# Structures of the Aminoglycoside Antibiotics 66-40B and 66-40D produced by *Micromonospora inyoensis*

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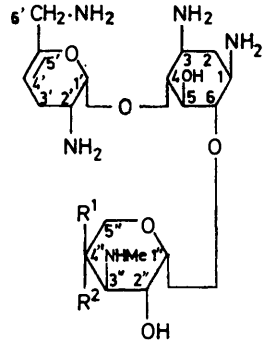
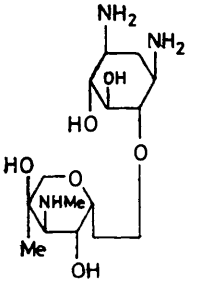
The novel amino-glycoside antibiotics 66–40B and 66–40D produced as minor components by fermentation of *Micromonospora inyoensis* have been shown to be *O*-2,6-diamino-2,3,4,6-tetradeoxy-α-D-*glycero*-hex-4-enopyranosyl-(1→4)-*O*-[3-deoxy-3-methylamino-α-D-xylopyranosyl-(1→6)]-2-deoxy-D-streptamine and *O*-2,6-diamino-2,3,4,6-tetradeoxy-α-D-*glycero*-hex-4-enopyranosyl-(1→4)-*O*-[3-deoxy-3-methylamino-β-L-arabinopyranosyl-(1→6)]-2-deoxy-D-streptamine, respectively. The novel 3-deoxy-3-methylamino-β-L-arabinopyranosyl sugar unit of 66–40D has not previously been found in any amino-glycoside antibiotic. Both 66–40B and D exhibit broad spectrum antibacterial activity.

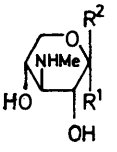
SUBMERGED fermentation of *Micromonospora inyoensis* (NRRL 3292) produces sisomicin (1),[1] a novel unsaturated aminoglycoside antibiotic,[2-6] as the principal



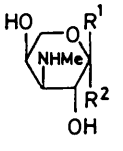
(1) $R^1$ = OH, $R^2$ = Me
(3) $R^1$ = H, $R^2$ = OH
(4) $R^1$ = OH, $R^2$ = H



(2)



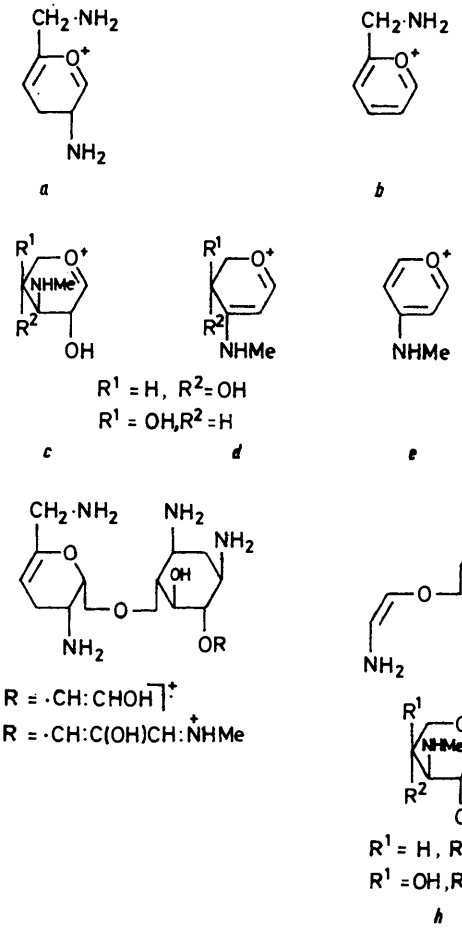
(5) $R^1$ = OMe, $R^2$ = H
(6) $R^1$ = H, $R^2$ = OMe



(7) $R^1$ = OMe, $R^2$ = H
(8) $R^1$ = H, $R^2$ = OMe

product. Minor components co-produced in the fermentation include garamine (2),[5] antibiotic 66-40B (3),[7] and antibiotic 66-40D (4).[7] We present in this paper details of the isolation and complete structural elucidation of the components (3) and (4).

