Carbohydrate Research, 109 (1982) 73–88
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

NOVEL SYNTHESIS OF 1- AND 3-*epi*-TOBRAMYCIN AND 1-*epi*-KANAMYCIN A*

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(Received January 7th, 1982; accepted for publication, May 24th, 1982)

ABSTRACT

Electrochemical reduction of 1-deamino-3,2′,6′,3″-tetra-*N*-formyl-1-hydroxyiminotobramycin (**4**) and 1-deamino-3,6′,3″-tri-*N*-formyl-1-hydroxyiminokanamycin A (**5**) (prepared in good yields by oxidation of the corresponding amines with hydrogen peroxide in the presence of a catalytic amount of sodium tungstate), by using a mercury cathode at −1.85 volt (*vs.* a saturated calomel electrode) gave principally 1-*epi*-tobramycin (**6**) and -kanamycin A (**7**) derivatives. Similar results were obtained by catalytic hydrogenation of **4** and **5** over Raney nickel. The electrochemical reduction or catalytic hydrogenation of 3-deamino-1,2′,6′,3″-tetra-*N*-formyl-3-hydroxyiminotobramycin (**17**) gave mainly the corresponding tobramycin derivative. Reduction of **4** and **17** with sodium cyanoborohydride gave principally the corresponding 1-*epi*-1- and 3-*epi*-3-hydroxyamino derivatives. Hydrogenation of these over Raney nickel gave the corresponding amines in quantitative yields. Hydrolysis of 1- and 3-*epi*-tobramycins with 48% hydrobromic acid under reflux gave (1L)-1,3,4/2,6-4,6-diaminocyclohexanetriol, $[\alpha]_D^{21.5}$ +39.4° and (1D)-1,3,4/2,6-4,6-diaminocyclohexanetriol, $[\alpha]_D^{24}$ −37.0°, respectively.

INTRODUCTION

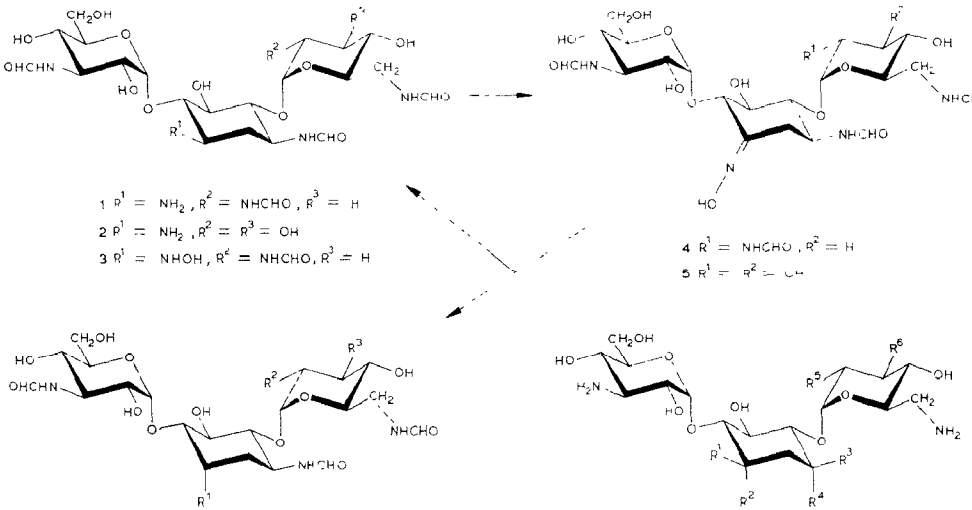
Mallams *et al.*[1] have reported the preparation of 1-*epi*-sisomicin and 1-*epi*-gentamicin C_1 by reductive amination of appropriately protected 1-deamino-1-oxo derivatives of sisomicin and gentamicin C_1. However, yields of the 1-*epi* derivatives were low and chromatographic purification of the products is tedious because of the formation of such by-products as the 1-deamino-1-hydroxy derivatives.

*Dedicated to Professor Sumio Umezawa on the occasion of his 73rd birthday and the 25th anniversary of the Microbial Chemistry Research Foundation.

We describe here the preparation of 1- and 3-*epi*-tobramycins and 1-*epi*-kanamycin A by electrochemical reduction or by reductions with sodium cyanoborohydride or Raney nickel of 1(or 3)-deamino-1(or 3)-hydroxyimino derivatives of tobramycin and kanamycin A.

RESULTS AND DISCUSSION

3,2′,6′,3″-Tetra-*N*-formyltobramycin[2] (**1**) was treated with 30% hydrogen peroxide in the presence of a catalytic amount of sodium tungstate[3] for 4 h at 20° to give 1-deamino-3,2′,6′,3″-tetra-*N*-formyl-1-hydroxyiminotobramycin (**4**) in 85% yield. Electrochemical reduction[4] of **4** by using a mercury cathode at −1.85 V (*vs.* a saturated calomel electrode) in the presence of M lithium chloride and M hydrochloric acid, and chromatography of the product on Amberlite CG-50 (NH_4^+) resin, gave two ninhydrin-positive products (**1** and **6**) in 1.9 and 49.8% yields, respectively. The minor compound was identical with authentic **1** by comparative rotations, i.r. and ^{1}H-n.m.r. spectra, and t.l.c. Deformylation of **6** with hydrochloric acid in aqueous methanol for 24 h at 35°, and chromatography of the product on Amberlite CG-50 (NH_4^+) resin gave compound **9** in 72% yield. The ^{1}H-n.m.r. spectrum of the sulfate of **9** revealed two doublets centered at δ 5.72 and 6.47 (H-1″ and H-1′). The ^{13}C-n.m.r. chemical shifts (Table I) of C-1, C-2, C-3, C-5, C-6, and C-1″ in the sulfate of **9** occur at higher field than those in tobramycin sulfate and these shift differences are similar



