THE STRUCTURE OF ANTIBIOTIC G-52, A NEW AMINOCYCLITOL-AMINOGLYCOSIDE ANTIBIOTIC PRODUCED BY *MICROMONOSPORA ZIONENSIS*

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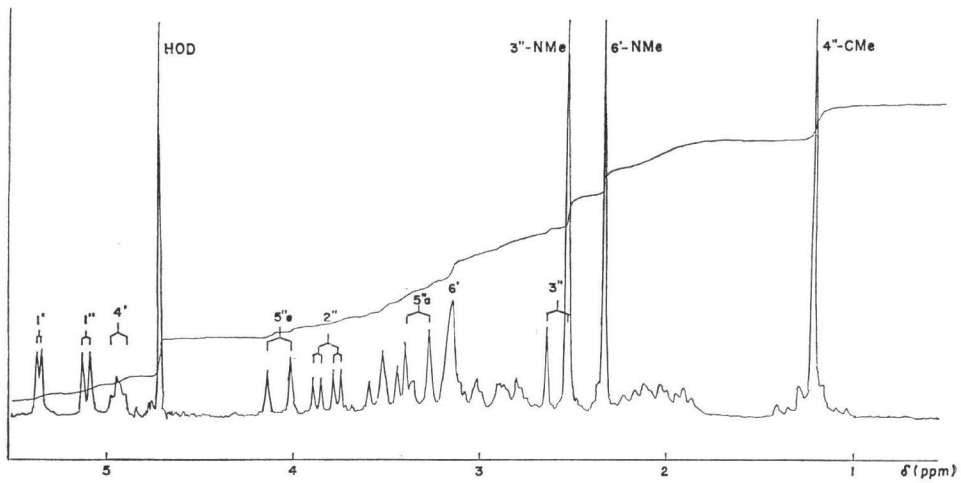
Antibiotic G-52, a new aminocyclitol-aminoglycoside antibiotic produced in the fermentation of *Micromonospora zionensis*, has been shown to be 6′-*N*-methylsisomicin on the basis of its spectral characteristics. This assignment was confirmed by synthesis of the antibiotic from sisomicin.

Fermentation of *Micromonospora zionensis* produces a complex of aminocyclitol-aminoglycoside antibiotics from which sisomicin (**1**) and a new, less polar material which has been named antibiotic G-52 has been isolated.[1] The preliminary characterization of antibiotic G-52, its *in vitro* antibacterial activity, and the identification of the major coproduced antibiotic as sisomicin are described in the preceding paper.[1] The structure of sisomicin (**1**) and some related antibiotics have been rigorously established.[2,3,4]

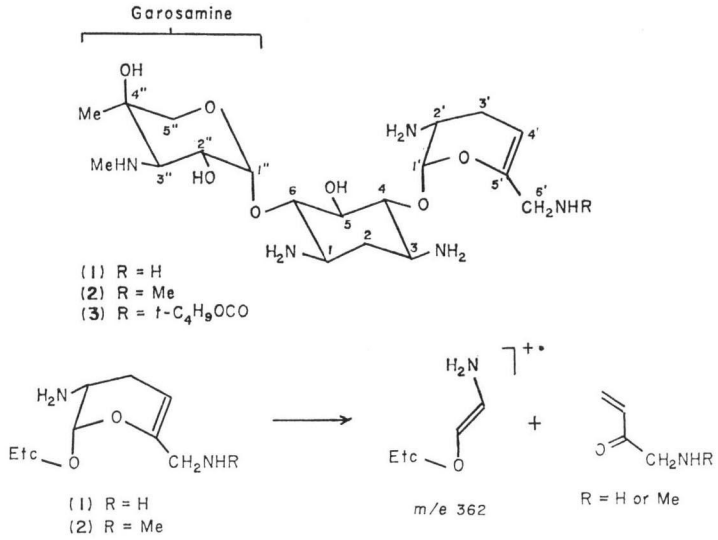
The pmr spectrum of antibiotic G-52, shown in Fig. 1, was markedly similar to that of sisomicin[2] except for an additional 3 proton singlet at δ 2.30 ppm assignable to an extra *N*-methyl group. The proton assignments in Fig. 1 were made on the basis of the close similarity to the resonances of sisomicin (**1**) and clearly showed the anomeric (1′), olefinic (4′), and allylic methylene (6′) protons of an unsaturated sugar, as well as the resonances expected for a garosamine unit.