These antibiotics were best separated by column chromatography. Microanalyses and high resolution mass spectra were in agreement with a composition of $C_{18}H_{35}N_5O_7$ for both (3) and (4), and both were isolated as monohydrates. The mass spectral cracking patterns of (3) and (4) were identical except for minor intensity differences. The spectra exhibited an ion *a* at *m/e* 127 which lost ammonia to give the ion *b* at *m/e* 110. Glycosyl cleavage of the 3-deoxy-3-methylaminopentopyranoside unit followed by successive losses of two molecules of water gave rise to ions *c*—*e* at *m/e* 146, 128, and 110. The formation of the ions *f* and *g* at *m/e* 330 and 372, respectively, indicated that the antibiotics contained 3-deoxy-3-methylaminopentopyranosyl units. Retro-Diels–Alder cleavage of the enopyranoside system[2,6] gave



*a* *b* *c* *d* *e*

$R^1$ = H, $R^2$ = OH
$R^1$ = OH, $R^2$ = H

*f* R = ·CH:CHOH
*g* R = ·CH:C(OH)CH:NHMe

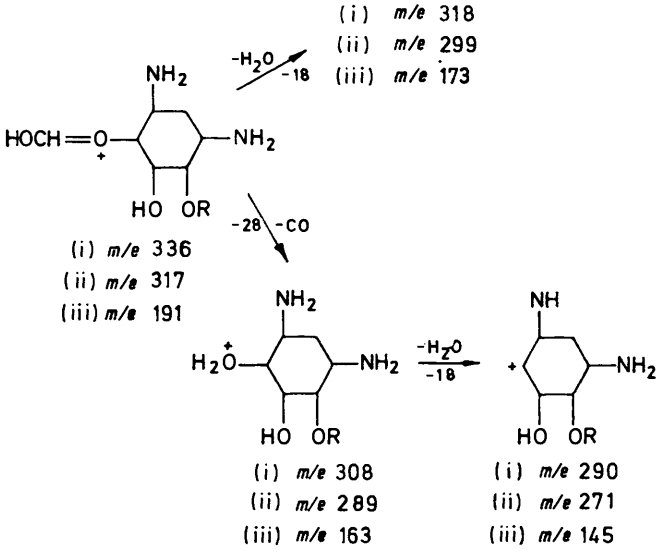
*h*

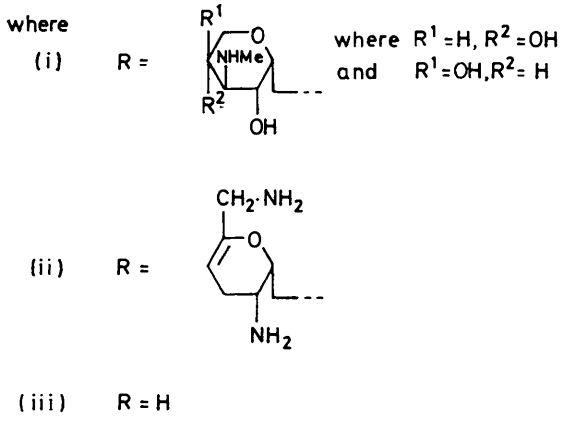
$R^1$ = H, $R^2$ = OH
$R^1$ = OH, $R^2$ = H

rise to a prominent ion *h* at *m/e* 348, supporting the location of the double bond at C(4′)—C(5′). The charac-

[1] M. J. Weinstein, J. A. Marquez, R. T. Testa, G. H. Wagman, E. M. Oden, and J. A. Waitz, *J. Antibiotics*, 1970, **23**, 551.
[2] D. J. Cooper, R. S. Jaret, and H. Reimann, *Chem. Comm.*, 1971, 285.
[3] R. D. Guthrie and G. J. Williams, *Chem. Comm.*, 1971, 923.
[4] H. Reimann, R. S. Jaret, and D. J. Cooper, *Chem. Comm.*, 1971, 924.
[5] M. Kugelman, A. K. Mallams, and H. F. Vernay, *J. Antibiotics*, 1973, **26**, 394.
[6] H. Reimann, D. J. Cooper, A. K. Mallams, R. S. Jaret, A. Yehaskel, M. Kugelman, H. F. Vernay, and D. Schumacher, *J. Org. Chem.*, 1974, **39**, 1451.
[7] D. H. Davies, D. Greeves, A. K. Mallams, J. B. Morton, and R. W. Tkach, 14th ICAAC, San Francisco, California, September 11—13th, 1974.

teristic protonated formyl ions formed in the mass spectra of aminoglycoside antibiotics [8,9] by initial cleavage of the C(1)—C(2) bonds in the sugars followed by losses of carbon monoxide and water are shown in the Scheme for 66-40B (3) and -40D (4). The compositions of all ions were determined by high resolution mass spectrometry.




SCHEME

The i.r. spectra of 66-40B (3) and -40D (4) showed absorptions, at 1690 and 1680 $cm^{-1}$, respectively, consistent with the presence of a vinyl ether group.