1 R^1 = NH_2, R^2 = NHCHO, R^3 = H
2 R^1 = NH_2, R^2 = R^3 = OH
3 R^1 = NHOH, R^2 = NHCHO, R^3 = H
4 R^1 = NHCHO, R^2 = H
5 R^1 = R^2 = OH
6 R^1 = NH_2, R^2 = NHCHO, R^3 = H
7 R^1 = NH_2, R^2 = R^3 = OH
8 R^1 = NHOH, R^2 = NHCHO, R^3 = H
9 R^1 = R^4 = R^6 = H, R^2 = R^3 = R^5 = NH_2
10 R^1 = R^4 = H, R^2 = R^3 = NH_2, R^5 = R^6 = OH
11 R^1 = NHOH, R^2 = R^4 = R^5 = H, R^3 = R^5 = NH_2
12 R^1 = R^4 = R^6 = H, R^2 = NHOH, R^3 = R^5 = NH_2
13 R^1 = R^5 = NH_2, R^2 = R^4 = R^6 = H, R^3 = NHOH
14 R^1 = R^5 = NH_2, R^2 = R^3 = R^6 = H, R^4 = NHOH

TABLE I

^{13}C-N.M.R. CHEMICAL SHIFTS

	Sisomicin	1-epi-*Sisomicin*	*Tobramycin (Sulfate)*	**9** *(Sulfate)*	*Kanamycin A (Sulfate)*	**10** *(Sulfate)*	**11** *(Sulfate)*	**12** *(Sulfate)*	**13** *(Sulfate)*	**14** *(Sulfate)*	**20** *(Sulfate)*	*2-Deoxystreptamine (Sulfate)*	**21** *(Sulfate)*	*Nebramine (Sulfate)*	**25** *(Sulfate)*
C-1	51.8	47.4	50.6	47.5[a]	50.7	47.5	59.5	56.5	50.6	50.5	50.0	51.0	49.6	50.7	48.7
C-2	36.4	34.1	28.6	27.5	28.4	27.2	25.1	25.7	25.5	25.9	27.9	29.0	27.5	29.0	26.3
C-3	50.4	47.7	48.7	47.9[a]	48.7	47.5	49.4	49.1	58.0	58.7	49.3	51.0	50.5	48.8	48.7
C-4	85.3	85.3	77.7	77.6[b]	78.4	78.2	77.4	77.5[c]	75.3	74.4	75.2	73.2	73.0[d]	77.6	75.4
C-5	75.4	72.7	75.1	72.1	73.7	70.8	75.0	73.4	73.5	73.5	72.4	75.6	73.3[d]	76.1	71.6
C-6	87.8	78.9	84.5	77.3[b]	84.6	78.2	80.9	77.8[c]	84.5	84.7	84.6	73.2	70.2	73.4	67.7
C-1′	100.6	100.7	94.3	94.2	96.9	97.2	94.3	94.2	94.3	95.9	95.9			94.4	93.8
C-2′	47.6	47.7	49.4	48.7	71.9	71.9	48.7	48.8	48.7	48.8	48.9			49.5	48.7
C-3′	25.6	25.3	30.2	30.2	73.7	73.3	30.1	30.2	30.3	30.4	30.5			30.2	30.5
C-4′	96.5	97.8	65.9	65.9	71.7	71.8	65.9	65.8	65.8	66.0	66.0			65.9	65.9
C-5′	150.4	150.2	71.1	70.6	69.6	69.6	71.1	71.0	70.9	70.3	70.3			70.8	70.6
C-6′	43.5	43.1	41.0	41.1	41.4	41.3	41.0	41.1	41.1	41.1	41.2			41.1	41.1
C-1″	101.5	95.8	101.4	97.5	101.3	97.8	101.4	98.0	101.4	101.2	101.3				
C-2″	70.0	69.6	69.0	68.4	69.1	68.4	69.1	68.4	69.0	69.0	69.1				
C-3″	64.3	64.0	55.8	55.7	55.8	55.7	55.7	55.7	55.8	55.8	55.8				
C-4″	73.0	73.1	66.3	66.4	66.3	66.3	66.3	66.4	66.3	66.3	66.4				
C-5″	68.5	68.2	73.6	73.3	72.8	72.7	73.5	73.4	73.5	73.5	73.5				
C-6″			60.8	60.7	60.8	60.6	60.7	60.7	60.8	60.7	60.8				
3″-NCH_3	37.9	37.7													
4″-CH_3	22.9	22.4													

[a,b,c,d]These values may be reversed.

TABLE II

^{13}C-N.M.R. CHEMICAL SHIFTS OF CYCLOHEXYLAMINE HYDROCHLORIDE[6] AND *N*-CYCLOHEXYLHYDROXYLAMINE SULFATE

	Cyclohexylamine (hydrochloride)	N-*Cyclohexylhydroxylamine (sulfate)*	Δ *(Cyclohexylamine − N-cyclohexylhydroxylamine)*
C-1	50.2	61.1	10.9
C-2	30.3	27.2	−3.1
C-3	23.5	24.3	0.8
C-4	23.9	25.4	1.5
C-5	23.5	24.3	0.8
C-6	30.3	27.2	−3.1

to those between sisomicin and 1-*epi*-sisomicin[1]. From these results, the structure of **9** was established as 1-*epi*-tobramycin.

