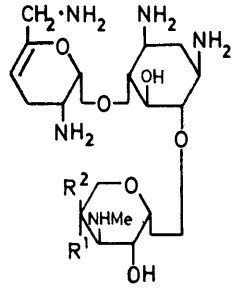
Structure of Aminoglycoside 66-40 C, a Novel Unsaturated Imine Produced by *Micromonospora inyoensis*

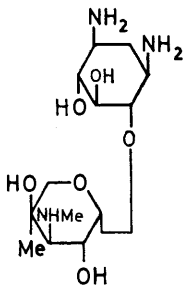
By D. Huw Davies, Alan K. Mallams,* James McGlotten, James B. Morton, and Richard W. Tkach, Research Division, Schering Corporation, Bloomfield, New Jersey 07003, U.S.A.

The aminoglycoside 66-40 C, produced as a minor component in the fermentation of *Micromonospora inyoensis*, has been shown by spectroscopic and chemical degradative studies to have the novel dimeric structure (5), containing αβ-unsaturated imine groups not previously encountered in any aminoglycoside antibiotic.

SUBMERGED fermentation of *Micromonospora inyoensis* (NRRL 3292) produces sisomicin (1),[1] a novel unsaturated aminoglycoside antibiotic,[2-5] as the principal product. Minor co-products include garamine (4),[4,5] antibiotic 66-40 B (2),[6,7] and antibiotic 66-40 D (3),[6,7] the structures of which we have described in detail previously, as well as aminoglycoside 66-40 C (5). We now present



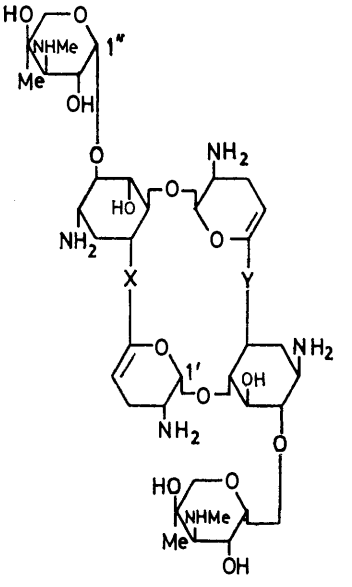
(1) R^1 = Me, R^2 = OH
(2) R^1 = OH, R^2 = H
(3) R^1 = H, R^2 = OH

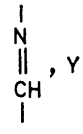
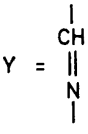


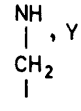
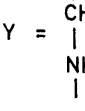
(4)

evidence leading to the assignment of total structure and absolute stereochemistry to aminoglycoside 66-40 C.

Column chromatographic separation of the minor components of the fermentation gave 66-40 C as a colourless amorphous solid. Microanalytical data were in agreement with a molecular formula $(C_{19}H_{32}N_4O_7)_n$. However, an electron impact mass spectrum showed no peaks above about *m/e* 300, suggesting that the molecule was not a normal pseudotrisaccharide. The low-mass region exhibited the characteristic fragment ions at *m/e* 160, 142, and 124 associated with the presence of a garosamine unit.[5] On flash ionization a molecular ion was obtained at *m/e* 856, indicating that the molecule was dimeric ($C_{38}H_{64}N_8O_{14}$). In view of the low volatility of 66-40 C a field desorption mass spectrum was run; this lent further support to a dimeric structure, showing ions at *m/e* 858 (MH_2^+) and 857 (MH^+), with a fragment ion at *m/e* 841 due probably to loss of ammonia from MH_2^+. Chemical ionization mass spectrometry gave the ion $[(M/2)H^+ - H_2O]$ at *m/e* 411, but no molecular ion



(5) X =  , Y = 

(6) X =  , Y = 

corresponding to a dimeric structure.† Osmometric molecular weight determinations carried out in duplicate at three different concentrations in pyridine on rigorously

† The QM^+ (quasimolecular ion) peaks are usually much weaker than those due to $(M/2)H^+$ in head-to-tail dimers,[8] consistent with observations on 66-40 C.