The n.m.r. spectrum of 66-40B (3) showed the presence of one *N*-methyl group (δ 2·43). The anomeric proton of the enopyranoside unit gave rise to a doublet at δ 5·32 with $J_{1',2'}$ 2·2 Hz, consistent with the structure shown. The olefinic H-4′ signal occurred as a multiplet at δ 4·84. The relative stereochemistry of the 3-deoxy-3-methylaminopentopyranoside unit was consistent with a *xylo*-configuration. The anomeric H-1″ signal occurred as a doublet at δ 5·00 ($J_{1'',2''}$ 4 Hz), H-2″ gave a doublet of doublets at δ 3·60 ($J_{2'',3''}$ 10 Hz), and H-3″ gave a doublet of doublets at δ 2·66 ($J_{3'',4''}$ *ca.* 8 Hz). The above assignments were confirmed by application of decoupling and INDOR.

The n.m.r. spectrum of 66-40D (4) also revealed one *N*-methyl group at δ 2·35 and the anomeric proton of the enopyranoside unit gave rise to a doublet at δ 5·31 with *J* 2·2 Hz, consistent with the structure shown. The olefinic H-4′ signal occurred as a multiplet at δ 4·82. Irradiation at the frequency of H-1′ caused the H-2′ signal at δ 3·04 to collapse to a doublet of doublets with *J* 6 and 9·5 Hz; irradiation at the frequency of H-2′ caused the H-1′ signal to collapse to a singlet. Irradiation at the frequency of H-3′ at δ *ca.* 2·05 caused the H-2′ signal to collapse to a doublet (*J* 2·2 Hz) and that of H-4′ to collapse to a broadened singlet. When the signal due to the 6′-protons at δ 3·12 was irradiated the H-4′ signal simplified to a doublet of doublets (*J* 2·5 and 4 Hz). The assignment of the *arabino*-configuration to the 3-deoxy-3-methylaminopentopyranosyl unit was deduced from the n.m.r. spectrum of 66-40D (4) as follows. The anomeric H-1″ signal occurred as a doublet at δ 5·05 ($J_{1'',2''}$ 4 Hz), and H-2″ gave rise to a doublet of doublets at δ 3·79 with $J_{2'',3''}$ 10 Hz, which on irradiation at the frequency of H-1″ collapsed to a doublet. The H-3″ signal occurred as a doublet of doublets at δ 2·78 ($J_{3'',4''}$ 3 Hz), which on irradiation at the frequency of H-2″ collapsed to a doublet with $J_{3'',4''}$ 3 Hz and on irradiation at the frequency of H-4″ collapsed to a doublet with $J_{2'',3''}$ 10 Hz. The signal due to H-4″ at δ 4·10 was obscured by one leg of the broadened H-5″*eq* doublet at δ 4·15 ($J_{gem}$ 12·5 Hz). Owing to the proximity of the H-4″ and -5″*eq* resonances, the H-5″*ax* signal also appeared as a broadened doublet at δ 3·64. Simultaneous irradiation at the frequencies of H-4″ and -5″*eq* caused the signal due to H-5″*ax* to collapse to a singlet, and irradiation at the frequency of H-5″*ax* caused both the H-5″*eq* and -4″ signals to collapse to broad singlets.

The $^{13}C$ n.m.r. spectra of 66-40B (3) and -40D (4) in $D_2O$ were recorded (Table 1). The assignments for C(1—6) and C(1′—6′) were in excellent agreement with those for the corresponding carbon atoms in sisomicin (1).[10] The assignments for the pentose sugars were based in part on analogy with the corresponding values reported [10-12] for the methyl glycosides of garosamine, D-xylose, and L-arabinose, as well as with the results of single-frequency off-resonance (SFOR) and pH studies on the monosaccharides (5)—(8).[13] In each case doublets characteristic of methine carbons were observed for C(1—4) in the SFOR spectra, and the C-5 methylene carbons gave rise to the expected triplets. The $OCH_3$

[8] P. J. L. Daniels, M. Kugelman, A. K. Mallams, R. W. Tkach, H. F. Vernay, J. Weinstein, and A. Yehaskel, *Chem. Comm.*, 1971, 1629.
[9] P. J. L. Daniels, A. K. Mallams, J. Weinstein, and J. J. Wright, in preparation.
[10] J. B. Morton, R. C. Long, P. J. L. Daniels, R. W. Tkach, and J. H. Goldstein, *J. Amer. Chem. Soc.*, 1973, **95**, 7464.
[11] A. S. Perlin, B. Casu, and H. J. Koch, *Canad. J. Chem.*, 1970, **48**, 2596.
[12] D. E. Dorman and J. D. Roberts, *J. Amer. Chem. Soc.*, 1970, **92**, 1355.
[13] D. J. Cooper, D. H. Davies, A. K. Mallams, and A. S. Yehaskel, preceding paper.

