The Gentamicin Antibiotics. Isolation and Characterisation of Methyl Garosaminide, a Novel Aminohexopyranoside

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GENTAMICIN C,† a broad-spectrum antibiotic complex, along with gentamicin A whose structure has been elucidated by Schaffner and Maehr,[1] has been isolated[2] from fermentations of *Micromonospora*. The complex has been shown[3] to consist of at least two, closely related, nonreducing pseudotrisaccharides.

Methanolysis of gentamicin C in refluxing methanol, saturated with hydrogen chloride, gave the methyl glycoside of a monosaccharide (named garosamine) in addition to unidentified disaccharide fragments. The glycoside was isolated from the methanolysis mixture by chromatography on silica gel using the lower phase of the system methanol–chloroform–17% ammonia (1 : 2 : 1) as eluent. The oily product was an anomeric mixture of pyranosides as indicated by the ^{1}H n.m.r. spectrum (deuterochloroform, 60 Mc./sec.) which showed the anomeric protons as doublets, ratio 1 : 2, at δ 4·75 (J = 6·5 c./sec.) and 4·18 (J = 3·6 c./sec.). Integration of the n.m.r. spectrum was consistent with seventeen protons, three of which were exchanged when the n.m.r. spectrum was re-run in deuterium oxide. The mass spectrum (determined by Dr. T. Traubel) showed the molecular ion at m/e 191, in agreement with the formula $C_8H_{17}NO_4$.

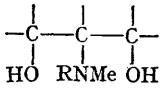
Acetylation of the anomeric mixture with acetic anhydride in methanol gave the crystalline *N*-acetate [ν_{max} ($CHCl_3$) 6·09] that was fractionally crystallized from ethanol to give essentially a single anomer, m.p. 190—194°, $[\alpha]_D^{25}$ + 184° (1% in methanol), M (ebullioscopic in methanol) 226, analyzing correctly for the expected formula $C_{10}H_{19}NO_5$ (M 233).

Periodate oxidation of methyl garosaminide resulted in the rapid consumption of two moles of oxidant; no additional uptake was noted in the

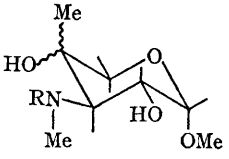
† Garamycin[R]

ensuing 48 hr. Under the same conditions methyl *N*-acetylgarosaminide consumed no periodate. These results show that the molecule contains the fragment (I). The ready cleavage of the sugar from the parent antibiotic precludes the presence of of an amino function at C-2,[4] thus C-2 must carry a hydroxy-substituent.

The ^{1}H n.m.r. spectrum (benzene, 60 Mc./sec.) of methyl garosaminide, obtained as largely one anomer by deacetylation of the *N*-acetate in refluxing hydrazine hydrate,[5] supports unequivocally structure (IIa) or its enantiomer. Observed signals are as follows:
δ 1·00 (4-CH_3, s); 2·40 (H-3, d, $J_{2,3}$ 10·5 c./sec.); 2·43 (NCH_3, s); 2·85 (O*H* and N*H*, slightly broadened s, 3H); 3·22 (OCH_3, s); δ 3·46 (H-5, s, 2H); 3·74 (H-2, q, $J_{1,2}$ 3·7 c./sec., $J_{2,3}$ 10·5 c./sec.); and 4·75 (H-1, d, $J_{1,2}$ 3·6 c./sec.).‡ Assignment of the mobile protons was confirmed by observing the variation of chemical shift with concentration;



(I)



(II) a; R=H
b; R=Ac

exchange with deuterium oxide could not be observed as the glycoside partitioned completely into the aqueous phase.

The splitting patterns and magnitudes of the coupling constants[6] establish unequivocally the relative stereochemistry at C-1, C-2, and C-3. The large value of $J_{2,3}$ 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 (2 protons) signal. It is not possible at this time to assign the relative stereochemistry at C-4.

The ^{1}H n.m.r. spectrum of methyl *N*-acetylgarosaminide (D_2O, 100 Mc./sec.) is more complex since there is evidence for a 2 : 1 mixture of rotational isomers[7]. However, splitting patterns and coupling constants indicate clearly that the *N*-acetyl derivative is essentially a single anomer and that the mixture is not due to a slowly established equilibrium between conformational isomers. The n.m.r. assignments which are in accord with (IIb) are as follows:
δ 1·02, 1·14 (4-CH_3, s); 2·15, 2·18 (NCO·CH_3, s); 2·96, 3·10 (NCH_3, s); 3·32, 3·35 (H-5 *ax.*, splittings masked by OCH_3 resonance); 3·44 (OCH_3, s); 3·79, 3·82 (H-5 *eq.*, d, J = 12 c./sec.); 4·16 (H-2, q, $J_{1,2}$ = 3·8 c./sec., $J_{2,3}$ = 11·5 c./sec.); 3·86, 4·65 (H-3, d, $J_{2,3}$ = 11·5 c./sec.); 4·90, 4·93 (H-1, d, $J_{1,2}$ = 3·7 c./sec.).

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‡ The integrals agreed with the recorded assignments.

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