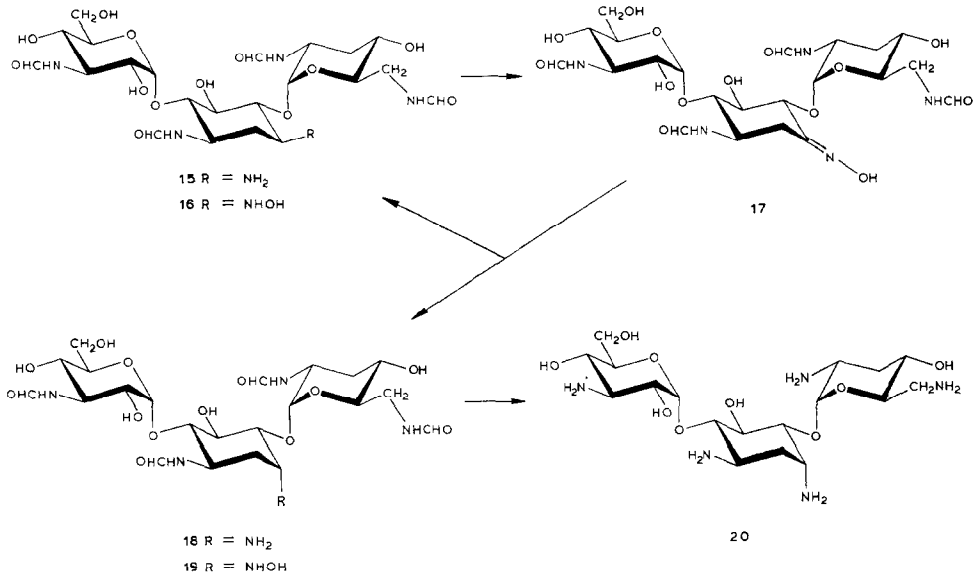
Catalytic hydrogenation of **4** over Raney nickel in aqueous ethanol at a pressure of 5 kg/cm^2 for 9 h at 41 °, and chromatography of the product, gave **1** and **6** in 12.6 and 34.8% yields, respectively.

As the yield of **6** by the aforementioned methods was not satisfactory, an alternative method was developed. Reduction of **4** with 1.5 molar equivalents of sodium cyanoborohydride in acetic acid for 2 h at room temperature and chromatography of the product on Amberlite CG-50 (H^+) resin under nitrogen, gave two products (**3** and **8**) in 21 and 57.7% yields, respectively. Compounds **3** and **8** were relatively stable in acidic media, but were unstable in neutral medium and oxidized readily with air to the hydroxyimino derivative **4**. Deformylation of **3** and **8** with hydrochloric acid in aqueous methanol, and treatment of the products with Amberlite IRA-400 ($CH_3CO_2^-$) resin followed by 0.05M sulfuric acid, gave the sulfates of compounds **11** and **12** in 85 and 86% yields, respectively. The ^{1}H-n.m.r. spectra of both **11** and **12** showed two doublets centered at δ 5.73 and 6.40 in **11**, and 5.77 and 6.43 in **12** (H-1″ and H-1′), respectively. The ^{13}C-n.m.r. data for **11** and **12** are shown in Table I. The C-1 resonances appeared at 59.5 p.p.m. in **11** and 56.5 p.p.m. in **12**, and the C-2 and C-6 resonances in both compounds appeared at higher field than those in the sulfates of tobramycin and **9**. These observations were compatible with those of cyclohexylamine hydrochloride[6] and *N*-cyclohexylhydroxylamine sulfate, as shown in Table II. Furthermore, the differences of the chemical shifts of C-1, C-3, C-4, C-5, C-6, and C-1″ (but not C-2), in **11** and **12** were similar to those in the sulfates of tobramycin and **9**. Catalytic hydrogenation of **3** and **8** over Raney nickel in aqueous ethanol gave **1** and **6** in quantitative yields, respectively. From these results, the structures of **11** and **12** were assigned as 1-*N*-hydroxytobramycin and 1-*epi*-1-*N*-hydroxytobramycin, respectively.

By analogy with the case of **4**, electrochemical reduction of 1-deamino-3,6′,3″-tri-*N*-formyl-1-hydroxyiminokanamycin A (**5**), which was prepared from 3,6′,3″-tri-

N-formylkanamycin A[5] (**2**) in 75.8% yield, gave two products (**2** and **7**) in 11 and 44.5% yields, respectively. The minor compound was identical with an authentic sample of **2** by comparative specific rotations, i.r. and ^{1}H-n.m.r. spectra, and t.l.c. Deformylation of **7** gave compound **10** in 71.7% yield. The ^{1}H-n.m.r. spectrum of the sulfate of **10** revealed two doublets centered at δ 5.73 and 6.27 (H-1″ and H-1′), respectively. The differences of ^{13}C chemical shifts of C-1, C-2, C-3, C-5, C-6, and C-1″ in the sulfates of kanamycin A and **10** (Table I) were similar to those in the sulfates of tobramycin and **9**. From these results, compound **10** appeared to be 1-*epi*-kanamycin A.

