CHEMISTRY OF THE GENTAMICINS. III. A COMMENT ON THE STRUCTURE OF GENTAMICIN A[1]

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(Received for publication August 28, 1980)

The absolute configuration of 2-deoxystreptamine (**5**) in the neomycins and paromomycins, and hence in neamine (**2**) and paromamine (**1a**), has long been determined by two independent methods, both based on the analysis of a simple, chiral derivative of **5**, in which the two enantiotopic hydroxyl groups at positions 4 and 6 are differentiated. Derived from neomycin and paromomycin, N,N′-diacetyl-6-O-methyl-2-deoxystreptamine (**4**) served for the assignment of chirality by REEVE's cuprammonium method,[2] whereas 5,6-di-O-methyl-2-deoxystreptamine (**6**) prepared from **1a** and **2** and degraded to di-O-methyl-D-(+)-tartaric acid established the absolute configuration of neomycins and paromomycins by an alternative procedure[3]. Studying one chelation site, the chirality of **5** in kanamycin was ascertained by analysis of **8** derived from kanamycin A[4] and **7** obtained from **8**[5,6] using the cuprammonium and tetraaminecopper methods, respectively.

Paromamine, obtained by hydrolysis of paromomycin, is thus firmly established as **1a**, and unsymmetrically substituted derivatives of 2-deoxystreptamine, such as **4** ($[\alpha]_D$ 15°), exhibit significant optical rotations so that the diastereomers **1a** and **1b** were expected to show significantly different optical rotations. Implications of this argument led to the assignment of complete structures for kanamycin C[7,2] and gentamicin A,[8,1] both of which liberated an O-(2-amino-2-deoxy-α-D-glucopyranosyl-2-deoxystreptamine)[9,8] known to be of either structure **1a** or **1b**, but having the same optical rotation as authentic **1a** from paromomycin. Thus, with position 4 in 2-deoxystreptamine assigned, the absolute configuration of kanamycin C and gentamicin A were consequently also specified.

The resulting structural assignments were further corroborated by comparisons with closely related, co-produced antibiotics revealing the linkage of specific amino sugar types to certain 2-deoxystreptamine positions. Direct proof of this pattern was provided for kanamycin A by determining the kanosamine-attachment-site as C6 of 2-deoxystreptamine. Similar studies involving cuprammonium complexing of N-acetylated degradation products of the gentamicin-C members (tetra-N-acetylgentamines) established C4 of 2-deoxystreptamine as the purpurosamine-attachment-site.[10] In antibiotics with 4,6-disubstituted 2-deoxystreptamine, therefore, 3-amino-3-deoxy-amino sugars are generally attached to C6 of 2-deoxystreptamine, whereas 2-amino-2-deoxy-amino sugars are located at C4.

In the course of a chemical synthesis of paromamine by S. UMEZAWA,[11] the diastereomer **1b** was obtained for the first time and, much to our concern, exhibited the same optical rotation as **1a** so that the argument of differentiability between **1a** and **1b** on the basis of their $[\alpha]_D$ values was no longer valid.

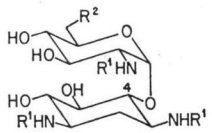
We have very recently found a strain of *Micromonospora echinospora* producing, inter alia, an aminoglycoside which was indistinguishable from gentamicin A.[12] To confirm the assigned glycoside-linkage loci 4 and 6 in 2-deoxystreptamine of gentamicin A isolated from this and from the commercial gentamicin fermentations, both gentamicin A samples, as well as paromomycin, were subjected to methanolysis.[1,13] The resulting crystalline aminoglycoside trihydrochlorides were converted to the free bases and N-acetylated. The paromamine synthesis of HASEGAWA[14] made available $[\alpha]_D$ values for tri-N-acetylparomamine (**3a**) and the diastereomer **3b** which differ significantly from each other. As evident from Table 1, we found positive optical rotations for the gentamicin A derivatives whose magnitudes are in better agreement with **3a** than with **3b**, thus conforming with the previously proposed gentamicin A structure.

The additivity principle of the rotational increments, $\Delta[M]_{436}$, in Cupra B and related complexing solvents has been implied and demonstrated in the analysis of compounds with two[4,15] and as many as five[16] chelation sites per mole-

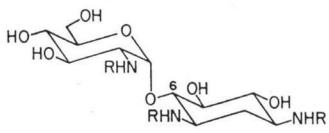
Table 1.

		Tri-N-acetylparomamine from		
		Paromomycin	Gentamicin A[a]	Gentamicin A[b]
$[\alpha]_D$	Water	+ 99.6°[c]	+105.5°	+101.1°
$[\alpha]_{436}$	Water	+199.8°	+211.9°	+202.8°
$[\alpha]_{436}$	Cupra B	+204.7°	+204.3°	+202.0°
$\Delta[M]_{436}$	Cupra B	+ 22°	− 35°	− 4°

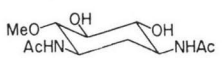
[a] from commercial gentamicin fermentation
[b] from *Micromonospora echinospora* sp. X-14847
[c] other reported values: +108°[13] and +92°[14]



1a: $R^1 = H$, $R^2 = OH$ (Paromamine)
2: $R^1 = H$, $R^2 = NH_2$ (Neamine)
3a: $R^1 = Ac$, $R^2 = OH$



1b: R = H
3b: R = Ac



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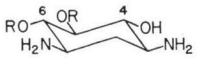
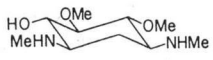


5: R = H (2-Deoxystreptamine)
6: R = Me



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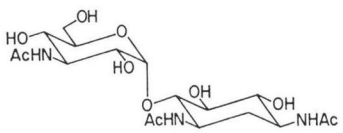

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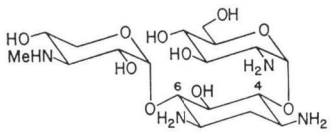

9 (Gentamicin A)

cule. Since the rotatory contribution of the 2-acetylamino-2-deoxy-D-glucopyranosyl portion is known,[16] a value of $\Delta[M]_{436}$ Cupra B = +2064° would be expected for **3a** or **3b** where only the sugar glycol grouping engages in cuprammonium complexing. The two vicinal diols in **3a** are of opposite sign and, assuming equal rotatory increment contributions, should yield $\Delta[M]_{436}$ Cupra B = 0°, whereas **3b**, with two positive dihedral angles between the vicinal glycol groupings, would be expected to give $\Delta[M]_{436}$ Cupra B = +4128°. Table 1 shows the observed values to be close to 0° so that the presence of the paromamine moiety **1a** in gentamicin A is beyond doubt. The major amino glycoside produced by *Micromonospora echinospora* sp. X-14847 is therefore identical with gentamicin A, which is confirmed to be **9** as originally proposed.[1]

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