and $NCH_3$ resonances occurred as quartets. The SFOR spectrum of the α-D-*xylo*-monosaccharide (5) clearly enabled the signal at δ 62·8 to be assigned to C-3 and that at δ 62·4 to C-5, on the basis of their multiplicities. The corresponding chemical shifts of the trisaccharide 66-40B (3) were reversed. Thus C-3″ gave rise to a signal at δ 62·7 and C-5″ a signal at δ 63·0, the latter having an enhanced linewidth relative to the methine resonances, consistent with the short relaxation time associated with a methylene group. Solutions of the monosaccharides (5)—(8) were very sensitive to atmospheric carbon dioxide and upon brief exposure to the atmosphere, or to carbon dioxide, gave rise to what are probably carbamates.[10] The $^{13}C$ n.m.r. spectra of these compounds showed lower-field resonances for C-1 and higher-field resonances for the $NCH_3$ relative to the parent amino-sugar in each instance. The $^{13}C$ n.m.r. spectra of (5), (6), 66-40B (3), and -40D (4) at pH 4 showed the expected up-field shifts of the β-carbon signals associated with *N*-protonation.[14]

The relative chemical shift differences Δ(*xylo* ⟶ *arabino*), for 66-40B (3) and -40D (4) and for the corresponding monosaccharides (5) and (8), and (6) and (7), are given in Table 2. The shift differences between the α-D-*xylo*- (5) and β-L-*arabino*- (8) monosaccharides were in excellent agreement with those recorded for 66-40B (3) and -40D (4). The shift differences in Table 2 are in general agreement with those reported for hydroxypentoses by Perlin *et al.*[11] The $^{13}C$ n.m.r. spectra of (3) and -40D (4) was assigned as α-D-*xylo* and β-L-*arabino*, respectively. Thus 66-40B (3) showed $[\theta]_{290}$ + 2530 (TACu) and +1935 (Cupra A), consistent with predominant complex formation between the 3″-methylamino-group and the vicinal 4″-hydroxy-group leading to a weak positive extremum. If the glycosidic linkage had been β, the predominant complexing would have occurred between the 2″-hydroxy- and 3″-methylamino-groups, leading to a negative extremum.[13] The c.d. spectrum of 66-40D showed $[\theta]_{290}$ − 8590 (TACu) and −6270 (Cupra A), clearly supporting a β-L-*arabino*-configuration. The c.d. data were in accord with a 4,6-substitution pattern about the deoxystreptamine ring in both compounds. It remained at this stage to establish the absolute stereochemistry about the deoxystreptamine ring.

Catalytic reduction of 66-40B (3) and -40D (4) over Perlman's catalyst gave the dihydro-derivatives (9) and (10), respectively. In both instances the reduction occurred exclusively from the 'top' face of the enopyranoside to give products having the 5′-$CH_2$·$NH_2$ substituent axial; this subsequently underwent conformational inversion from the $^4C_1$ to the more stable $^1C_4$ conformation. A similar conformational inversion was observed[4,6] when sisomicin (1) was reduced to the dihydro-derivative. The conformational change was evident from the n.m.r. signals of H-1′ in (9) and (10), which occurred as doublets at δ 4·70 and 4·76 with $J_{1',2'}$ 2 Hz, respectively, consistent with the axial orientation of these protons in the $^1C_4$ conformation. Both dihydro-derivatives showed no vinylic proton signals in the n.m.r. spectra and no vinylic ether absorption in the i.r. spectra. The mass spectra were consistent with structures (9) and (10).

TABLE 1

$^{13}C$ Chemical shifts (p.p.m. downfield from $Me_4Si$)