[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] H. Reimann, R. S. Jaret, and D. J. Cooper, *Chem. Comm.*, 1971, 924.
[4] M. Kugelman, A. K. Mallams, and H. F. Vernay, *J. Antibiotics*, 1973, **26**, 394.
[5] 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.
[6] A. K. Mallams, D. H. Davies, R. W. Tkach, D. Greeves, and J. B. Morton, 14th ICAAC, San Francisco, California, September 11—13th, 1974, Paper 168.
[7] D. H. Davies, D. Greeves, A. K. Mallams, J. B. Morton, and R. W. Tkach, *J.C.S. Perkin I*, 1975, 814.
[8] H. Ziffer, H. M. Fales, G. W. A. Milne, and F. H. Field, *J. Amer. Chem. Soc.*, 1970, **92**, 1597.

dried, unsolvated material,* gave a molecular weight of 847 for 66-44 C, close to that (856) for a dimeric structure. Sisomicin (1), run under identical conditions as a control, gave a molecular weight of 453 (theory 447).

The u.v. spectrum of 66-40 C in methanol exhibited a single maximum at 248 nm (ε 22 000) which on acidification underwent a bathochromic shift to 281 nm ($\varepsilon \xrightarrow{\text{slow}} 0$), characteristic of an αβ-unsaturated imine.[9] Further evidence for this chromophore was obtained from the i.r. spectrum (KCl disc) ($\nu_{max.}$ 1 670 and 1 640 cm^{-1}).

The ^1H n.m.r. spectrum at 100 MHz (D_2O) showed line broadening consistent with a dimeric structure. The presence of garosamine was indicated by the following bands. Two singlets corresponding to the 4''-methyl and 3''-*N*-methyl groups were present at δ 1.22 and 2.52, respectively. The anomeric proton (H-1'') signal occurred as a doublet at δ 5.15, $J_{1'',2''}$ 4 Hz. A doublet of doublets at δ 3.84 due to H-2'', $J_{2'',3''}$ 11 Hz, collapsed to a doublet, $J_{1'',2''}$ 4 Hz, on irradiation at the frequency of H-3'', and irradiation at the frequency of H-1'' produced a doublet, $J_{2'',3''}$ 11 Hz. The 3''-proton gave a doublet at δ 2.61. Doublets at δ 3.36 and 4.10, $J_{5a'',5e''}$ 12.5 Hz, were evident, due to H-5a'' and -5e'', respectively. The ^1H n.m.r. spectrum also supported the presence of an enopyranoside moiety. A doublet at δ 5.50, $J_{1',2'}$ 2 Hz, due to the anomeric proton H-1', was consistent with that observed for the enopyranoside moiety of sisomicin (1).[5] The 2'-proton signal occurred as a multiplet at δ 2.95 consistent with the presence of a 2'-amino-substituent, and on irradiation at the frequency of this signal the doublet due to H-1' collapsed to a singlet. At the same time the broad multiplet at δ *ca.* 2.26 due to the 3'-protons sharpened considerably. Irradiation at the frequency of the $C(3')H_2$ signal caused the multiplet due to H-2' to collapse to a doublet, $J_{1',2'}$ 2 Hz. The chemical shift of the 3'-protons was in agreement with an allylic orientation. The vinylic H-4' signal occurred as a multiplet at δ *ca.* 5.48, which collapsed to a singlet on irradiation at the frequency of the 3'-protons. The H-4' signal was partially obscured by that of H-1' at δ 5.50. A low field singlet at δ 7.56 was assigned to the 6'-imine proton. The above n.m.r. evidence together with the u.v. data clearly supported the proposed structure for the enopyranoside moiety. The n.m.r. data were in agreement with the sugars having axial glycosidic linkages. The presence of a signal due to the imine proton and the absence of a $C(6')H_2$ singlet, which in the spectrum of sisomicin (1) occurs at δ 3.09, coupled with the information that the imine was conjugated with a vinylic ether double bond from the u.v. data, clearly located the imine carbon atom at position 6'. The presence of the H-2a signal of the deoxystreptamine ring as a doublet of doublet of doublets with $J_{1,2a} = J_{2a,3} = J_{2a,2e} = 12.5$ Hz at δ 1.81 [deshielded relative to the corresponding proton in sisomicin (1)],[5] indicated that one of the amino-groups in the deoxystreptamine was involved in the imine bond. The c.d. spectrum of 66-40 C in methanol showed a negative extremum at 280 nm, consistent with the location of the imine nitrogen atom on a chiral carbon atom having an *S*-configuration.[10] C-3 of deoxystreptamine fulfils this requirement, indicating that the imines are formed from the 3-amino-groups and not from the 1-amino-groups, which have *R*-chirality.