The E.I. mass spectrum of antibiotic G-52 exhibited an $M^{+\cdot}$ peak at *m/e* 461, consistent with a homolog of sisomicin. Intense peaks at *m/e* 118 and 160 were consistent with the presence of garosamine, and at *m/e* 191, 173, 163 and 145 confirmed the presence of 2-deoxystreptamine.[5,6] The un-

Fig. 1



Scheme 1



saturated sugar gave rise to an intense ion at *m/e* 141, 14 mass units higher than for the corresponding sugar in sisomicin, localizing the extra *N*-methyl group in this moiety. A prominent peak at *m/e* 362 could be assigned to the ion shown in Scheme 1. The presence of this ion, appearing at the same mass to charge ratio as sisomicin,[6,7] located the extra *N*-methyl group in antibiotic G-52 on the 6′-nitrogen atom. A characteristic feature of the mass spectrum was a peak at *m/e* 430, corresponding to loss of methylamine (31 mass units) from the molecular ion. This peak was absent in the mass spectrum of sisomicin and therefore arose from loss of the 6′-methylamino group. In contrast, in the mass spectrum of gentamicin C_{2b},[8] the 4′,5′-dihydro analog of G-52, a peak corresponding to a loss of 30 mass units from the molecular ion is observed, this probably results from loss of methylamine from the MH^+ ion. The mass spectrum of G-52 and related antibiotics is discussed in greater detail elsewhere.[6]

Table 1. ^{13}C Chemical shifts* of sisomicin and antibiotic G-52

Carbon	Sisomicin (**1**)[9]	Antibiotic G-52 (**2**)	$\Delta\delta$
1	51.8	51.6	
2	36.4	36.2	
3	50.4	50.2	
4	85.3	85.7	
5	75.4	75.4	
6	87.8	87.9	
1′	100.6	100.9	
2′	47.6	47.3	
3′	25.6	25.6	
4′	96.5	99.0	+2.5
5′	150.4	147.7	−2.7
6′	43.5	52.6	+9.1
6′-*N*-Me		34.7	
1″	101.5	101.5	
2″	70.0	70.2	
3″	64.3	64.2	
4″	73.0	73.2	
5″	68.5	68.6	
3″-*N*-Me	37.9	37.7	
4″-*C*-Me	22.9	22.5	

* Downfield from TMS

The proton decoupled cmr spectrum of antibiotic G-52 is shown in the Table 1 in comparison to that of sisomicin. The cmr resonances of sisomicin have been assigned previously.[9] Resonances for the garosamine and 2-deoxystreptamine carbon atoms were practically identical in both compounds. The extra *N*-methyl group of antibiotic G-52 resonated at δ 34.7 ppm, whilst carbons 5′ and 6′ were shifted 2.7 ppm upfield and 9.1 ppm downfield respectively from analogous resonances in sisomicin,

consistent with the presence of an extra methyl substituent on the 6′-nitrogen atom. Carbon-4′ also experienced an appreciable deshielding effect of 2.5 ppm compared to the comparable resonance in sisomicin.

On the basis of these spectral parameters the structure of antibiotic G-52 can be confidently assigned as 6′-*N*-methylsisomicin (**2**). This assignment was confirmed by conversion of **1** to **2**. Reaction of sisomicin (**1**) with *t*-butoxycarbonyloxyphthalimide afforded the 6′-*N*-*t*-butoxycarbonyl derivative (**3**)* which was reduced with lithium aluminum hydride to antibiotic G-52 (**2**).

The presence of the 6′-*N*-methyl substituent in antibiotic G-52 gives this antibiotic significant activity against sisomicin-resistant bacteria possessing R-factor specified enzymes which inactivate this latter antibiotic by 6′-*N*-acetylation.[1] This result was to be expected in view of the similar activity of other 6′-*N*-methyl substituted aminoglycosides.[10]

Experimental

For general condition see Reference 8.

Antibiotic G-52

The antibiotic was isolated as described by MARQUEZ *et al.*[1] Prior to determination of physical constants the antibiotic was decarbonated by passage down a column of Amberlite IR 401S resin (OH^- cycle). The eluate was collected under nitrogen and lyophilized. The cmr spectrum of antibiotic G-52 is given in Table 1. The pmr spectrum (100 MHz, D_2O) is shown in Fig. 1, chemical shifts are δ 5.31 (1H, d, J=2.25 Hz, 1′-H), 5.06 (1H, d, J=4 Hz, 1″-H), 4.90 (1H, br mult, 4′-H), 4.03 (1H, d, J=12.25 Hz, 5_e″-H), 3.77 (1H, q, J=4, 10.5 Hz, 2″-H), 3.29 (1H, d, J=12.25 Hz, 5_a″-H), 3.11 (2H, br s, 6′-CH_2), 2.54 (1H, d, J=10.5 Hz, 3″-H), 2.50 (3H, s, 3″-*N*Me), 2.30 (3H, s, 6′-*N*Me), 1.18 (3H, s, 4″-*C*Me) ppm. Mass spectral peaks at *m/e* (% of base peak): 462 (2) {MH^+}, 461 (5) {$M^{+\cdot}$}, 444 (18) {M–NH_3}, 430 (2) {M–CH_3NH_2}, 362 (10) see Scheme 1, 350 (2), 332 (9), 322 (10), 304 (32) {garosamine-2-deoxystreptamine series}, 311 (17), 313 (10), 303 (32), 285 (57) {unsaturated sugar-2-deoxystreptamine series}, 191 (27), 173 (22), 163 (26), 145 (60) {2-deoxystreptamine series}, 141 (80) {unsaturated sugar}, 160 (100), 118 (92) {garosamine}.