Treatment of 1,2′,6′,3″-tetra-*N*-formyltobramycin (**15**), obtained by partial acid hydrolysis of penta-*N*-formyltobramycin with hydrochloric acid in aqueous methanol[2], with 30% hydrogen peroxide in the presence of a catalytic amount of sodium tungstate and an equimolar amount of sodium acetate for 4 h at 20°, and chromatography of the product on silica gel, gave 3-deamino-1,2′,6′,3″-tetra-*N*-formyl-3-hydroxyiminotobramycin (**17**) in 76.2% yield. Electrochemical reduction of **17** under conditions similar to those for **4** gave two products (**15** and **18**) in 36 and 4.5% yields, respectively. The major compound was identical with authentic **15** by comparative specific rotations, i.r. and ^{1}H-n.m.r. spectra, and t.l.c. Deformylation of **18** with hydrochloric acid in aqueous methanol and chromatography of the product gave compound **20** in 89.9% yield. The ^{1}H-n.m.r. spectrum of the sulfate of **20** revealed two doublets centered at δ 5.73 and 6.19 (H-1″ and H-1′), respectively. The

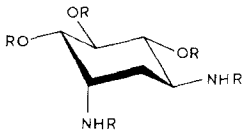


^{13}C-n.m.r. data of the sulfate of **20** revealed upfield shifts of 0.6, 0.7, 2.5, and 2.7 p.p.m. for C-1, C-2, C-4, and C-5, respectively, and downfield shifts of 0.6 and 1.6 p.p.m. for C-3 and C-1′, respectively, as compared with those of tobramycin (Table I).

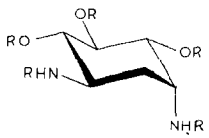
Boxler *et al.*[1b] reported that epimerization of the 3-amino group in going from gentamicin C_{1a} to 3-*epi*-gentamicin C_{1a} resulted in shielding at C-1, C-2, C-3, C-4, and C-5, with slight deshielding at C-6. The ^{13}C-n.m.r. data of the sulfate of **20** are compatible with those of 3-*epi*-gentamicin C_{1a}, and the structure of **20** thus appears to be 3-*epi*-tobramycin.

Catalytic hydrogenation of **17** under conditions similar to those used for **4** gave **15** and **18** in 22.6 and 1.7% yields, respectively. Furthermore, reduction of **17** with sodium cyanoborohydride in acetic acid and chromatography of the product on Amberlite CG-50 (H^+) resin gave two products (**16** and **19**) in 15.2 and 66.4% yields, respectively. Compounds **16** and **19** were also unstable in neutral medium and oxidized in air to the hydroxyimino derivative **17**. Deformylation of **16** and **19** under conditions similar to those used for **3** and **8** gave the sulfates of compounds **13** and **14** in 99.2 and 88.5% yields, respectively. The ^{1}H-n.m.r. spectra of both **13** and **14** showed two doublets centered at δ 5.72 and 6.43 in **13** and 5.70 and 6.22 in **14** (H-1″ and H-1′), respectively. As shown in Table I, the C-3 resonances appeared at 58.0 p.p.m. in **13** and 58.7 p.p.m. in **14**, and the differences of the chemical shifts of C-1, C-3, C-4, C-6, and C-1′ (but not of C-2 and C-5) in **13** and **14** were similar to those in the sulfates of tobramycin and **20**. Catalytic hydrogenation of **16** and **19** over Raney nickel under conditions similar to those used for **3** and **8** gave **15** and **18**, respectively, in quantitative yields. From these results, the structures of **13** and **14** were assigned as 3-*N*-hydroxytobramycin and 3-*epi*-3-*N*-hydroxytobramycin, respectively.

Hydrolysis of **9** and **20** for 20 h with boiling 48% hydrobromic acid, and chromatography of the product on Amberlite CG-50 (NH_4^+) resin, gave (1L)-1,3,4/2,6-4,6-diaminocyclohexanetriol (**21**) and (1D)-1,3,4/2,6-4,6-diaminocyclohexanetriol (**23**) in 85.4 and 86.4% yields, respectively. Compounds **21** and **23** had identical elemental compositions and melting points, and their i.r. and ^{1}H-n.m.r. spectra were superposable. The optical rotations of **21**, $[\alpha]_D^{21.5}$ +39.4° and **23**, $[\alpha]_D^{24}$ −37.0°, were equal in magnitude but opposite in sign. These physicochemical properties clearly indicate that **21** and **23** are enantiomers. As shown in Table I, the ^{13}C-n.m.r. spectrum of the sulfate of **21** showed six, separated carbon-signals, whereas that of 2-deoxystreptamine sulfate showed only four.



21 R = H
22 R = Ac



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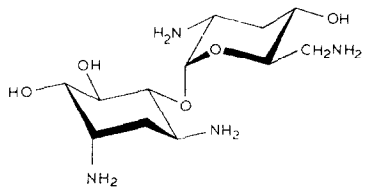
24 R = Ac



25

When **9** was boiled for 4 h with 6M hydrochloric acid and the product chromatographed on Amberlite CG-50 (NH_4^+) resin, the hydrolyzed product **25** was obtained in 57.2% yield. The ^{1}H-n.m.r. spectrum of the sulfate of **25** showed a doublet centered

TABLE III

ANTIMICROBACTERIAL ACTIVITY OF TOBRAMYCIN, 1-*epi*- (**9**), AND 3-*epi*-TOBRAMYCINS (**20**)

Organism	*Minimum inhibitory concentration (μg/mL)*		
	9	**20**	*Tobramycin*
Escherichia coli W 677/JR 88	3.13	>100	1.56
Escherichia coli W 677/JR 225	25	>100	100
Staphylococcus aureus APO 1	0.78	>100	0.2
Staphylococcus aureus FDA 209P JC-1	0.39	6.25	0.2
Klebsiella pneumoniae ATCC 27736	1.56	50	0.78
Klebsiella pneumoniae K1-184	50	>100	100
Pseudomonas aeruginosa ATCC 9721	0.78	25	0.39
Pseudomonas aeruginosa PP-6	25	>100	>100
Serratia marcescens ATCC 13880	6.25	>100	3.13
Enterobacter cloacae C1-126	50	>100	50
Proteus rettgeri Ret-29	0.78	25	0.39
Proteus inconstans In 23	1.56	25	0.78
Proteus morganii Morg-74	0.78	25	0.78
Proteus vulgaris ATCC 6390	1.56	25	0.39
Proteus mirabilis Pm-112	6.25	>100	12.5
Shigella sonnei ATCC 11060	3.13	50	0.78
Salmonella typhimurium ATCC 13311	1.56	50	0.39
Staphylococcus epidermidis ATCC 14990	0.2	1.56	0.1

