Synthesis of 1-*N*-Ethylsisomicin: A Broad-spectrum Semisynthetic Aminoglycoside Antibiotic

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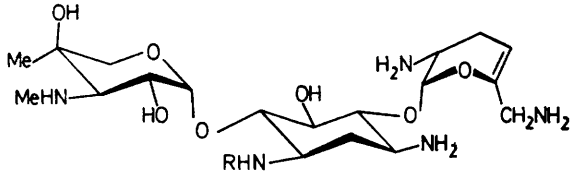
Summary Site-selective reductive *N*-alkylation of aminoglycoside antibiotics related to gentamicin is described and the structure of the products and of related compounds is unambiguously established using circular dichroism and mass spectrometry.

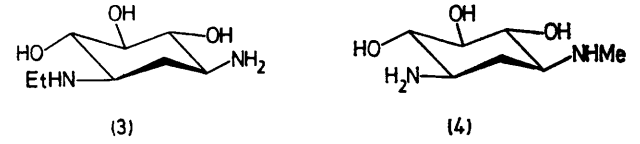
THE aminoglycoside antibiotics are important therapeutic agents because of their activity against gram-negative bacteria not readily susceptible to other antibiotics. Their widespread clinical use has led to a growing number of resistant bacterial strains, whose resistance usually results from enzymic modification of the antibiotic. In the case of sisomicin (**1**),[1] such modes of inactivation include acetylation of one of the amine groups attached to carbons 2′, 6′ and 3, and adenylylation of the hydroxy group attached to carbon 2″.[2] There is thus considerable importance attached to the preparation of aminoglycoside antibiotics which are active against a high proportion of these resistant organisms. Amikacin, the 1-*N*-(*S*)-4-amino-2-hydroxybutyryl derivative of kanamycin A has been demonstrated to possess such desirable properties.[3] We now report the novel synthesis, from the gentamicin–sisomicin class of antibiotics, of 1-*N*-alkyl derivatives which also possess an improved resistance spectrum.

We have found that the relative reactivity of the amine groups of the gentamicin antibiotics towards reductive alkylation in the presence of an aldehyde and a hydride-donor reducing agent is pH-dependent. Although the C-6′

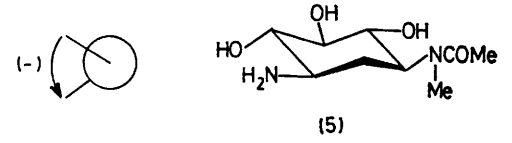
amino group is in general more reactive, under conditions of low pH the C-1 amino group is selectively alkylated. This selectivity is lost upon raising the pH of the reaction mixture. This procedure appears to be general within the gentamicin–sisomicin series and has enabled a wide range of derivatives to be prepared from a variety of substrates without the conventional protection of other amine groups. The high reactivity of the C-1 amine group at low pH suggests that it is the least basic in the molecule and initial acid-base titration studies using ^{13}C n.m.r. are in agreement.[4] The selectivity observed is considered to reflect the relative equilibrium concentrations of free amine under acidic conditions. Similar considerations have led to the development of a selective acylation process.[5]



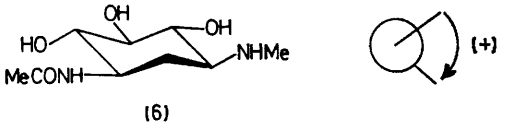
(1); R = H
(2); R = Et



(3) (4)



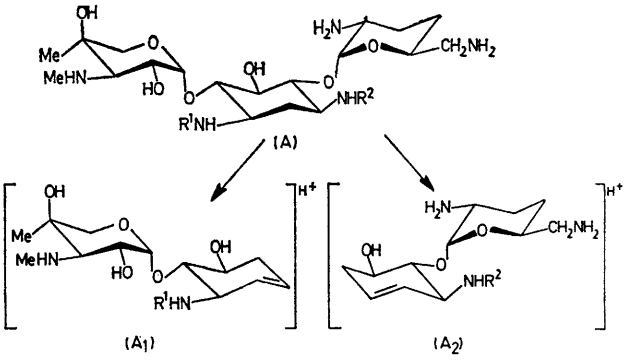
(5)



(6)