Further support for structure (5) for 66-40 C was obtained from the ^{13}C n.m.r. spectrum (Table and Figure). The spectrum exhibited the expected resonances for the garosamine units.[11] The absence of a C-6' methylene carbon signal in the spectrum of 66-40 C (5) [*cf.* sisomicin (1)] and the presence of a characteristic imine carbon resonance at δ_C 161.0 was consistent with structure (5). The chemical shift differences between 66-40 C (5) and sisomicin (1) [11] (Table) are in accord with structure (5), in which the imine groups involve C-6' and the 3-amino-group of the deoxystreptamine ring in each case. The presence of the imine group caused a down-

^{13}C Chemical shifts [a]

Carbon	(1) [11]	(5)	Δ [(1) → (5)]	(1) (pH 1.5) [12]	(7) (pH 1)	(6)	(6) (pH 1)	Δ [(6) Base → pH 1]	Δ [(1) → (6)]	(10)	(10) (pH 1)	Δ [(10) Base → pH 1]
1	51.8	51.3	−0.5	50.7	50.6	51.4	50.6	−0.8	−0.4 (δ)	51.7	50.7	−1.0
2	36.4	36.0	−0.4	28.2	28.3	33.3	27.7	−5.6	−3.1 (γ)	36.2	28.3	−7.9
3	50.4	65.9	+15.5	49.2	49.0	55.4	55.8	+0.4	+5.0 (β)	50.1	49.1	−1.0
4	85.3	80.8	−4.5	79.8	80.5	84.5	81.8	−2.7	−0.8 (γ)	84.8	79.6	−5.2
5	75.4	76.7	+1.3	74.5	74.6	75.6	74.3	−1.3	+0.2 (δ)	75.5	74.6	−0.9
6	87.8	87.4	−0.4	83.8	83.9	87.7	83.5	−4.2	−0.1	87.7	84.1	−3.6
1'	100.6	99.8	−0.8	97.8[d]	97.7	101.7	99.5	−2.2	+1.1	100.5	97.5	−3.0
2'	47.6	47.1	−0.5	47.1	46.8	47.0	47.0		−0.6	46.8	47.0	+0.2
3'	25.6	25.6		23.9	24.8	26.5	25.1	−1.4	+0.9	25.9	23.5	−2.4
4'	96.5	115.5	+19.0	101.7[d]	102.0	98.6	101.4	+2.8	+2.1 (δ)	110.4	107.6	−2.8
5'	150.4	146.4	−4.0	144.3	149.5	148.1	143.5	−4.6	−2.3 (γ)	141.5	142.8	+1.3
6'	43.5	161.0	+117.5	41.5	189.9/123.8	48.3	49.4	+1.1	+4.8 (β)	*b*	*b*	
1''	101.5	101.3		101.7	102.0	101.5	101.9	+0.4		101.4	101.9	+0.5
2''	70.0	70.0		67.1	67.2	70.2	67.2	−3.0	+0.2	70.0	67.4 [c]	−2.6
3''	64.3	64.3		64.4	64.2	64.3	64.3			64.2	64.2	
4''	73.0	73.1		70.8	70.8	73.2	70.8	−2.4	+0.2	73.1	70.8	−2.3
5''	68.5	68.6		68.6	68.6	68.6	68.6		+0.1	68.5	68.7	+0.2
3''-NCH_3	37.9	37.8		35.5	35.4	37.5	35.5	−2.2	−0.2	37.6	35.5	−2.1
4''-CH_3	22.9	22.7		22.0	21.8	22.5	21.9	−0.6	−0.4	22.4	21.9	−0.5

[a] P.p.m. downfield from Me_4Si; solvent D_2O. [b] Not recorded under spectral conditions used. [c] Obscured by dioxan reference peak. [d] The assignments for C-1' and -4' at pH 1 for sisomicin were originally thought [12] to be the opposite of those quoted above. Titration studies [12] have subsequently revealed the correct assignments and it is therefore necessary to reverse the corresponding assignments for 66-40 B and D,[7] which were based on the original sisomicin assignments.