TABLE IV

ANTIMICROBACTERIAL ACTIVITY OF KANAMYCIN A AND 1-*epi*-KANAMYCIN A (**10**)

Organism	*Minimum inhibitory concentration (μg/mL)*	
	10	*Kanamycin* A
Escherichia coli W 677/JR 88	>100	6.25
Escherichia coli W 677/JR 225	50	6.25
Staphylococcus aureus APO 1	>100	25
Staphylococcus aureus FDA 209P JC-1	6.25	1.56
Klebsiella pneumoniae ATCC 27736	50	3.13
Pseudomonas aeruginosa Ps-24	12.5	50
Pseudomonas aeruginosa ATCC 9721	12.5	25
Pseudomonas aeruginosa PI-67	12.5	100
Serratia marcescens ATCC 13880	100	6.25
Enterobacter cloacae Cl-126	>100	100
Proteus rettgeri Ret-29	6.25	0.78
Proteus inconstans In-47	50	100
Proteus morganii Morg-102	50	100
Proteus vulgaris ATCC-6390	12.5	1.56
Proteus mirabilis Pm-71	12.5	3.13
Shigella sonnei ATCC 11060	50	3.13
Salmonella typhimurium ATCC 13311	100	6.25
Staphylococcus epidermidis ATCC 14990	1.56	0.39

at δ 6.18 (H-1′). The ^{13}C-n.m.r. spectrum of the sulfate of **25** showed twelve carbon signals (Table I). From these results, the structure of **25** was assigned as 1-*epi*-nebramine.

The antibacterial activities of **9**, **10**, **11**, **12**, **13**, **14**, and **20** were tested against several microorganisms. The activities of **9**, **10**, and **20** are shown in Tables III and IV. Compound **9** showed a slightly lower activity against most of the bacteria tested, as compared with that of tobramycin. Compound **20** showed marked decrease of activity against most of the bacteria tested. Compound **10** showed lower activity than that of kanamycin A against most of the bacteria tested, except for *Pseudomonas aeruginosa* Ps-24, ATCC 9721, and PI-67. Compounds **11**, **12**, **13**, and **14** were almost devoid of activity.

EXPERIMENTAL

General methods. — Melting points were measured with a Monoscope (H. Boch, Frankfurt, Germany) and were uncorrected. Evaporations were performed under diminished pressure, ^{1}H-N.m.r. spectra were recorded with a Varian T-60 n.m.r. spectrometer with tetramethylsilane as the external standard. ^{13}C-N.m.r. spectra were recorded in deuterium oxide with a Varian NV-14 FT n.m.r. spectrometer and 1,4-dioxane as an internal standard. Chemical shifts are reported in p.p.m. downfield from tetramethylsilane. Optical rotations were measured in water with a Perkin–Elmer Model 141 polarimeter, unless otherwise stated. T.l.c. was performed with precoated silica gel plates (E. Merck, AG, Darmstadt, Germany). Sulfates were prepared, unless otherwise stated, as follows: One part of the free base was dissolved in 10 parts of water and the solution was adjusted to pH 4.5 by addition of 0.05M sulfuric acid. The solution was concentrated to one-tenth volume and ethanol was added. The resulting precipitate was collected by filtration, washed with ethanol, and dissolved in a small amount of water. The solution was treated with active carbon and lyophilized. The lyophilizate was kept in a desiccator containing 200 g of sodium bromide and 100 mL of water until the weight became constant by absorption of moisture. For the preparation of 1- and 3-*N*-hydroxytobramycins, distilled water was boiled before use and cooled to room temperature in an atmosphere of nitrogen.

1-Deamino-3,2′,6′,3″-tetra-N-*formyl-1-hydroxyiminotobramycin* (**4**). — To a solution of 600 mg of **1** (ref. 2) and 10 mg of sodium tungstate in 2 mL of water was added dropwise 0.2 mL of 30% hydrogen peroxide with stirring at 20°, and stirring was continued for 4 h. To the solution was added 2.5 mL of methanol and the solution was kept for 30 min at 20°. Water (15 mL) was added, and the solution was passed through a column (15 mL) of Amberlite MB-3 resin. The resin was washed with 100 mL of water. The combined eluate and washings were evaporated. The residue was dissolved in 20 mL of water, treated with active carbon, and lyophilized to give 511 mg (85%) of **4** as a colorless foam, $[\alpha]_D^{22}$ +190.2 ±2.1° (*c* 1.078); δ_H 5.57 (d, 1 H, $J_{1',2'}$ or $J_{1'',2''}$ 4 Hz, H-1′ or H-1″) and 5.67 (d, 1 H, $J_{1'',2''}$ or $J_{1',2'}$ 3 Hz, H-1″ or H-1′).

Anal. Calc. for $C_{22}H_{35}N_5O_{14} \cdot 0.5\ H_2O$: C, 43.85, H, 6.02; N, 11.62. Found: C, 44.00; H, 6.15; N, 11.88.