As an example of the general procedure, an aqueous solution of sisomicin (**1**) sulphate (pH 5) with approximately one molar equivalent of sodium cyanaborohydride in the presence of an excess of acetaldehyde gives, after chromatography, 1-*N*-ethylsisomicin (**2**) (25%) together with some starting material (20%). The major product of the reaction was shown to be (**2**), rather than the 3-*N*-alkyl isomer in the following manner. Acid hydrolysis gave the unknown (−)-*N*-ethyl-2-deoxystreptamine (**3**) ($[\alpha]_D$ −40°). To determine the absolute configuration of (**3**) we needed to compare its optical rotation with that of (+)-*N*-methyl-2-deoxystreptamine (**4**) ($[\alpha]_D$ +39°) obtained from hygromycin B by acid hydrolysis.[6] However, as separate investigations into the absolute stereochemistry of this compound and its enantiomer derived from destomycin were incompatible,[7] an unambiguous assignment was necessary. (**4**) was partially *N*-acetylated with acetylimidazole to give the mono-*N*-acetates (**5**) and (**6**). ^1H n.m.r. established that (**5**) (δ 2·75, N-CH_3) was the tertiary amide and (**6**) (δ 2·26, N-CH_3; δ 7·65, N$HCOCH_3$) was the isomeric secondary amide. The absolute stereochemistry of (**5**) and (**6**) was determined by c.d. measurements of their cuprammonium complexes[8] (TaCu) in solution. Only vicinal amino-alcohols can form strong complexes with tetrammine copper and the sign of the Cotton effect at 280 nm is diagnostic for the chirality of the complex. The value for $[\theta]_{280}$ of +4,550 obtained for (**5**) is associated with a λ chelate conformation with a negative torsional angle. Similarly the value for $[\theta]_{280}$ of −6,210 obtained for (**6**) corresponds to a δ chelate conformation with a positive torsional angle. These results establish that the structure of the hygromycin B hydrolysis product is 3-*N*-methyl-2-deoxystreptamine (**4**). This is in agreement with the assignment made by Kurihara and his co-workers and also with a recent independent assignment by Rinehart.[9] The hydrolysis product of (**2**) having the opposite sign of rotation is therefore 1-*N*-ethyl-2-deoxystreptamine (**3**), and (**2**) is the 1-*N*-ethyl derivative of sisomicin.

Using this assignment we have since discovered a specific mass spectral fragmentation of *N*-alkyl gentamicin derivatives which permits the position of alkylation to be determined from the mass spectrum alone. From the alkylation of gentamicin C_{1a}, two products were obtained which were alkylated on the 2-deoxystreptamine grouping. Degradation to (−)-*N*-ethyl-2-deoxystreptamine established that the major product was the 1-*N*-ethyl derivative of gentamicin C_{1a}, and that the minor product was the 3-



R^1, R^2	A	A_1 *m/e*	A_2 *m/e*
$R^1=R^2=H$	Gentamicin C_{1a}	289	258
$R^1=Et$, $R^2=H$	1-*N*-Ethylgentamicin C_{1a}	317	258
$R^1=H$, $R^2=Et$	3-*N*-Ethylgentamicin C_{1a}	289	286

isomer. Examination of the mass spectra of these two derivatives revealed a specific fragmentation in which an amino group on the deoxystreptamine ring is lost together with the glycosyloxy group vicinal to it. There is a hydrogen transfer to the pseudo-disaccharide ion associated with the fragmentation. Gentamicin C_{1a} itself gives rise to ions A_1 and A_2 at *m/e* 289 and 258 respectively. In 1-*N*-ethylgentamicin C_{1a}, ion A_1 appears at *m/e* 317, an increase of 28 mass units corresponding to the addition of an ethyl group, while ion A_2 remains unchanged. 3-*N*-Ethylgentamicin C_{1a} gives rise to complementary ions. This is an extremely useful fragmentation and has been used to define the structure of a large number of alkyl derivatives

we have prepared. Other physical measurements such as ^{1}H n.m.r. and ^{13}C n.m.r. do not give such unequivocal information.

1-*N*-Ethylsisomicin is active against many 2″-*O*-adenylylating and 3-*N*-acetylating strains. This compound also exhibits low chronic toxicity in relation to gentamicin and sisomicin.[10] The 3- and 3″-alkyl isomer isolated as minor by-products in the reductive alkylation have very weak antibacterial activity.

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