* Microanalytical data (C, H, N) for the samples used (Alfred Bernhardt, Bonn) indicated that they were not hydrated.

[9] Z. Badr, R. Bonnett, T. R. Emerson, and W. Klyne, *J. Chem. Soc.*, 1965, 4503.

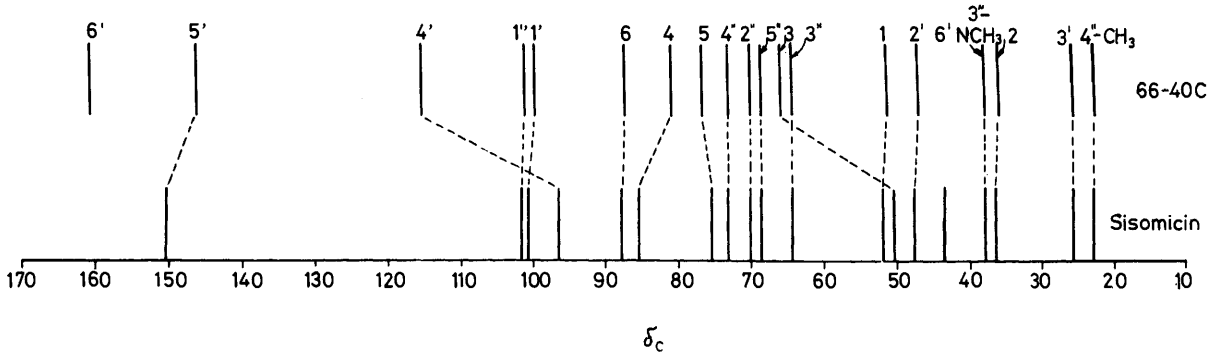
[10] Z. Badr, R. Bonnett, W. Klyne, R. J. Swan, and J. Wood, *J. Chem. Soc.* (*C*), 1966, 2047.

[11] J. B. Morton, R. C. Long, P. J. L. Daniels, R. W. Tkach, and J. H. Goldstein, *J. Amer. Chem. Soc.*, 1973, **95**, 7464.

[12] P. J. L. Daniels, unpublished observations.

field shift of 15.5 p.p.m. for the β-carbon (C-3) signal. Upfield γ shifts of −4.5 and −0.4 p.p.m. were observed for C-4 and C-2, respectively. The shifts for the δ-carbon atoms of deoxystreptamine were +1.3 and −0.5 p.p.m. for C-5 and C-1, respectively. The ^{13}C n.m.r. data were also in agreement with glycosidic linkages to the 4- and 6-positions of the deoxystreptamine units.

The c.d. spectra of 66-40 C (5) in TACu and in Cupra A exhibited negative extrema at 290 nm, in agreement with the expected absolute stereochemistry with respect to vicinal amino-alcohol groups in the garosamine portions. The only remaining point of uncertainty about structure (5) was the absolute stereochemistry of the sugar units about the deoxystreptamine rings, and this was proved as follows.



^{13}C N.m.r. spectra of aminoglycoside 66—40 C (5) and sisomicin (1)

