THE STRUCTURE OF AMINOGLYCOSIDE ANTIBIOTIC 66-40G PRODUCED BY *MICROMONOSPORA INYOENSIS*

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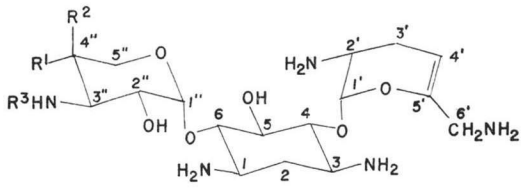
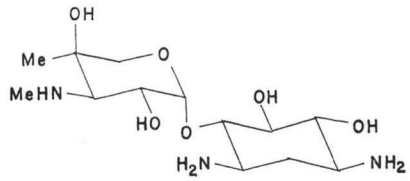
Aminoglycoside antibiotic 66-40G is a minor component produced in the fermentation of *Micromonospora inyoensis*. Its structure has been established as 3''-de-*N*-methyl-sisomicin (**4**) by spectroscopic means and by direct comparison with an authentic sample obtained from photochemical oxidative de-*N*-methylation of sisomicin (**1**).

Fermentation of *Micromonospora inyoensis* (NRRL 3292) produces sisomicin (**1**)[2] as the major aminoglycoside aminocyclitol antibiotic[3~6]. A number of minor components which are coproduced in the fermentation of sisomicin (**1**) have been isolated and their structures established[5~8]. These include garamine (**5**)[5,6], antibiotic 66-40B (**2**)[7,8], antibiotic 66-40C (**6**)[9], and antibiotic 66-40D (**3**)[7,8].

Examination of a concentrate of sisomicin related substances, which had been obtained from the mother liquors of a sisomicin crystallization, revealed a new biologically active component which was designated 66-40G. The 66-40G (**4**) component which was admixed with 66-40B (**2**) and 66-40D (**3**) was readily separated from these two by ion-exchange chromatography on Amberlite CG–50 resin in the NH_4^+ cycle using stepwise elution with dilute NH_4OH. The antibiotic thus obtained, after concentra-



(1)	R^1 = Me	R^2 = OH	R^3 = Me
(2)	R^1 = OH	R^2 = H	R^3 = Me
(3)	R^1 = H	R^2 = OH	R^3 = Me
(4)	R^1 = Me	R^2 = OH	R^3 = H



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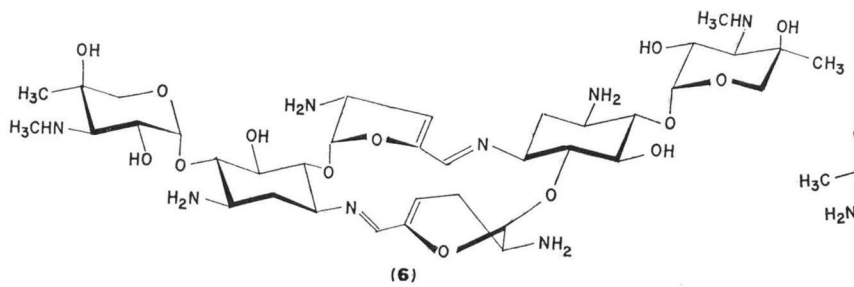
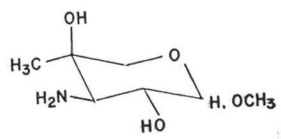


(6)



(7)

tion and lyophilization of the appropriate fractions, was found to have an elemental composition and a mass spectral fragmentation pattern similar to 66-40B (**2**) and 66-40D (**3**). The pmr spectrum of 66-40G (**4**) did not show a signal in the region of δ 2.4 where the other members of this series show a singlet for the 3″-*N*-methyl protons. The presence of a 4″-*C*-methyl group however was indicated by a singlet at δ 1.20. It was apparent, therefore, that 66-40G was 3″-de-*N*-methylsisomicin (**4**).