*1-Deamino-3,6',3''-tri-*N*-formyl-1-hydroxyiminokanamycin A* (**5**). — To a solution of 1.0 g of **2** (ref. 5) and 17 mg of sodium tungstate in 3.4 mL of water was added 0.34 mL of 30% hydrogen peroxide with stirring, and the solution was stirred for 4 h at 20°. To the solution was further added 0.5 mL of 30% hydrogen peroxide and the solution was refrigerated overnight. Methanol (5 mL) was added, the solution was kept for 30 min at room temperature, and then passed through a column (20 mL) of Amberlite MB-3 resin. The resin was washed with 100 mL of water. The combined eluate and washings were evaporated, the residue was dissolved in 12 mL of hot methanol, and 150 mL of ethyl acetate was added. The precipitate was filtered off and washed with ethyl acetate. The precipitate was dissolved in a small amount of water, treated with active charcoal, and lyophilized to give 822 mg (75.8%) of **5** as a colorless foam. $[\alpha]_D^{22}$ +155.9 ±1.9° (*c* 1.02); δ_H 5.55 (d, 1 H, $J_{1',2'}$ or $J_{1'',2''}$ 3 Hz, H-1' or H-1'') and 5.82 (d, 1 H, $J_{1'',2''}$ or $J_{1',2'}$ 3 Hz, H-1'' or H-1').

Anal. Calc. for $C_{21}H_{34}N_4O_{15} \cdot 3\ H_2O$: C, 39.62; H, 6.33; N, 8.80. Found: C, 39.41; H, 6.04; N, 8.65.

*3-Deamino-1,2',6',3''-tetra-*N*-formyl-3-hydroxyiminotobramycin* (**17**). — To a solution of 2.0 g of **15** {$[\alpha]_D^{22}$ +130.0 ±1.7° (*c* 1)}, 66 mg of sodium tungstate, and 274.5 mg of sodium acetate in 7 mL of water was added 3.6 mL of 30% hydrogen peroxide dropwise with stirring. The solution was stirred for 4 h at 20°. To the solution was added successively 15 mL of methanol and 500 mL of ethyl acetate. The resulting precipitate was filtered off and washed with ethyl acetate. The precipitate was dissolved in water and evaporated. The residue was dissolved in a mixture of 5 mL of acetonitrile, 1.5 mL of methanol, and 3.5 mL of water, adsorbed onto a column of 400 g of silica gel, and eluted with 10:1:1 (v/v) acetonitrile–methanol–water, collecting 20-mL fractions. From fractions 231–370, 1.457 g (71.2%) of pure **17** was obtained as a colorless foam. From fractions 180–230 and 371–500, 236 mg of crude **17** was obtained. Rechromatography of the crude product gave 105 mg (5%) of pure **17**, (total 1.562 g, 76.2%), $[\alpha]_D^{23}$ +98.2 ±1.4° (*c* 1); δ_H 5.57 (d, 1 H, $J_{1',2'}$ or $J_{1'',2''}$ 4 Hz, H-1' or H-1'') and 5.67 (d, 1 H, $J_{1'',2''}$ or $J_{1',2'}$ 4 Hz, H-1'' or H-1'').

Anal. Calc. for $C_{22}H_{35}N_5O_{14} \cdot H_2O$: C, 43.21; H, 6.10; N, 11.45. Found: C, 43.21; H, 6.16; N, 11.36.

Electrochemical reduction of **4**. — A solution of 2.47 g of **4** in 50 mL of M lithium chloride, adjusted to pH 6.4 by the addition of M hydrochloric acid, was electrochemically reduced at a stirring-mercury cathode supplying a constant voltage of −1.85 V (*vs.* a saturated calomel electrode). As the solution became basic with the progress of the reduction, the pH of the solution was adjusted to 6.4 by addition of the appropriate amount of M hydrochloric acid at intervals of 15 min. The reaction was monitored by polarography of pipetted samples. The reduction was complete in 4 h. The mixture was evaporated, methanol (10 mL) was added, the mixture was filtered, and the filtrate was evaporated. The residue was dissolved in 7 mL of methanol and 250 mL of acetone was added. The resulting precipitate was filtered off

and washed with acetone. This procedure was repeated again. The precipitate was dissolved in 15 mL of water and adsorbed onto a column of 0.4 L of Amberlite CG-50 (NH_4^+) resin, which was eluted with water, collecting 5-mL fractions. Fractions 34–40 gave 1.153 g of crude **6**, and fractions 41–47 gave 917 mg (39.7%) of pure **6**. Rechromatography of the crude product gave 130 mg (5.6%) of pure **6** as a colorless foam, $[\alpha]_D^{22}$ +141.0 ±1.8° (*c* 1); δ_H 5.58 (d, 1 H, $J_{1',2'}$ or $J_{1'',2''}$ 3.5 Hz, H-1′ or H-1″) and 5.75 (d, 1 H, $J_{1'',2''}$ or $J_{1',2'}$ 4 Hz, H-1″ or H-1′).

Anal. Calc. for $C_{22}H_{37}N_5O_{13}$: C, 45.59; H, 6.44; N, 12.09. Found: C, 45.30; H, 6.35; N, 12.20.

Fractions 48–58 gave 172 mg of a mixture of **6** and **1**. Rechromatography of the mixture gave 105 mg (4.5%) of pure **6** (total 1.152 g, 49.8%) and 45 mg (1.9%) of pure **1**, $[\alpha]_D^{22}$ +126.1 ±1.5° (*c* 1); δ_H 5.60 (d, 1 H, $J_{1',2'}$ or $J_{1'',2''}$ 4 Hz, H-1′ or H-1″) and 5.72 (d, 1 H, $J_{1'',2''}$ or $J_{1',2'}$ 4 Hz, H-1″ or H-1′).

