Structure of Sisomicin, a Novel Unsaturated Aminoglycoside Antibiotic from *Micromonospora inyoensis*†

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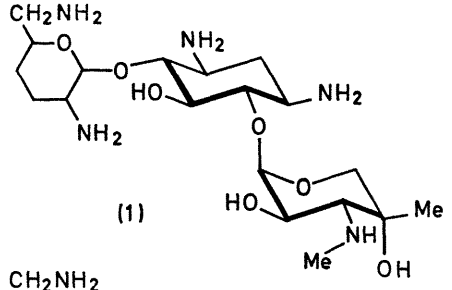
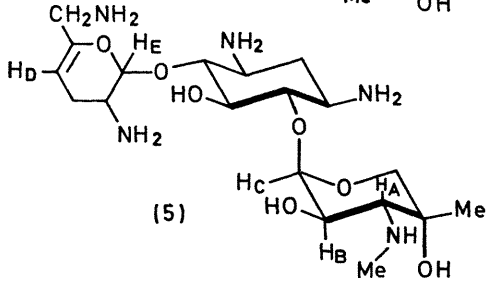
Summary The gross structure (**5**) is assigned to a new aminoglycoside antibiotic consisting of deoxystreptamine, garosamine, and a 2,6-diamino-2,3,4,6-tetradeoxyhex-4-enose.

SUBMERGED fermentations of the new species *Micromonospora inyoensis* (NRRL 3292) have been shown [1a-c] to produce a new gentamicin-like aminoglycoside antibiotic designated sisomicin. Isolation of the antibiotic was achieved[2a,b] *via* adsorption on to Amberlite® IRC-50 ion exchange resin. The biology of sisomicin has been discussed;[3a,b] the compound is of particular interest in that it shows great activity *versus* a number of Gram-negative organisms, especially *Pseudomonas aeruginosa*.

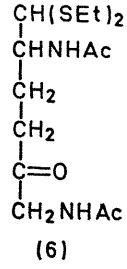
The crude antibiotic complex consisted[2] of one major component and several minor contaminants. Column chromatography on silica gel using the lower phase of the system $CHCl_3$–MeOH–NH_4OH (1 : 1 : 1) as developer/eluant afforded the pure antibiotic as a syrup that crystallised from ethanol or propanol as a hemihydrate, m.p., 198—201°, $[\alpha]_D^{26}$ + 189° (0·3, H_2O), M^+ 447·262 (calculated for $C_{19}H_{37}N_5O_7$, M^+ 447·269). The molecular weight suggests that sisomicin is a dehydro-derivative of gentamicin C_{1a}[4,5] (**1**) or an isomer thereof. Further examination



(1)

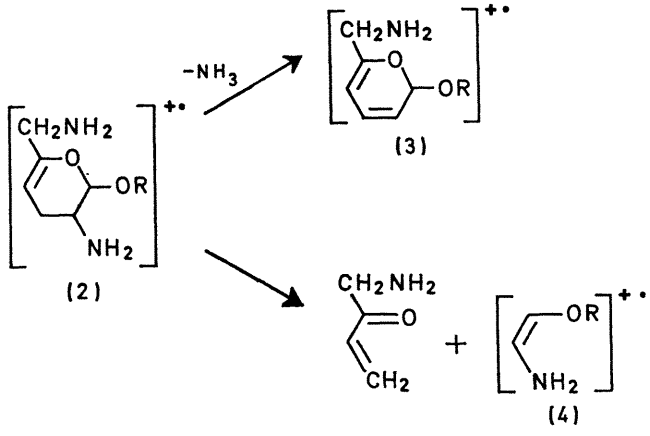


(5)



(6)

of the high resolution mass spectrum of sisomicin lends support to this hypothesis and enables localisation of the site of unsaturation. The base peak at *m/e* 160·100 corresponds to the garosamine moiety[6,7] (calculated for $C_7H_{14}NO_3$, 160·097). An intense peak (77%) at *m/e* 127·087 agrees well with the presence of a dehydro-purpurosamine C^4 unit (calculated for $C_6H_{11}N_2O$, 127·087). The initial fragmentation of the molecular ion involves loss of ammonia to give an ion *m/e* 430·243 (calculated for $C_{19}H_{34}N_4O_7$, 430·243). Such an unusual degradation is readily explicable if the double bond is placed at the Δ^4 position in the purpurosamine ring as in (**2**); loss of ammonia then gives the resonance-stabilised ion (**3**) (see Scheme). Another significant fragmentation not observed with gentamicin C_{1a} and supporting the above assignment of the double bond is the retro-Diels–Alder decomposition of the molecular ion, giving an intense peak (45%) at *m/e* 362·212 (calculated for $C_{15}H_{30}N_4O_6$, 362·216) corresponding to (**4**).