| Carbon | (1)[10] | (3) | (3) pH 4 | Δ(base ⟶ pH 4) | (4) | (4) pH 4 | Δ(base ⟶ pH 4) | (5) [a] | (5), $CO_2$ [a,b] | (6) [a] | 6), $CO_2$ [a,b] | (6) pH 4 [a] | (7) [a] | (8) [a] | (8), $CO_2$ [a,b] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C-1 | 51·8 | 51·7 | 51·2 | −0·5 | 51·6 | 50·8 | −0·8 | | | | | | | | |
| C-2 | 36·4 | 36·2 | 30·7 | −5·5 | 36·3 | 28·3 | −8·0 | | | | | | | | |
| C-3 | 50·4 | 50·2 | 49·3 | −0·9 | 50·2 | 49·1 | −1·1 | | | | | | | | |
| C-4 | 85·3 | 85·4 | 81·2 | −4·2 | 85·5 | 79·9 | −5·6 | | | | | | | | |
| C-5 | 75·4 | 75·3 | 74·7 | −0·6 | 75·4 | 74·4 | −1·0 | | | | | | | | |
| C-6 | 87·8 | 88·1 | 85·2 | −2·9 | 88·0 | 84·0 | −4·0 | | | | | | | | |
| C-1′ | 100·6 | 100·9 | 101·5 | +0·6 | 100·9 | 102·0 | +1·1 | | | | | | | | |
| C-2′ | 47·6 | 47·4 | 47·2 | −0·2 | 47·4 | 47·1 | −0·3 | | | | | | | | |
| C-3′ | 25·6 | 25·5 | 23·6 | −1·9 | 25·5 | 24·0 | −1·5 | | | | | | | | |
| C-4′ | 96·5 | 96·7 | 97·6 | +0·9 | 96·7 | 97·9 | +1·2 | | | | | | | | |
| C-5′ | 150·4 | 150·6 | 144·1 | −6·5 | 150·6 | 144·4 | −6·2 | | | | | | | | |
| C-6′ | 43·5 | 43·3 | 41·6 | −1·7 | 43·4 | 41·6 | −1·8 | | | | | | | | |
| C-1″ | 101·5 | 100·9 | 101·3 | +0·4 | 101·3 | 101·5 | +0·2 | 99·3(d) | 100·1 | 104·5(d) | 105·4(d) | 104·0(d) | 105·6(d) | 99·6 | 100·3 |
| C-2″ | 70·0 | 70·8 | 67·4 | −3·4 | 68·7 | 66·4 | −2·3 | 70·4(d) | 70·5 | 71·6(d) | 71·6(d) | 68·7(d) | 70·6(d) | 68·2 | 68·2 |
| C-3″ | 64·3 | 62·7 | 61·9 | −0·8 | 59·3 | 59·2 | −0·1 | 62·8(d) | 62·8 | 65·7(d) | 65·6(d) | 65·2(d) | 63·6(d) | 59·4 | 59·2 |
| C-4″ | 73·0 | 68·7 | 64·6 | −4·1 | 64·3 | 63·3 | −1·0 | 68·5(d) | 68·6 | 68·3(d) | 68·3(d) | 64·9(d) | 64·9(d) | 64·0 | 63·9 |
| C-5″ | 68·5 | 63·0 | 63·1 | +0·1 | 65·5 | 64·7 | −0·8 | 62·4(t) | 62·3 | 67·0(t) | 66·9(t) | 66·8(t) | 68·7(t) | 65·5 | 65·3 |
| 3″-$NCH_3$ | 37·9 | 34·3 | 30·6 | −3·7 | 32·8 | 30·9 | −1·9 | 34·9(q) | 34·3 | 34·8(q) | 33·9(q) | 32·2(q) | 32·9(q) | 33·4 | 32·5 |
| 4″-$CCH_3$ | 22·9 | | | | | | | | | | | | | | |
| 1-$OCH_3$ | | | | | | | | 56·2(q) | 55·9 | 57·9(q) | 57·7(q) | 58·4(q) | 57·8(q) | 56·3 | 56·0 |

[a] The double prime notation of carbons does not apply to these monosaccharides. The symbols in parentheses represent the multiplicities of the signals obtained in the SFOR spectra. [b] Formed by brief exposure of the solution to $CO_2$.

TABLE 2

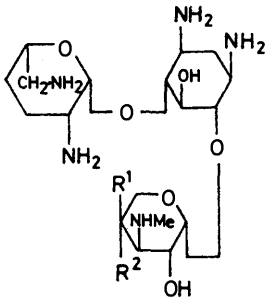
Relative $^{13}C$ chemical shift differences

| Carbon | | Δ(α-D-*xylo* ⟶ β-L-*arabino*) (3) ⟶ (4) | (5) ⟶ (8) | Δ(β-D-*xylo* ⟶ α-L-*arabino*) (6) ⟶ (7) |
|---|---|---|---|---|
| C-1 | (δ) | +0·4 | +0·3 | +1·1 |
| C-2 | (γ) | −2·1 | −2·2 | −1·0 |
| C-3 | (β) | −3·4 | −3·4 | −2·1 |
| C-4 | (α) | −4·4 | −4·5 | −3·4 |
| C-5 | (β) | +2·5 | +3·1 | +1·7 |
| 3-$NCH_3$ | (δ) | −1·5 | −1·5 | −1·9 |

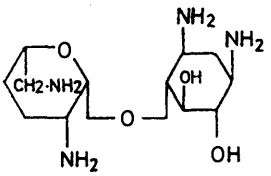
From the c.d. spectra the absolute stereochemistry[15,16] of the 3-deoxy-3-aminopentopyranoside units in 66-40B (3) and 66-40D (4) clearly demonstrated that both the glycosidic linkages in each compound were axial and that the sites of glycosylation were 4 and 6 in the deoxystreptamine ring in both instances.