Treatment of 66-40 C (5) with dilute aqueous methanolic hydrogen chloride at 25 °C for 20 h followed by reduction with sodium cyanoborohydride gave the known pseudodisaccharide garamine (4),[4,5] identical with an authentic sample prepared from sisomicin (1). The garosamine units were therefore glycosidically attached to the 6-positions of the deoxystreptamine rings, thus establishing the absolute stereochemistry of 66-40 C as indicated in structure (5). When 66-40 C (5) was treated with the same concentration of dilute aqueous methanolic hydrogen chloride at 25 °C for 20 h then passed over Amberlite IRA45 resin, unchanged 66-40 C (5) [and no garamine (4)] was obtained. The latter finding indicated that the garamine (4) was not simply being produced by acidic hydrolysis of the vinylic ether system. In order to rationalize the formation of garamine in the above reaction a sample of 66-40 C was dissolved in D_2O in a ^{13}C n.m.r. tube, and the solution was acidified to pH 1 with hydrogen chloride. The ^{13}C n.m.r. spectrum was recorded at intervals; after 24 h complete hydrolysis of the dimeric imine to the monomeric aldehyde (7) had occurred. The ^{13}C n.m.r. data for (7) (Table) showed that the molecule was no longer dimeric, having resonances for the garosamine unit as well as for C-1—6 and for C-1'—4' in close agreement with the corresponding values [12] for sisomicin (1) at pH 1.5. The vinylic C-5' gave a band at δ_C 144.3 and C-6' gave two signals at δ_C 189.9 and 123.8 in the ratio *ca.* 1 : 2 due to the free aldehyde and its hydrated form, respectively. The formation of garamine upon addition of sodium cyanoborohydride to the aldehyde (7) may readily be explained by initial reduction to the 6'-alcohol (8),[13] which on stirring in the reaction medium at pH 1 underwent hydrolysis to garamine. The hydrolysis was demonstrated to occur by treating an authentic sample of the 6'-alcohol (8) [13] with aqueous acid at pH 1 to give garamine (4).

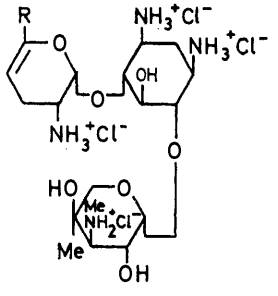
Reduction of 66-40 C (5) either with sodium borohydride or directly with sodium cyanoborohydride at pH 1 gave tetrahydro-66-40 C (6), which showed no u.v. or c.d. absorption in methanol solution above 200 nm. A band at 1 680 cm^{-1} in the i.r. spectrum indicated that the vinylic ether groups had not undergone reduction. The ^1H n.m.r. spectrum of (6) showed no imine proton resonances, the only olefinic proton resonances being those due to H-4', which gave a multiplet at δ 4.82. Spin decoupling and the use of INDOR techniques on H-2', the 3'-protons, and H-4' confirmed the presence of the vinylic ether systems. Another striking feature of the spectrum of (6) was the position of the multiplet due to H-2a (δ 1.13), the deshielding by the imine groups in 66-40 C (5) having been removed. The spectrum showed line broadening characteristic of a dimer, and no molecular ion was obtained in an electron impact mass spectrum. Flash ionization mass spectrometry, however, gave the expected molecular ion at *m/e* 860, and chemical ionization mass spectrometry gave an $[(M/2)H^+ - H_2O]$ ion at *m/e* 413. The c.d. spectra of tetrahydro-66-40 C (6) in TACu and in Cupra A showed negative extrema at 290 nm having decreased amplitude relative to 66-40 C (5) owing to removal of the contributions from the αβ-unsaturated imine chromophores.

^{13}C N.m.r. spectra of tetrahydro-66-40 C (6) were recorded for the free base as well as at pH 1 (Table). The spectra clearly showed the vinylic C-4' and C-5' signals at δ_C 98.6 and 148.1, respectively. The imine carbon signals were no longer present, and a signal at δ_C 48.3 due to the C-6' methylene groups confirmed the fact that the imine groups had been reduced. Protonation of the amino-groups in tetrahydro-66-40 C (6) produced the

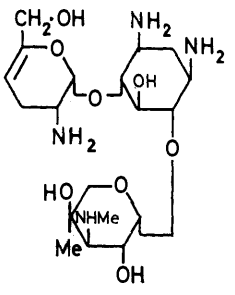
[13] D. H. Davies, A. K. Mallams, M. Counelis, D. Loebenberg, E. L. Moss, and J. A. Waitz, *J. Medicin. Chem.*, in the press.

expected [14] upfield shifts in signals of carbon atoms β to the amino-groups. The chemical shift differences between tetrahydro-66-40 C (6) and sisomicin (1) were consistent with the proposed structure having the bridging between C-6′ and C-3 (Table).