SCHEME

The ^1H n.m.r. spectrum of sisomicin (D_2O, 60 MHz) was consistent with structure (**5**). Observed signals are: δ 1·21 (4-CH_3, s), 2·52 (NCH_3, s), 2·56 (H_A, d, $J_{2,3}$ 10·5 Hz), 3·79 (H_B, q, $J_{1,2}$ 4·0, $J_{2,3}$ 10·5 Hz), 4·88 (H_D, br t), 5·09 (H_C, d, $J_{1,2}$ 4·0 Hz), and 5·35 (H_E, d, $J_{1',2'}$ 2·0 Hz).‡

Methanolysis of sisomicin afforded a complex mixture from which methyl garosaminide could be isolated by chromatography. The glycoside was characterised *via* its *N*-acetyl-derivative, m.p. 190—193°, identical in all respects with the compound obtained from the gentamicin C components.[6]

Acetylation of sisomicin with MeOH–Ac_2O gave the penta-*N*-acetyl derivative that precipitated from MeOH–Et_2O as an amorphous, white powder m.p. 185—190°, $[\alpha]_D^{26}$ + 200° (0·3, H_2O). Treatment of this compound with EtSH–conc. HCl for 16 h at 5° gave a mixture of thioacetals and *NN'*-diacetyl-2-deoxystreptamine from which the thioacetals were removed by chloroform extraction. Subsequent fractional crystallisation of the thioacetals from ethanol afforded colourless needles (36%) of the ketone (**6**) m.p. 153—154°, $[\alpha]_D^{26}$ + 33·7° (0·3, $CHCl_3$), λ_{max} ($CHCl_3$) 5·81, 5·99 μm, M^+ (1%) 334, M — Et (12%) 205, M — $AcNH_2$ (33%) 275, M — $CH(SEt)_2$ (50%) 199, $CH(SEt)_2$ (base peak) 135. The n.m.r. spectrum of (**6**) ($CDCl_3$, 60

† Sisomicin was formerly known as rickamicin and as antibiotic 6640.
‡ The integrals agreed with recorded assignments.

MHz) supports unequivocally the postulated structure. Observed signals are:§ δ 1·28, 1·30 (2 × t, SCH_2CH_3, J 7·0 Hz), 1·98, 2·02 (2 × s, $NHCOCH_3$), 2·69, 2·70 (2 × q, SCH_2CH_3, J 7·0 Hz), 3·92 (d, 1-H, $J_{1,2}$ 4·0 Hz), 4·11 (d, 6-H, $J_{6,NH}$ 5·0 Hz), 6·00 (d, NH, $J_{2,NH}$ 9·0 Hz), and 6·38 (br m, NH). The resonance due to the C-2 proton appeared as a broad multiplet *ca.* δ 4·2 partially masked by 1-H and 2-H. The C-3, C-4 methylenes appeared in the region δ 2·0 (masked by $NCOCH_3$) and 2·5 (masked by SCH_2CH_3), respectively. Exposure of the sample to D_2O for 48 h resulted in exchange of the amide protons and simplification of the 6-H doublet to a singlet.

Sisomicin can thus be tentatively assigned structure (**5**). The absolute stereochemistry and the positions of linkage of the two sugars to the 2-deoxystreptamine moiety have been assigned by biogenetic analogy to gentamicin C_{1a} (**1**); formal proof of these assignments has been undertaken[8] and will be reported elsewhere. Satisfactory microanalytical data were obtained for all new compounds.

We thank Dr. Milton Yudis and his staff for performing the physical measurements.

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§ Integrals agreed with the recorded assignments and with the required total of twenty-six protons.

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