[14] G. Kotowycz and R. U. Lemieux, *Chem. Rev.*, 1973, **73**, 669.
[15] S. T. K. Bukhari, R. D. Guthrie, A. I. Scott, and A. D. Wrixon, *Chem. Comm.*, 1968, 1580.
[16] S. T. K. Bukhari, R. D. Guthrie, A. I. Scott, and A. D. Wrixon, *Tetrahedron*, 1970, **26**, 3653.

Methanolysis of dihydro-66-40B (9) with hydrogen chloride in methanol gave the pseudodisaccharide, 5′-epigentamine $C_{1a}$ (11), identical with an authentic sample



(9) $R^1$ = H, $R^2$ = OH
(10) $R^1$ = OH, $R^2$ = H



(11)

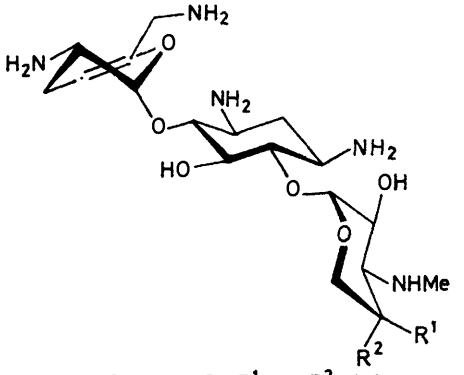
prepared from dihydrosisomicin [4,6] in a similar manner. The physical constants for 5′-epigentamine $C_{1a}$ (11) rigorously established the location of the enopyranoside in 66-40B (3) as the 4-position of the deoxystreptamine ring, and the positive extrema at 290 nm in the c.d. spectra of (11) recorded in TACu and Cupra A proved the absolute stereochemistry about that ring. The other product of the methanolysis was an anomeric mixture of methyl α- and β-gentosaminides [(5) and (6)], which were identical with authentic samples prepared from gentamicin A [17] and by synthesis.[13] The pure α-anomer (5) was obtained as a crystalline solid by chromatography and was identical with the synthetic α-anomer.[13] This established the sugar at the 6-position of the deoxystreptamine as being the known 3-deoxy-3-methylamino-D-xylopyranoside (gentosamine).[17]

When dihydro-66-40D (10) was subjected to a similar methanolysis, 5′-epigentamine $C_{1a}$ (11) was again obtained and was shown to be identical with an authentic sample prepared from dihydrosisomicin.[4,6] This defined the location of the enopyranoside in 66-40D (4) as the 4-position of the deoxystreptamine and established the absolute stereochemistry about the deoxystreptamine ring. The other product of the methanolysis was an anomeric mixture of methyl 3-deoxy-3-methylamino-L-arabinopyranosides [(7) and (8)] which were identical with synthetic samples.[13] This amino-sugar appears not to have been encountered previously in any antibiotic.

The solution conformations in $D_2O$ of 66-40B (3) and -40D (4) could readily be shown from the $^{13}C$ n.m.r. spectra to be similar to those of the kanamycins,[14,18] sisomicin (1),[10] the gentamicins,[10] and tobramycin.[19] The difference between substituent γ-effects on carbon atoms in a cyclitol or pyranose ring has been postulated to be indicative of rotamer populations about the ether bond [20,21] in such molecules. Thus shielding of the γ-carbons of the deoxystreptamine ring by the 4-*O*-glycosyl and 6-*O*-glycosyl units would be expected, from work [20,21] on vicinal diols, to amount to *ca.* 1·4 ± 0·2 p.p.m. The 4-*O*-glycosyl units of sisomicin (1),[10] the gentamicins,[10] and tobramycin [19] have been demonstrated to produce upfield γ-shifts at C-3 of this order of magnitude, and the 6-*O*-glycosyl unit produces a similar γ-shift at C-5, negligible shielding being observed at C-1. The preferred rotamer populations about the O–C(4) and O–C(6) bonds have been shown from the above work [10,19] and from $^1H$ n.m.r. studies [18] to be as depicted in Figure 1. Similarly 66-40B (3) shows upfield γ-shifts for C-3 of 1·4 and for C-5 of 1·3 p.p.m., while C-1 shows negligible γ-shielding. Also 66-40D (4) shows upfield γ-shifts for C-3 of 1·4 and for C-5 of 1·2 p.p.m., while C-1 shows no γ-shielding. These results indicate that the preferred glycosyl rotamer populations are as shown in Figure 1 and that 66-40B (3) and -40D (4) have the solution conformations shown in Figure 2.

