the parent methyl garosaminide. The hydroxy-group at C-4 must therefore be *cis* to the 3-methylamino-substituent and the sugar is thus a member of the arabinose series.

Evidence for the absolute stereochemistry of methyl garosaminide was obtained from measurements (made on the anomeric mixture) of the change of molecular rotation upon complexing in Cupra B solution.[9] The result gave $[M]_{\text{CupraB}}^{436} - [M]_{H_2O}^{436} = -748°$. If the two possible enantiomers (III) and (IV) are considered in the conformations consistent with the n.m.r. spectra, it can be seen that compound (III) would be expected to exhibit a negative rotational shift in Cupra B and (IV) a positive shift. Thus the latter can be ruled out, and methyl garosaminide must have the absolute stereochemistry shown in (III), *i.e*, it is methyl 3-deoxy-4-*C*-methyl-3-methylamino-L-arabinopyranoside.

EXPERIMENTAL

N.m.r. spectra were determined with a Varian A60A (60 MHz) or HA100 spectrometer (100 MHz), with tetramethylsilane as internal standard. Mass spectra were determined with a Perkin-Elmer–Hitachi RMU6 spectrometer (direct inlet system). M.p.s were determined with a Kofler apparatus. T.l.c. was performed on silica gel plates (8 × 4 in.); spots were located with iodine vapour. Column chromatography was performed with chromatographic grade silica gel (J. T. Baker and Co.) on high resolution columns obtained from Glenco (Houston).

Methyl Garosaminide (*Anomeric Mixture*).—Gentamicin C (1·0 g.) was treated with a saturated solution of hydrogen chloride in anhydrous methanol (50 ml.) and refluxed for 1·5 hr. The solvent was removed *in vacuo* below 40° and the residue was dissolved in water (25 ml.) and applied to a column (1·5 cm. i.d.) of Amberlite IRA-401S (OH^-) resin (10 g.). The column was eluted and washed with distilled water (100 ml.) and the aqueous solution of free bases was freeze-dried, yielding a syrup (0·95 g.). T.l.c. [chloroform–methanol–concentrated ammonia (1 : 1 : 1; lower phase)] showed the presence of the three gentamines (R_F *ca.* 0·2—0·4) and methyl garosaminide (R_F *ca.* 0·80). The compounds were readily separated on a column (2·5 cm. i.d.) of silica gel (100 g.) by use of the same solvent system; fractions (3 ml.) were collected automatically and assayed by t.l.c. The appropriate fractions gave syrupy methyl garosaminide (270 mg.) and a mixture of the gentamines (390 mg. of an amorphous foam). A satisfactory elemental analysis could not be obtained for the hygroscopic methyl garosaminide; *m/e* 191 (M^+, $C_8H_{17}NO_4$) and 160 (M — OMe, base peak); $[M]_{\text{Cupra B}}^{436} = -342°$, $[M]_{H_2O}^{436} = +406°$.

Methyl N-*Acetyl*-β-*garosaminide*.—Methyl garosaminide (2·40 g.) was dissolved in methanol (50 ml.), acetic anhydride (10 ml.) was added, and the mixture was left overnight at room temperature. It was then evaporated under reduced pressure and the residual oil was crystallized from absolute ethanol (15 ml.). Five further recrystallizations from ethanol afforded colourless prisms (800 mg.) of *methyl* N-*acetyl*-3-*deoxy*-4-C-*methyl*-3-*methylamino*-β-L-*arabinopyranoside* (methyl *N*-acetyl-β-garosaminide), m.p. 190—196° (softens at 180—187°), $[\alpha]_D^{26}$ +217° (*c* 0·3 in H_2O), $\nu_{max.}$ ($CHCl_3$) 6·09 μm., *M* (mass spectrometry) 233 (Found: C, 51·65; H, 8·35; N, 5·95. $C_{10}H_{18}NO_5$ requires C, 51·45; H, 8·2; N, 6·0%).

[8] (*a*) L. D. Hall, *Adv. Carbohydrate Chem.*, 1964, **19**, 51; (*b*) R. U. Lemieux, J. D. Stevens, and R. R. Fraser, *Canad. J. Chem.*, 1962, **40**, 1955.

[9] R. E. Reeves, *Adv. Carbohydrate Chem.*, 1951, **6**, 107.

Methyl β-Garosaminide.—Methyl *N*-acetyl-β-garosaminide (150 mg.) was treated with hydrazine hydrate (1·5 ml.) and refluxed for 24 hr. The excess of hydrazine and its *N*-acetate were removed *in vacuo* at 100°, and the syrupy residue was purified by chromatography as described for the anomeric mixture. The product, *methyl β-garosaminide*, was a colourless, hygroscopic syrup (74 mg.), $[\alpha]_D^{26}$ +209° (*c* 0·3 in H_2O) (Found: C, 49·6; H, 8·9; N, 7·35. $C_8H_{17}NO_4$ requires C, 50·0; H, 8·95; N, 7·3%).

Methyl 3,4-NO-*Benzylidene*-3-*deoxy*-4-C-*methyl*-3-*methylamino*-β-L-*arabinopyranoside*.—Methyl β-garosaminide (1·5 g.) in ethanol (5 ml.) was treated with benzaldehyde (1 ml.) and warmed gently in a hot water bath. A vigorous exothermic reaction occurred, maintaining the mixture at reflux for several min.; the mixture was then left overnight at room temperature. The solvent and excess of benzaldehyde were removed *in vacuo* at 60° and the residue was crystallized from benzene–hexane giving colourless needles (1·9 g.) of the *oxazolidine*, m.p. 135—139° (softens at 110—115° with change of crystalline form), $[\alpha]_D^{27}$ +89° (*c* 0·3% in $CHCl_3$) (Found: C, 64·35; H, 7·55; N, 5·2. $C_{15}H_{21}NO_4$ requires C, 64·5; H, 7·6; N, 5·0%), δ ($CDCl_3$; 60 MHz), 1·43 (4-CH_3, s), 2·33 (*N*-CH_3, s), 3·12 (H-3, d, $J_{2,3}$ 7·5 Hz), 3·46 (*O*-CH_3, s), 3·68 (H-5, s, 2H), 3·92 (H-2, q, $J_{1,2}$ 4, $J_{2,3}$ 7·5 Hz), 4·75 (H-1, d, $J_{1,2}$ 4 Hz), 5·08 (PhC*H*, s), and 7·2—7·6 (aromatic m, 5H).

Periodate Oxidations.—The method described by Gardell [10] was used to determine the uptake of periodate by methyl garosaminide (anomeric mixture) and its *N*-acetate. The results are shown in the Table.

	Periodate consumed (mol.)	
Time (hr.)	Methyl garosaminide	Methyl *N*-acetylgarosaminide
3·0	1·55	0·00
26·0	1·67	0·00
66·0	1·80	0·00

[10] S. Gardell, *Methods of Biochemical Analysis*, 1958, **3**, 289.

[0/1518 *Received, September 4th*, 1970]