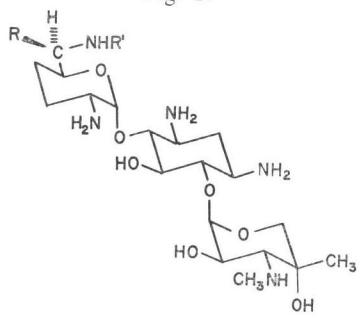
GENTAMICIN DERIVATIVES MODIFIED AT THE 2″-POSITION. THE PREPARATION OF 2″-EPI-GENTAMICIN C_1 AND 2″-DEOXYGENTAMICIN C_2

Sir:

Gentamicin C complex (Garamycin ®), comprising gentamicins C_1, C_2 and C_{1a}, is active against a variety of kanamycin-resistant strains of bacteria which inactivate kanamycin by 3′-*O*-phosphorylation and 6′-*N*-acetylation. Recently, however, three new inactivation mechanisms have been found in gentamicin-resistant bacterial strains. Thus gentamicin has been shown to be inactivated by certain resistant strains of *Pseudomonas aeruginosa* by 3-*N*-acetylation;[1] by certain *Providencia* strains by 2′-*N*-acetylation[2] and by strains of *Escherichia coli* and *Klebsiella pneumoniae* by *O*-adenylylation.[3] The latter two modes of inactivation also affect tobramycin and 3′,4′-dideoxykanamycin B. Gentamicin-resistant strains of *E. coli*, *Klebsiella*, *Serratia* and *Enterobacter* reported recently by WITCHITZ[4] have also been shown to inactivate the closely related sisomicin by 3-*N*-acetylation.[5] In the case of inactivation by adenylylation, it has been shown by BENVENISTA and DAVIES (personal communication) on the basis of substrate studies, and by NAGANAWA *et al.*[6] by chemical studies with 3′,4′-dideoxykanamycin B inactivated by the same enzyme, that adenylylation takes place at the 2″-hydroxyl group. Modification of gentamicins at the 2″-position was therefore undertaken with the objective of preparing an antibiotic with potential for activity against adenylylating strains of bacteria.

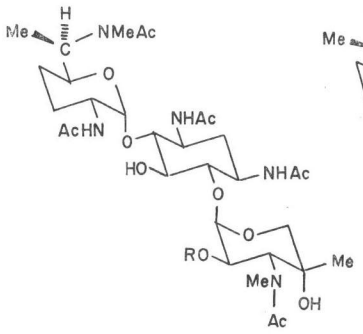
The first modification carried out was inversion of stereochemistry of the 2″-hydroxyl group. This particular epimerisation of an aminoglycoside has been reported previously in some kanamycin derivatives modified at the 6″-position,[7] and 2″-epi-kanamycin has been reported in a patent[8] to be an antibiotic with reduced toxicity. Penta-*N*-acetylgentamicin C_1[9] (**1**), dried by repeated evaporation with dry pyridine, was reacted with 1.05 equivalents of methanesulfonyl chloride in pyridine for 3 hours at room temperature to give, after chromatography over silica gel, eluting with the lower phase of a chloroform-methanol-15% ammonium hydroxide (2:1:1) solvent system, the 2″-*O*-mesyl derivative (**2**) in 66% yield: PMR (60 MHz, D_2O) δ 3.26 ppm (3H, singlet, $\underline{CH_3}$-SO_3). Solvolysis of the mesylate, with participation of the *trans* vicinal acetamido group, was effected by warming in 10% aqueous DMF for 1 hour at 90°C affording penta-*N*-acetyl-2″-epi-gentamicin C_1 (**3**): $[\alpha]_D^{26}+123°$ (*c* 0.45, H_2O). The PMR spectrum (60 MHz, D_2O) of **3** was complex due to amide rotamers but showed two absorptions in the anomeric region, a broad absorption at δ 5.6 ($W\frac{1}{2}=7$ Hz) assigned to H-1′ and a sharper resonance at δ 5.04 ppm ($W\frac{1}{2}=3$ Hz) assigned to H-1″. Hydrolysis of **3** with refluxing 2.5 N sodium hydroxide for 96 hours gave 2″-epi-gentamicin C_1 (**4**) as a white amorphous solid: $[\alpha]_D^{26}+102°$ (*c* 0.38, H_2O); PMR (100 MHz, D_2O) δ 5.1 (2 H, overlapping doublets, H-1′ and H-1″),

Fig. 1.