Anal. Calc. for $C_{22}H_{37}N_5O_{13} \cdot 0.5\ H_2O$: C, 44.89; H, 6.51; N, 11.90. Found: C, 45.08; H, 6.62; N, 11.94.

The product was identical with authentic 3,2′,6′,3″-tetra-*N*-formyltobramycin by comparative t.l.c., and i.r. and ^{1}H-n.m.r. spectra.

Electrochemical reduction of **5**. — A solution (pH 6.90) of 700 mg of **5** in 50 mL of M lithium chloride containing M hydrochloric acid, was electrochemically reduced with a stirring mercury cathode supplying a constant voltage of −1.85 V (*vs.* a saturated calomel electrode) for 4.5 h and treated as already described to give 287 mg (44.5%) of **7** and 78 mg (11%) of **2**. Compound **7** had $[\alpha]_D^{22}$ +134.1 ±4.9° (*c* 0.35); δ_H 5.62 (d, 1 H, $J_{1',2'}$ or $J_{1'',2''}$ 4 Hz, H-1′ or H-1″) and 5.90 (d, 1 H, $J_{1'',2''}$ or $J_{1',2'}$ 4 Hz, H-1″ or H-1′).

Anal. Calc. for $C_{21}H_{36}N_4O_{14} \cdot 2.5\ H_2O$: C, 41.11; H, 6.74; N, 9.13. Found: C, 41.17; H, 6.48; N, 9.15.

Compound **2** had $[\alpha]_D^{22.5}$ +118.0 ±1.5° (*c* 1.04); δ_H 5.65 (d, 1 H, $J_{1',2'}$ or $J_{1'',2''}$ 4 Hz, H-1′ or H-1″) and 5.83 (d, 1 H, $J_{1'',2''}$ or $J_{1',2'}$ 4 Hz, H-1″ or H-1′).

Anal. Calc. for $C_{21}H_{36}N_4O_{14} \cdot 2.5\ H_2O$: C, 41.11; H, 6.74; N, 9.13. Found: C, 40.81; H, 6.43; N, 9.06.

This product was identical with authentic **2** by comparative t.l.c., and i.r. and ^{1}H-n.m.r. spectra.

Electrochemical reduction of **17**. — A solution (pH 6.45) of 450 mg of **17** in 50 mL of M lithium chloride containing M hydrochloric acid was electrochemically reduced with a stirring mercury cathode supplying a constant voltage of −1.85 V (*vs.* a saturated calomel electrode) for 9 h and treated as already described to give 20 mg (4.5%) of **18** and 158.5 mg (36%) of **15**. Compound **15** had $[\alpha]_D^{22.5}$ +130.1 ±1.7° (*c* 1.03); δ_H 6.67 (d, 2 H, $J_{1',2'}$ and $J_{1'',2''}$ 3 Hz, H-1′ and H-1″).

Anal. Calc. for $C_{22}H_{37}N_5O_{13} \cdot 1.5\ H_2O$: C, 43.56; H, 6.65; N, 11.55. Found: C, 43.84; H, 6.91; N, 11.45.

This compound was identical with authentic **15** by comparative t.l.c., and i.r. and ^{1}H-n.m.r. spectra.

Compound **18** had $[\alpha]_D^{22}$ +133.2 ±1.7° (*c* 1.03); δ_H 5.58 (d, 1 H, $J_{1',2'}$ or $J_{1'',2''}$ 3 Hz, H-1′ or H-1″) and 5.65 (d, 1 H, $J_{1'',2''}$ or $J_{1',2'}$ 3 Hz, H-1″ or H-1′).

Anal. Calc. for $C_{22}H_{37}N_5O_{13} \cdot H_2O$: C, 44.21; H, 6.58; N, 11.72. Found: C, 44.08; H, 6.82; N, 11.39.

1-epi-*Tobramycin* (**9**). — A solution of 800 mg of **6** in a mixture of 0.5 mL of water, 11.8 mL of methanol, and 2.4 mL of concentrated hydrochloric acid was stirred for 24 h at 36°. The solution was made neutral with Amberlite IR-45 (OH^-) resin and the resin was filtered off and washed with water. The combined filtrate and washings were evaporated. The residue was dissolved in 20 mL of water and adsorbed onto a column of 200 mL of Amberlite CG-50 (NH_4^+) resin which was gradient-eluted with 1 L of water and 1 L of M ammonium hydroxide. Each fraction was 12 mL. Fractions 89–113 gave 534 mg of crude **9**. Rechromatography of the product on a column of 100 mL of Amberlite CG-50 (NH_4^+) resin gave 510 mg of pure **9** as a colorless foam. The sulfate of **9** had $[\alpha]_D^{22.5}$ +116.6 ±1.5° (*c* 1); δ_H 5.72 (d, 1 H, $J_{1'',2''}$ 4 Hz, H-1″) and 6.47 (d, 1 H, $J_{1',2'}$ 4 Hz, H-1′).

Anal. Calc. for $C_{18}H_{37}N_5O_9 \cdot 2.5\ H_2SO_4 \cdot 7\ H_2O$: C, 25.77; H, 6.42; N, 8.35; S, 9.56. Found: C, 25.47; H, 6.42; N, 8.20; S, 9.67.