Catalytic hydrogenation of 66-40 C (5) over 20% palladium hydroxide on carbon gave tetrahydro-66-40 C (6) together with unchanged 66-40 C (5) and some garamine (4). No perhydro-derivative corresponding to reduction of both imine and vinylic ether systems was observed. Similar results were obtained when 66-40 C (5) was hydrogenated in glacial acetic acid over 30% palladium–carbon, the major product being tetrahydro-66-40 C (6).



(7) R = CHO and $CH(OH)_2$ (1:2)



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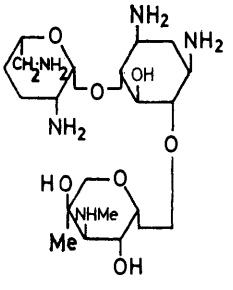

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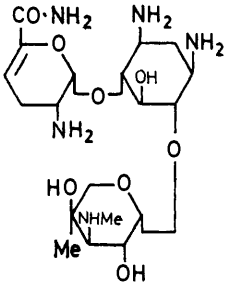

(10)

When tetrahydro-66-40 C (6) was hydrogenated over 20% palladium hydroxide on carbon under similar conditions in methanol for 24 h, the sole material isolated was unchanged tetrahydro-66-40 C (6). Thus the vinylic ether groups of 66-40 C (5) are resistant to catalytic hydrogenation under conditions which would reduce the corresponding vinylic ether group of sisomicin (1) to give the dihydro-derivative (9).[15]

When kept for lengthy periods in a vial containing air, gradual oxidative degradation of 66-40 C (5) occurred leading to garamine (4) and *O*-2-amino-2,3,4-trideoxy-α-D-*glycero*-hex-4-enopyranuronamidosyl-(1 ⟶ 4)-garamine (10). The amide (10) showed a vinylic ether i.r. absorption at 1 680 cm^{-1} and an allylic amide absorption at 1 645 cm^{-1}. The mass spectrum of (10) showed the expected molecular ion at *m/e* 461, and the presence of the D_9 ion (terminology of ref. 15) at *m/e* 362 due to a retro-diene cleavage indicated that the only changes were in the 6′-position of the molecule. The ^1H n.m.r. spectrum of (10) showed no $C(6')H_2$ signal; the ^{13}C n.m.r. parameters (Table) also revealed the absence of a $C(6')H_2$ system.

[14] G. Kotowycz and R. U. Lemieux, *Chem. Rev.*, 1973, **73**, 669.

[15] P. J. L. Daniels, A. K. Mallams, J. Weinstein, J. J. Wright, and G. W. A. Milne, *J.C.S. Perkin I*, 1976, 1078.

EXPERIMENTAL

Unless otherwise stated, optical rotations were recorded at 26 °C for solutions in water (*c* 0.3%). I.r. spectra were recorded for KCl discs with a Perkin-Elmer 221 spectrometer. N.m.r. spectra were obtained at 100 MHz for solutions in D_2O with a Varian XL 100-15 spectrometer (internal or external sodium 4,4-dimethyl-4-silapentane-1-sulphonate standard). ^{13}C N.m.r. spectra were recorded for solutions in D_2O with an internal dioxan standard [$\delta_C(Me_4Si) = \delta_C(\text{dioxan}) + 67.4$], and shifts are reported in p.p.m. downfield from Me_4Si. The spectra were obtained with a Varian XL 100-12 spectrometer by Fourier transform with a Varian 620L–16K computer. C.d. spectra were recorded with a Cary 61 spectrometer. Mass spectra were recorded with a Varian MAT CH 5 spectrometer.