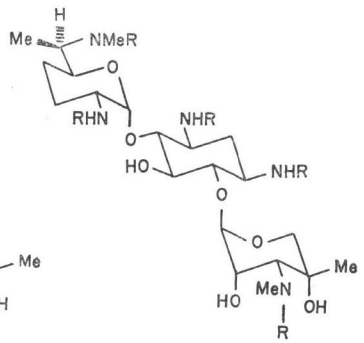


Gentamicin C_1 R = R′ = CH_3
Gentamicin C_2 R = CH_3, R′ = H
Gentamicin C_{1a} R = R′ = H

Fig. 2.



(1) R = H
(2) R = SO_2Me



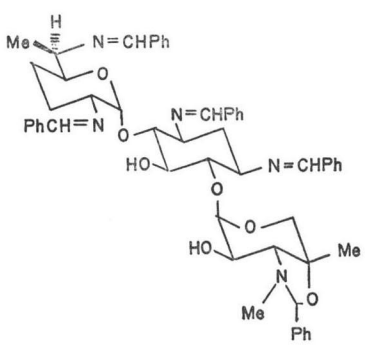
(3) R = Ac
(4) R = H

4.10, 4.01 (2 H, narrow multiplet, H-2″, overlapping with doublet, J=12.5 Hz, H-5″$_{eq}$), 3.9~3.1 (5 H, complex, 5×H-C-O), 2.95~2.73 (4H, complex, 4×H-C-N), 2.66 (1 H, doublet, J=3 Hz, H-3″), 2.40 (3H, singlet, CH_3-N), 2.37 (3 H, singlet, CH_3-N), 2.05~1.3 (5H, complex, 2×H-3′, 2×H-4′ and H-2$_{eq}$), 1.16 (3H, singlet CH_3-C), 1.1 ppm (3 H, doublet, J=7 Hz, CH-CH_3). Irradiation of H-3″ simplified H-2″ at δ 4.10 to a singlet overlapping one arm of the H-5″$_{eq}$ doublet; irradiation of H-2″ simplified H-3″ and the anomeric protons to singlets at δ 2.66 and 5.1 ppm respectively. The mass spectrum of **4** was identical to the parent gentamicin C_1. The spectral data cited are in agreement with the *β*-L-*ribo* configuration assigned to 2″-epi-gentamicin C_1. Dr. J. DAVIES (University of Wisconsin) examined this compound and found that it was not a substrate for gentamicin adenylyl synthetase; the compound however was without antibacterial activity *in vitro* at levels up to 25 mcg/ml.