6′-*N*-*t*-Butoxycarbonylsisomicin (**3**)

A solution of sisomicin (1.8 g, 4.02 mmole) in tetrahydrofuran (80 ml) and methanol (11 ml) was cooled in an ice-water bath. *t*-Butoxycarbonyloxyphthalimide (1.26 g, 4.8 mmole) was added, in one portion, with stirring. After 1 hour at ambient temperature, the mixture was diluted with 90 ml ice-cold water and stirred with Amberlite IRA-401S (OH^-) ion-exchange resin until the pH of the solution was 10.3. The resin was removed by filtration and washed with water. The combined filtrate was concentrated to 100 ml and lyophilized to give 1.7 g of product which was chromatographed on 50 g silica gel (60~100 mesh) on a 2.7 cm×3 cm column using a solvent system composed of chloroform, methanol and ammonium hydroxide (30: 10: 1) as the eluant, 6-ml fractions were taken. Fractions 80~250 which contained the desired product were combined, concentrated and lyophilized to give (**3**) (0.579 g, 26%) $[\alpha]_D^{26}$ +155.5° (*c* 0.1, water). The pmr spectrum (60 MHz, D_2O) gave peaks at δ 5.38 (1H, d, J=2.2 Hz, 1′-H), 5.13 (1H, d, J=4.0 Hz, 1″-H), 4.91 (1H, m, 4′-H), 2.64 (1H, d, J=10.0 Hz, 3″-H), 2.56 (3H, s, *N*-Me), 1.49 (9H, s, *C*-Me_3) and 1.26 (3H, s, *C*-Me). Mass spectral peaks at *m/e*: 547 (M^+), 530 (M–NH_3), 417, 399, 389, 371 (unsaturated sugar-2-deoxystreptamine series), 350, 322, 304 (garosamine-2-deoxystreptamine series), 191, 173, 163, 145 (2-deoxystreptamine series), 227 (unsaturated sugar), 160 (garosamine), 362 (see Scheme 1).

Anal. Calcd. for $C_{24}H_{45}O_9N_5 \cdot {}^1/_2H_2O$: C, 51.78; H, 8.32; N, 12.58%.
Found: C, 51.75, H, 7.99; N, 12.56%.

* The yield in this process was quite high according to tlc examination of the reaction mixture. After work-up however only 26% of **3** was isolated, together with much recovered starting material. It is apparent therefore that the *t*-BOC group of **3** can be cleaved by silica gel chromatography.

Antibiotic G-52 from 6′-*N*-*t*-butoxycarbonylsisomicin

To a solution of 6′-*N*-*t*-butoxycarbonylsisomicin (0.3 g, 0.55 mmole) in dry tetrahydrofuran (30 ml) was added lithium aluminum hydride (0.15 g) and the mixture was heated under reflux in an atmosphere of argon. After 4 hours the reduction was essentially complete as monitored by tlc on silica gel using chloroform, methanol and ammonium hydroxide (2: 1: 0.35) as the developing system. The mixture was cooled to 0°C and methanol (10 ml) was added with stirring, followed by water (10 ml). The solids were removed by filtration through a pad of Celite and washed with water. The combined filtrate was concentrated then redissolved in water (50 ml). The product was absorbed on to Amberlite IR-120 (H^+) form ion-exchange resin by stirring the aqueous solution with the resin until the pH of the solution was 3.0. The resin was removed by filtration, washed with water and stirred with 7% ammonium hydroxide solution. The ammonium hydroxide solution was evaporated *in vacuo* and the residue lyophilized to give 183 mg of crude antibiotic G-52. This material was chromatographed on a column (1.3 cm × 14 cm) of silica gel (60~100 mesh, 5 g) and eluted with the solvent system mentioned above. The homogeneous fractions containing antibiotic G-52 were pooled, concentrated and lyophilized to give 60 mg (24%) of pure G-52. The mass spectrum, the pmr spectrum and chromatographic mobility of synthetic G-52 were identical with natural antibiotic G-52.

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