The carbon-13 chemical shifts of 66-40G (**4**) are shown in Table 1 along with those of sisomicin (**1**)[10] and are consistent with the structure. Thus, the shielding of C-3″ by 8.7 ppm along with the absence of a resonance at 37.9 ppm relative to sisomicin indicates the presence of a primary amine function at C-3″.

Table 1. ^{13}C-Chemical shifts (downfield from TMS) of sisomicin and antibiotic 66-40G

Carbon	Sisomicin (**1**)[10]	Antibiotic 66-40G (**4**)
1	51.8	51.6
2	36.4	36.3
3	50.4	50.2
4	85.3	85.4
5	75.4	75.4
6	87.8	88.0
1′	100.6	100.8
2′	47.6	47.4
3′	25.6	25.5
4′	96.5	96.6
5′	150.4	150.4
6′	43.5	43.3
1″	101.5	101.4
2″	70.0	71.0
3″	64.3	55.6
4″	73.0	72.3
5″	68.5	68.5
3″–N–Me	37.9	
4″–C–Me	22.9	21.8

Methanolysis of 66-40G (**4**) yielded on chromatography, α and β anomers (**7**) (in the ratio of about 1: 1) of methyl 3-de-*N*-methyl-garosaminide in place of the methyl garosaminide which is the product when sisomicin is methanolized[6].

Furthermore, when examined on tlc, the compound was identical with a sample of 3″-de-*N*-methyl-sisomicin which had been prepared from sisomicin by a photochemical oxidative de-*N*-methylation procedure[1].

Experimental Section

For general conditions see Ref. 9

Isolation of Antibiotic 66-40G (**4**)

A 5.5 g sample of sisomicin related substances, which was obtained by counter-current distribution of the mother liquors from a sisomicin crystallization, was dissolved in water and after adjustment to pH 6.1, applied to the top of a 3.3 cm × 142 cm Amberlite CG–50 column in the NH_4^+ cycle. The column was subjected to stepwise elution beginning with water and then changing successively to 0.05 N NH_4OH, 0.1 N NH_4OH and 0.18 N NH_4OH, collecting 20-ml fractions and monitoring by thin-layer chromatography using Analtech silica gel GF plates (250 microns) in the system chloroform - methanol - 7% ammonium hydroxide (1: 2: 1). This gave 66-40G (**4**) in 1.6 g yield on concentration and lyophilization. Changing the eluant to 0.2 N NH_4OH and finally 0.24 N NH_4OH yielded 66-40B (**2**) (1.8 g) and 66-40D (**3**) (1.6 g) respectively. These were identical with authentic samples of these compounds previously[8] obtained (t.l.c., n.m.r., mass spectrometry).

The 66-40G (**4**) thus obtained had m.p. 168~178°C; $[\alpha]_D^{26}$ +158.4° (H_2O); δ 5.33 (1H, d, $J_{1',2'}$ = 2.5 Hz, H-1′), 5.09 (1H, d, $J_{1'',2''}$ = 4 Hz, H-1″), 4.88 (1H, broad t, H-4′), 4.05 (1H, d, J_{gem} = 12 Hz, H-5″e), 3.67 (1H, dd, $J_{1'',2''}$ = 4 Hz, $J_{2'',3''}$ = 12 Hz, H-2″), 3.37 (1H, d, J_{gem} = 12 Hz, H-5″a), 3.14 (2H, s, 6′-CH_2), 2.79 (1H, d, $J_{3'',2''}$ = 12 Hz, H-3″a), 2.10 (2H, m, H-3′), 2.01 (1H, dt, H-2e), 1.22 (1H, m, H-2a), 1.20 (3H, s, 4″-CH_3); θ_{280} −6260 (TACu). Found: C, 47.11; H, 7.32; N, 14.07%;

M^+ 433. $C_{18}H_{35}N_5O_7 \cdot CO_2 \cdot 1/2\ H_2O$ requires C, 46.91; H, 7.46; N, 14.40.