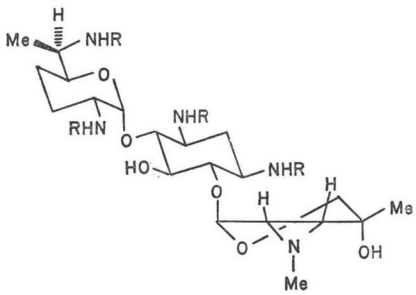
Attention was next turned to removal of the 2″-hydroxyl group. Pentabenzylidene gentamicin C_2 (**5**)[10] was reacted with 1.05 equivalents of methane sulfonyl chloride in pyridine affording the 2″-*O*-mesyl derivative. After removal of the benzylidene groups with 0.01 N hydrochloric acid the intermediate 2″-*O*-mesyl gentamicin C_2 cyclised during work-up, which included chromatography over silica gel using a chloroform-methanol-ammonium hydroxide solvent system as eluant, to give the epimino compound (**6**) in 63% overall yield from **5**. The epimine (**6**) was a white amorphous solid, $[\alpha]_D^{26}$+56° (*c* 0.4, MeOH) analysing as a hemihydrate. The mass spectrum of **6** was characterized by intense ions for aziridine-containing fragments, the base peak in the spectrum arose from glycosidic cleavage of the epimino sugar at *m/e* 142 and the remainder of the spectrum fitted well with patterns observed for other underivatised aminoglycoside antibiotics.[11] High resolution mass spectrometry supported the assigned composition of the compound (Found $M^{+\cdot}$ *m/e* 445·291, $C_{20}H_{39}N_5O_6$ requires 445·290). The anomeric proton of the epimino sugar appeared as a sharp singlet at δ 5.19 ppm indicating an angle approaching 90° between H_1″ and H_2″. This is analogous to the situation observed for 2,3-epoxides of pyranose sugars in which J≃0 for epoxide ring protons and their *trans* neighbours. The 2″ and 3″ protons of **6** appeared as doublets at δ 1.77 and 1.87 ppm (J=6 Hz). The relatively high field positions and coupling constant observed for these resonances are consistent with *cis* protons on an epimine ring. Other PMR signals for **6** were as expected for the assigned structure.

Acetylation of **6** with acetic anhydride in methanol gave the *tetra-N*-acetyl derivative (**7**) which was hydrogenated in tetrahydrofuran solution at 140°C and 2500 p.s.i. for 30 hours over a RANEY nickel catalyst to give the expected mixture of products **8** and **9** as well as a small amount of recovered starting material. These compounds were separated by chromatography over silica gel and hydrolysed with aqueous sodium hydroxide to give the respective free bases **10** and **11**. The PMR spectrum of the tetra-*N*-acetyl derivative (**8**) showed two resonances in the anomeric region,

Fig. 3.



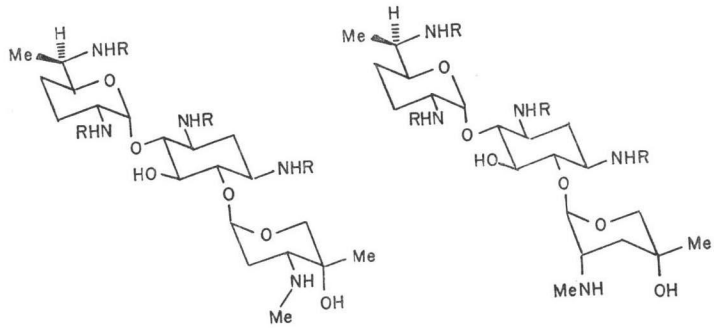
(5)



(6) R = H

(7) R = Ac

Fig. 4.



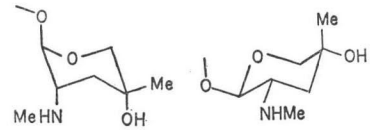
(8) R = Ac
(10) R = H

(9) R = Ac
(11) R = H

a doublet at δ 5.23 (J=3.5 Hz) assigned to H_1' and a broad absorption at δ 5.10 ppm assigned to H_1''. This latter signal simplified to a sharp singlet on irradiation of a multiplet at δ 1.6 ppm, indicating coupling to protons bound to carbon unsubstituted by an electronegative atom and therefore consistent with the 2-deoxysugar assignment. The free base 2″-deoxygentamicin C_2 (**10**) was an amorphous white solid, mp 118~136°C, $[\alpha]_D^{26}+118°$ (*c* 0.47. H_2O); PMR (100 MHz, D_2O) δ 5.18 (2H, overlapping H-1′ and H-1″), 3.93 (1H, doublet J=12 Hz, H-5_{eq}), 3.85~2.5 (*ca* 10 H, complex), 2.37 (3H, singlet, N-$\underline{CH_3}$), 2.1~1.25 (*ca* 7H, complex), 1.18 (3H, singlet, C-$\underline{CH_3}$), 1.08 ppm (1H, doublet, J=7.5 Hz, CH-$\underline{CH_3}$); irradiation at δ 1.7 ppm simplified the anomeric resonances to an overlapping singlet at δ 5.20 ppm and a doublet J=*ca* 3.5 Hz. The mass spectrum of **10** showed an $[M+1]^+$ ion at *m/e* 448, M^+ at *m/e* 447 and prominent fragment ions at *m/e* 191, 173, 163, 145 (2-deoxystreptamine ions) and 144, 143 (monosaccharide ions) in agreement with the proposed structure.[11]