Preferred 4-*O*-glycosyl rotamer  Preferred 6-*O*-glycosyl rotamer

FIGURE 1



66-40 B(3) $R^1$ = H, $R^2$ = OH
66-40 D(4) $R^1$ = OH, $R^2$ = H

FIGURE 2

Both antibiotics 66-40B (3) and -40D (4) exhibit broad spectrum antibacterial activity, which will be reported in detail elsewhere.

## EXPERIMENTAL

Unless otherwise stated optical rotations were recorded at 26° in water (*c* 0·3%). I.r. spectra were recorded for KCl discs on a Perkin-Elmer 221 spectrometer. $^1H$ N.m.r. spectra were obtained at 60 or 100 MHz for solutions in $D_2O$ on a Varian A60A or XL 100-15 spectrometer, with sodium 4,4-dimethyl-4-silapentane-1-sulphonate as internal or external standard. $^{13}C$ N.m.r. spectra were recorded for solutions in $D_2O$ with an internal dioxan reference and

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shifts are reported in p.p.m. downfield from $Me_4Si$ ($\delta_C$ for dioxan = −67·4). The spectra were obtained on a Varian XL100-12 spectrometer by Fourier transform with a Varian 620L-16K computer. C.d. spectra were recorded on a Cary 61 spectrometer. Mass spectra were recorded on either a Varian MAT CH5 or a CEC 21-100B spectrometer.

*Isolation of Antibiotics* 66-40B (3) *and* -40D (4).—The residual antibiotic complex produced by submerged fermentation of *Micromonospora inyoensis* (NRRL 3292),[1] after removal of sisomicin by crystallization and chromatography on a silica gel column with chloroform–methanol–15% ammonium hydroxide (3 : 5 : 3) as eluant, contained the minor components garamine (2) [5] and antibiotics 66-40B (3) and -40D (4). The crude mixture of fractions rich in 66-40B and -40D (8·3 g) was chromatographed repeatedly on a silica gel column (170 × 7·5 cm) with the same eluant to give, in order of elution, the isomeric antibiotics 66-40B (3) (1·6 g) and 66-40D (4) (0·8 g). Both were obtained as amorphous monohydrates after passage over Amberlite IRA 401S ($OH^-$) resin and lyophilization.

*Antibiotic* 66-40B (3) had m.p. 91—102° (Found: C, 47·9; H, 7·75; N, 15·3%; $M^+$, 433·2540. $C_{18}H_{35}N_5O_7,H_2O$ requires C, 47·9; H, 8·25; N, 15·5%; $C_{18}H_{35}N_5O_7$ requires $M$, 433·2536), $[\alpha]_D$ +152·8°, $\nu_{max.}$ 3340, 1690, 1050, and 1000 $cm^{-1}$, $\delta$ 2·43 (3H, s, 3″-$NCH_3$), 4·84 (1H, m, H-4′), 5·00 (1H, d, $J$ 4 Hz, H-1″), and 5·32 (1H, d, $J$ 2·2 Hz, H-1′), $[\theta]_{290}$ +2530 (TACu), $[\theta]_{290}$ +1935 (Cupra A).

*Antibiotic* 66-40D (4) had m.p. 92—103° (Found: C, 47·8; H, 8·15; N, 15·65%; $M^+$, 433·2565), $[\alpha]_D$ +147·3°, $\nu_{max.}$ 3330, 1680, 1075, and 1000 $cm^{-1}$, $\delta$ 2·35 (3H, s, 3″-$NCH_3$), 4·82 (1H, m, H-4′), 5·05 (1H, d, $J$ 4 Hz, H-1″), and 5·31 (1H, d, $J$ 2·2 Hz, H-1′), $[\theta]_{290}$ −8590 (TACu), $[\theta]_{290}$ −6270 (Cupra A).

*Dihydro-antibiotic* 66-40B (9).—Antibiotic 66-40B (3) (300 mg) dissolved in methanol (7 ml) was hydrogenated over 20% palladium hydroxide–carbon (50 mg) at 25° and 60 lb $in^{-2}$ for 5 days. More catalyst (50 mg) was added after 48 h. The catalyst was filtered off and the filtrate was evaporated and chromatographed on a silica gel column (110 × 2·5 cm) with the lower phase of the chloroform–methanol–concentrated ammonium hydroxide (1 : 1 : 1) system as eluant to give *dihydro*-66-40B (9) as an amorphous solid (140 mg) after passage over Amberlite IRA 401S ($OH^-$) resin and lyophilization; m.p. 135—145° (Found: $M^+$, 435·2683. $C_{18}H_{37}N_5O_7$ requires $M$, 435·2693), $[\alpha]_D$ +103·0°, $\nu_{max.}$ 3330, 1145, and 1040 $cm^{-1}$, $\delta$ 2·41 (3H, s, 3″-$NCH_3$), 4·70 (1H, d, $J$ 2 Hz, H-1′), and 4·95 (1H, d, $J$ 4 Hz, H-1″), $[\theta]_{290}$ +1675 (TACu), $[\theta]_{290}$ +1330 (Cupra A).