Methanolysis of Antibiotic 66-40G (**4**)

Antibiotic 66-40G (**4**) (300 mg) was dissolved in 4.5 N methanolic HCl (12 ml) and heated under reflux for 7.5 hours. The solution was cooled, concentrated *in vacuo* and chromatographed on a 2.5 cm × 56 cm silica gel column with the lower phase of the chloroform - methanol - concentrated ammonium hydroxide (1 : 1 : 1) system as eluant. This yielded, on concentration of the appropriate fractions, the amorphous aminohexopyranoside mixture of α and β anomers (**7**) (95 mg). The pmr spectrum (79.5 MHz, D_2O) gave peaks at δ 4.82 (1H, d, $J_{1,2}=4$ Hz, H-1(β)), 4.3 (1H, d, $J_{1,2}=8$ Hz, H-1 (α)), 3.75 (1H, d, J = 12.5 Hz, H-5e (α & β)), 3.54 (3H, s, O-Me (α)), 3.41 (3H, s, O-Me (β)), 3.39 (1H, d, J = 12.5 Hz, H-5a (α & β)), 3.02 (1H, d, $J_{3,2}=11$ Hz, H-3 (β)), 2.76 (1H, d, $J_{3,2}=10$ Hz, H-3 (α)), 1.21 (6H, s, C-CH_3 (α & β)) $(M+1)^+$ 178.1.

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References

1) NAGABHUSHAN, T. L.; J. J. WRIGHT, A. B. COOPER, W. N. TURNER & G. H. MILLER: Chemical modification of some gentamicins and sisomicin at the 3″-position. J. Antibiotics 31: 43～54, 1978
2) WEINSTEIN, M. J.; J. A. MARQUEZ, R. T. TESTA, G. H. WAGMAN, E. M. ODEN & J. A. WAITZ: Antibiotic 66-40, a new *Micromonospora*-produced aminoglycoside antibiotic. J. Antibiotics 23: 551～554, 1970
3) COOPER, D. J.; R. S. JARET & H. REIMANN: Structure of sisomicin, a novel unsaturated aminoglycoside antibiotic from *Micromonospora inyoensis*. Chem. Comm. 1971: 285～286, 1971
4) REIMANN, H.; R. S. JARET & D. J. COOPER: Sisomicin: Stereochemistry and attachment of the unsaturated sugar moiety. Chem. Comm. 1971: 924～925, 1971
5) KUGELMAN, M.; A. K. MALLAMS & H. F. VERNAY: The preparation of garamine, a novel pseudodisaccharide from sisomicin. J. Antibiotics 26: 394～395, 1973
6) REIMANN, H.; D. J. COOPER, A. K. MALLAMS, R. S. JARET, A. YEHASKEL, M. KUGELMAN, H. F. VERNAY & D. SCHUMACHER: The structure of sisomicin, a novel unsaturated aminocyclitol antibiotic from *Micromonospora inyoensis*. J. Org. Chem. 39: 1451～1457, 1974
7) MALLAMS, A. K.; D. H. DAVIES, R. W. TKACH, D. GREEVES & J. B. MORTON: Novel aminoglycoside antibiotics related to sisomicin. 14th ICAAC, Paper 168, San Francisco, Calif., Sept. 11～13, 1974
8) DAVIES, D. H.; D. GREEVES, A. K. MALLAMS, J. B. MORTON & R. W. TKACH: Structures of the aminoglycoside antibiotics 66-40B and 66-40D produced by *Micromonospora inyoensis*. J. Chem. Soc. Perkin I 1975: 814～818, 1975
9) DAVIES, D. H.; A. K. MALLAMS, J. MCGLOTTEN, J. B. MORTON & R. W. TKACH: Structure of aminoglycoside 66-40C, a novel unsaturated imine produced by *Micromonospora inyoensis*. J. Chem. Soc., Perkin I 1977: 1407～1411, 1977
10) MORTON, J. B.; R. C. LONG, P. J. L. DANIELS, R. W. TKACH & J. H. GOLDSTEIN: A carbon-13 magnetic resonance study of aminoglycoside pseudotrisaccharides. The gentamicin antibiotics. J. Am. Chem. Soc. 95: 7464～7469, 1973