Isolation of Aminoglycoside 66-40 C (5).—The residual antibiotic complex produced by fermentation of *Micromonospora inyoensis*, after removal of the sisomicin by crystallization and chromatography, contained antibiotic 66-40 B (2), antibiotic 66-40 D (3), aminoglycoside 66-40 C (5), and garamine (4) The crude mixture of minor components (9.65 g) was chromatographed on a silica gel column (160 × 5 cm) [chloroform–methanol–7% ammonium hydroxide (1 : 2 : 1) as eluant] to give *aminoglycoside 66-40 C* (5), which after passage down Amberlite IRA 401S (OH^-) resin followed by lyophilization was obtained as an amorphous solid (1.8 g), m.p. 185—205° (decomp.) (Found: C, 53.2; H, 7.65; N, 13.1. $C_{38}H_{64}N_8O_{14}$ requires C, 53.3; H, 7.5; N, 13.1%), *m/e* 856 ($M^{+\cdot}$), 847 (osmometry) (calc. 856), $[\alpha]_D$ +112.5°, $\nu_{max.}$ 3 300, 1 670, 1 640, 1 620, and 1 025 cm^{-1}, $\lambda_{max.}$ (MeOH) 248 nm (ε 22 000), $\lambda_{max.}$ (MeOH–HCl) 281 nm ($\varepsilon \xrightarrow{\text{slow}} 0$), $[\theta]_{280}$ −7 720 (MeOH), $[\theta]_{290}$ −23 400 (TACu), $[\theta]_{290}$ −18 620 (Cupra A), δ_H 1.22 (6 H, s, 4″-CH_3), 2.52 (6 H, s, 3″-NCH_3), 5.15 (2 H, d, *J* 4 Hz, H-1″), 5.48 (2 H, m, H-4′), 5.50 (2 H, d, *J* 2 Hz, H-1′), and 7.56 (2 H, s, H-6′).

Conversion of 66-40 C (5) *into Garamine* (4).—Aminoglycoside 66-40 C (5) (200 mg) was dissolved in a mixture of distilled water (15 ml) and 2N-hydrogen chloride in methanol (25 ml) and the solution was stirred at 25 °C for 20 h. Sodium cyanoborohydride (800 mg) was added and the mixture was stirred at 25 °C for 80 h. The solution was passed over Amberlite IRA 45 resin and the aqueous methanolic eluate was evaporated to dryness; the residue was chromatographed on a silica gel column (110 × 2.5 cm) [chloroform–methanol–7% ammonium hydroxide (1 : 2 : 1)] to give garamine (4), which was obtained as an amorphous solid after passage over Amberlite IRA 401S (OH^-) resin and lyophilization; yield 71 mg, $[\alpha]_D$ +129.7°, i.r., n.m.r., and c.d. (TACu and Cupra A) data identical with those of authentic material.[4,5]

Tetrahydro-66-40 C (6).—(i) *Sodium borohydride.* Aminoglycoside 66-40 C (5) (200 mg) was dissolved in dry methanol (10 ml) and sodium borohydride (500 mg) was added in portions. The solution was stirred at 25 °C for 112 h. Excess of borohydride was destroyed with acetic acid and the solution was evaporated to dryness. The residue was chromatographed on a silica gel column (110 × 2.5 cm)

[chloroform–methanol–7% ammonium hydroxide (1 : 2 : 1)] to give *tetrahydro-66-40 C* (6) as an amorphous solid after passage down Amberlite IRA 401S (OH^-) resin and lyophilization; yield 100 mg, m.p. 195—205° (decomp.) (Found: C, 52.9; H, 8.1; N, 12.8. $C_{38}H_{68}N_8O_{14}$ requires C, 53.0; H, 7.9; N, 13.0%), *m/e* 860 ($M^{+\cdot}$), $[\alpha]_D$ +188.4°, $\nu_{max.}$ 3 300, 1 680, 1 060, and 1 025 cm^{-1}, $[\theta]_{290}$ −13 000 (TACu), $[\theta]_{290}$ −10 480 (Cupra A), δ_H *ca.* 1.13 (2 H, ddd, $J_{1,2a} = J_{2a,3} = J_{2a,2e}$ = 12.5 Hz, H-2a), 1.21 (6 H, s, 4″-CH_3), 2.52 (6 H, s, 3″-NCH_3), 2.57 (2 H, d, $J_{2'',3''}$ 10.5 Hz, H-3″), 3.31 (2 H, d, $J_{5a'',5e''}$ 12.5 Hz, H-5″a), 3.80 (2 H, dd, $J_{1'',2''}$ 4, $J_{2'',3''}$ 10.5 Hz, H-2″), 4.05 (2 H, d, $J_{5''a,5''e}$ 12.5 Hz, H-5″e), 4.82 (2 H, m, H-4′), 5.08 (2 H, d, $J_{1'',2''}$ 4 Hz, H-1″), and 5.24 (2 H, d, $J_{1',2'}$ 2 Hz, H-1′).