*Dihydro-antibiotic* 66-40D (10).—Antibiotic 66-40D (4) (450 mg) dissolved in methanol (22 ml) was hydrogenated over 20% palladium hydroxide–carbon (75 mg) at 25° and 60 lb $in^{-2}$ for 24 h. The product was worked up as above and chromatographed on a silica gel column (110 × 2·5 cm) with chloroform–methanol–concentrated ammonium hydroxide (3 : 4 : 3) as eluant. Concentration of the relevant fractions followed by passage over Amberlite IRA 401S ($OH^-$) resin and lyophilization gave *dihydro*-66-40D (10) as an amorphous solid (270 mg), m.p. *ca.* 100° (forms gum), decomp. >180° (Found: $M^+$, 435·2693), $[\alpha]_D$ +151·9°, $\nu_{max.}$ 3300, 1075, and 1005 $cm^{-1}$, $\delta$ 2·35 (3H, s, 3″-$NCH_3$), 4·76 (1H, d, $J$ 2 Hz, H-1′), and 5·08 (1H, d, $J$ 4 Hz, H-1″), $[\theta]_{290}$ −10,140 (TACu), $[\theta]_{290}$ −9640 (Cupra A).

*Methanolysis of Dihydro*-66-40B (9).—Dihydro-66-40B (9) (70 mg) was dissolved in dry methanol saturated with hydrogen chloride (11 ml) and heated under reflux for 4 h. The solution was concentrated *in vacuo* and the residue was chromatographed on a silica gel column (20 × 2·5 cm) with the lower phase of the chloroform–methanol–concentrated ammonium hydroxide (1 : 1 : 1) system as eluant to give a mixture of methyl α- and β-gentosaminides [(5) and (6)], identical (t.l.c. and mass spectra) with authentic samples prepared from gentamicin A[17] and by synthesis.[13] The α- and β-anomers were separated by chromatography on a silica gel column (110 × 1 cm) with 40% methanol in chloroform as eluant to give the major α-anomer (5) as a crystalline solid (8 mg), m.p. 114—115° (synthetic α-anomer,[13] m.p. 110—112°).

The more polar hydrolysis product from the initial column chromatography was 5′-epigentamine $C_{1a}$ (11),[4,6] which was obtained as an amorphous solid (42 mg) after passage over Amberlite IRA 401S ($OH^-$) resin and lyophilization and was identical (t.l.c., n.m.r., high resolution mass spectrometry, c.d.) with samples prepared from sisomicin (1) [4,6] and dihydro-66-40D (10).

*Methanolysis of Dihydro*-66-40D (10).—Dihydro-66-40D (10) (200 mg) was dissolved in dry methanol saturated with hydrogen chloride (70 ml) and heated under reflux for 10 h. The solution was cooled, concentrated *in vacuo*, and passed over Amberlite IRA 401S ($OH^-$) resin; the aqueous methanolic eluate was evaporated and chromatographed on a silica gel column (110 × 1 cm) with the lower phase of the chloroform–methanol–concentrated ammonium hydroxide (1 : 1 : 1) system as eluant, to give methyl 3-deoxy-3-methylamino-α- and β-L-arabinopyranoside [(7) and (8)] as a gum (50 mg) which was identical (t.l.c., n.m.r.) with a synthetic mixture of anomers.[13] The natural anomeric mixture showed $[\alpha]_D$ +161·8°; an equilibrium mixture of the synthetic anomers [10] showed $[\alpha]_D$ +157·3°.

The more polar hydrolysis product from the initial column chromatography was 5′-epigentamine $C_{1a}$ (11),[4,6] obtained as an amorphous solid (50 mg) after passage over Amberlite IRA 401S ($OH^-$) resin and lyophilization; m.p. 135—138° [Found: $(M + 1)^+$, 291·2022. Calc. for $C_{12}H_{26}N_4O_4$: $(M + 1)$, 291·2032], $[\alpha]_D$ +42·6°, $\delta$ 4·76 (1H, d, $J$ 2 Hz, H-1′), $[\theta]_{290}$ +3970 (TACu), $[\theta]_{290}$ +2700 (Cupra A), identical (t.l.c., mass spectrometry, n.m.r., $[\alpha]_D$, c.d.) with samples prepared from sisomicin (1) [4,6] and dihydro-66-40B (9).

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