The major product of the hydrogenation reaction was the 2″-deoxy-3″-desmethylamino-2″-methylaminogentamicin C_2 derivative 9. The free base (**11**) of this compound was amorphous, mp 83 ~ 91°C, $[\alpha]_D^{26}+89°$ (*c* 0.37, H_2O); PMR (100 MHz, D_2O) δ 5.09 (1H, doublet J = 3.5 Hz, H-1′), 4.68 (1H, doublet J = 5.5 Hz, H-1″), 3.9 ~ 3.1 (6H, complex, 6×$\underline{H}$-C-O), 3.0~2.5 (5H, complex, 5×$\underline{H}$-C-N), 2.33 (3H, singlet, N-$\underline{CH_3}$), 2.15~1.1 (8H, complex), 1.24 (3H, singlet, C-$\underline{CH_3}$), 1.04 ppm (3H, doublet J=7 Hz, CH-$\underline{CH_3}$). The principal fragmentations in the mass spectrum of **11** were similar to those of **10** although the spectra were by no means identical. In the PMR spectrum of **11** in D_2O the signal assigned to H-1″ resonated at δ 4.68 ppm as a doublet, J=5.5 Hz. This absorption is intermediate in chemical shift and coupling constant between those which could be expected for conformers **12** and **13** and it consequently appears that in aqueous solution at ambient temperature the observed resonances for **11** represent weighted averages

Fig. 5.



(12) (13)

for these two conformers. The instability of conformer **12** is clearly due to the unfavorable 1,3-diaxial interaction between the methylamino group at 2″ and the hydroxyl group at 4″ which is only partially compensated for by the possibility of hydrogen bonding between these groups and by the anomeric effect. In anhydrous $CDCl_3$ solution the signal for H-1″ of **11** appears at δ 4.92 ppm as a doublet, J=2 Hz. This is clearly consistent with the presence of a greater proportion of conformer **12** and reflects the greater stabilizing effect of the intramolecular hydrogen bond in this conformer in a nona-

Table 1. *In vitro* antibacterial activity of 2″-deoxygentamicin C_2 and gentamicin

Organism	No. of strains	MIC (μg/ml)*	
		2″-Deoxy gentamicin C_2	Gentamicin
Escherichia coli G, T-adenyl	2	0.3~3.0	>50
Klebsiella pneumoniae G, T-adenyl	2	3.0~7.5	17.5
Staphylococcus aureus	4	0.3~17.5	0.03
Streptococcus pyogenes	2	>25	0.3~0.8
Bacillus subtilis	1	0.1	0.08
Escherichia coli G-sens.	1	3.0	0.03
Klebsiella pneumoniae K-phos.	3	0.8	0.3
Pseudomonas aeruginosa G-sens.	7	3.0~>25	0.1~0.3
G-acetyl	2	>25	>50
G, T. resist.	1	>25	>50
Providence sp G, T, resist.	1	>25	>50

* In MUELLER-HINTON broth. G=gentamicin, T=tobramycin

queous solvent.

The *in vitro* antibacterial activity of 2″-deoxygentamicin C_2 (**10**) is shown in the Table 1. The compound is a broad-spectrum antibacterial and, as predicted, shows good activity against gentamicin-resistant adenylylating strains of *E. coli* and *K. pneumoniae*. In other respects however **10** shows lower *in vitro* potency than gentamicin and is inactive against other types of gentamicin resistant organisms. Compound **11** shows no antibacterial activity of note.

From this, and other work,[13] it is concluded that antibiotics of the kanamycin-gentamicin class require a 2″-equatorial hydroxyl group for maximum activity against a wide range of organisms.

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