(ii) *Sodium cyanoborohydride at* pH 1. Aminoglycoside 66-40 C (5) (100 mg) was dissolved in a mixture of distilled water (6 ml) and 2N-hydrogen chloride in methanol (10 ml) containing sodium cyanoborohydride (200 mg), and the mixture was stirred at 25 °C for 100 h. After passage down Amberlite IRA 45 resin the solution was evaporated to dryness and chromatographed on a silica gel column (110 × 2.5 cm) [lower phase of chloroform–methanol–ammonium hydroxide (1 : 1 : 1)] to give tetrahydro-66-40 C (6) (53 mg), identical with that described in (i).

Catalytic Reduction of Aminoglycoside 66-40 C (5).—Aminoglycoside 66-40 C (5) (600 mg) was dissolved in methanol (30 ml) and hydrogenated over 20% palladium hydroxide on carbon (120 mg) at 55 lb in^{-2} and 25 °C for 4.5 h. Filtration, evaporation, and chromatography of the residue on a silica gel column (110 × 2.5 cm) [lower phase of chloroform–methanol–concentrated ammonium hydroxide (1 : 1 : 1)] gave (in order of elution) tetrahydro-66-40 C (6) (100 mg), unchanged 66-40 C (5) (150 mg), and garamine (4) (60 mg), all identical with authentic samples (t.l.c. and n.m.r.).

O-2-*Amino*-2,3,4-*trideoxy*-α-D-glycero-*hex*-4-*enopyranuronamidosyl*-(1 ⟶ 4)-*garamine* (10).—Aminoglycoside 66-40 C (5) (500 mg) was kept at 25 °C in a vial containing air for 2 years. The solid was then chromatographed on a silica gel column (160 × 2.5 cm) [lower phase of chloroform–methanol–concentrated ammonium hydroxide (2 : 1 : 1)] to give O-2-*amino*-2,3,4-*trideoxy*-α-D-glycero-*hex*-4-*enopyranuronamidosyl*-(1 ⟶ 4)-*garamine* (10) as an amorphous solid after passage over Amberlite IRA 401S (OH^-) resin followed by lyophilization; yield 26 mg (5%) (Found: $M^{+\cdot}$, 461.2497. $C_{19}H_{35}N_5O_8$ requires *M*, 461.2485), $[\alpha]_D$ +132.6°, $\nu_{max.}$ (KBr) 3 330, 1 680, 1 645, and 1 050 cm^{-1}, *m/e* 462 [$(M + 1)^+$], 461 ($M^{+\cdot}$), 331 (A_1), 313 (A_2), 303 (A_3), 285 (A_4), 350 (A_5), 332 (A_6), 322 (A_7), 304 (A_8), 191 (A_9), 173 (A_{10}), 163 (A_{11}), 145 (A_{10}), 141 (B_1), 160 (C_1), 434 (D_3), 285 (D_4), 362 (D_9), 203 (D_{10}), 386 (E_1), 260 (E_2), 344 (E_3), 270 (F_1), 289 (F_2), 130 (F_3), and 112 (F_4) (see ref. 15 for terminology), δ_H 1.19 (3 H, s, 4″-CH_3), 250 (3 H, s, 3″-NCH_3), 5.06 (1 H, m, H-4′), 5.08 (1 H, d, $J_{1'',2''}$ 4 H, H-1″), and 5.56 (1 H, d, $J_{1',2'}$ 2.5 Hz, H-1′). The more polar fractions from the column afforded 66-40 C (5) (350 mg, 70%) and garamine (4) (29 mg, 8%).

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