1-epi-*Kanamycin A* (**10**). — Compound **7** (256 mg) was hydrolyzed with a mixture of water, methanol, and concentrated hydrochloric acid and the product was treated as already described to give 186 mg of **10** as a colorless foam. The sulfate of **10** had $[\alpha]_D^{22.5}$ +116.6 ±1.5° (*c* 1); δ_H 5.73 (d, 1 H, $J_{1'',2''}$ 3 Hz, H-1″) and 6.27 (d, 1 H, $J_{1',2'}$ 3 Hz, H-1′).

Anal. Calc. for $C_{18}H_{36}N_4O_{11} \cdot 2\ H_2SO_4 \cdot 6\ H_2O$: C, 27.41; H, 6.65; N, 7.10; S, 8.13. Found: C, 27.41; H, 6.58; N, 6.92; S, 8.34.

3-epi-*Tobramycin* (**20**). — Compound **18** (250 mg) was deformylated with a mixture of water, methanol, and concentrated hydrochloric acid as already described to give 205 mg of **20** as a colorless foam. The sulfate of **20** had $[\alpha]_D^{23}$ +69.8 ±1.0° (*c* 1); δ_H 5.73 (d, 1 H, $J_{1'',2''}$ 3 Hz, H-1″) and 6.19 (d, 1 H, $J_{1',2'}$ 3 Hz, H-1′).

Anal. Calc. for $C_{18}H_{37}N_5O_9 \cdot 2.5\ H_2SO_4 \cdot 7\ H_2O$: C, 25.77; H, 6.73; N, 8.35; S, 9.56. Found: C, 25.57; H, 6.43; N, 8.13; S, 9.33.

(*1*L)-*1,3,4/2,6-4,6-Diamino-1,2,3-cyclohexanetriol* (**21**) *and its pentaacetate* (**22**). — A solution of 250 mg of **9** in 4.5 mL of 48% hydrobromic acid was heated under reflux for 20 h at 155°. The solution was evaporated and the excess of hydrobromic acid was removed by distillation of water 3 times from the residue. To the residue was added 20 mL of hot water and 1 g of Darco G-60. The mixture was filtered through a Millipore filter and the filtrate was evaporated. The residue was dissolved in 15 mL of water, adsorbed onto a column of 50 mL of Amberlite CG-50 (NH_4^+ form) resin, and the column was gradient-eluted (7-mL fractions) with 0.5 L of water and 0.5 L of 0.5M ammonium hydroxide. Fractions 57–72 gave 82 mg of the product, which was dissolved in 8 mL of water and the pH of the solution adjusted to 4.6 with 6.5 mL of 0.05M sulfuric acid. The solution was evaporated and the residue crystallized from aqueous methanol to give 88 mg (85.4%) of the sulfate of **21** as fine needles, m.p. >320° (dec.), $[\alpha]_D^{21.5}$ +39.4 ±1.0° (*c* 0.8).

Anal. Calc. for $C_6H_{14}N_2O_3 \cdot H_2SO_4$: C, 27.69; H, 6.20; N, 10.76; S, 12.32. Found: C, 27.39; H, 6.21; N, 10.65; S, 12.18.

A solution of the sulfate of **21** (48 mg) in 10 mL of water was passed through a column of 5 mL of Amberlite IRA-400 (OH^-) resin, and the column was washed with 50 mL of water. The combined eluate and washings were evaporated and the residue was crystallized from aqueous ethanol to give 28 mg of the free base **21** as colorless needles, m.p. 178 (darkened)–186° (dec.), $[\alpha]_D^{22}$ +53.2 ±0.9° (*c* 1).

Anal. Calc. for $C_6H_{14}N_2O_3 \cdot H_2O$: C, 39.99; H, 8.95; N, 15.55. Found: C, 40.02; H, 9.03; N, 15.59.

Compound **21** (25 mg) was dissolved in 2 mL of methanol and 0.095 mL of acetic anhydride. The solution was kept for 24 h at room temperature and then evaporated. The residue was dissolved in 1 mL of pyridine and 0.14 mL of acetic anhydride and the solution was kept for 24 h at room temperature, and the residue crystallized from ethanol–ether to give 45 mg (73.2%) of **22** as colorless needles, m.p. 164–167°, $[\alpha]_D^{22}$ +10.8 ±0.5° (*c* 1, ethanol).

Anal. Calc. for $C_{16}H_{24}N_2O_8 \cdot 0.5\ H_2O$: C, 50.38; H, 6.61; N, 7.35. Found: C, 50.07; H, 6.37; N, 7.27.

(1D)-1,3,4/2,6-4,6-Diamino-1,2,3-cyclohexanetriol (**23**) *and its pentaacetate* (**24**). — Compound **20** (266 mg) was hydrolyzed with 48% hydrobromic acid as already described. The product crystallized from aqueous methanol to give 89 mg (86.4%) of **23** sulfate as fine needles, m.p. >320° (dec.), $[\alpha]_D^{24}$ −37.0 ±0.7° (*c* 1.06).

Anal. Calc. for $C_6H_{14}N_2O_3 \cdot H_2SO_4$: C, 27.69; H, 6.20; N, 10.76; S, 12.32. Found: C, 27.52; H, 6.20; N, 10.76; S, 12.28.

The free base **23** (31.6 mg) was obtained from the sulfate (50 mg) as already described, and crystallized from aqueous ethanol as colorless needles, m.p. 178 (darkened)–186° (dec.), $[\alpha]_D^{24.5}$ −52.3 ±0.9° (*c* 1.04).

Anal. Calc. for $C_6H_{14}N_2O_3 \cdot H_2O$: C, 39.99; H, 8.95; N, 15.55. Found: C, 39.75; H, 8.91; N, 15.27.

Compound **23** (15 mg) was acetylated as already described, and the acetate crystallized from ethanol–ether to give 26 mg (75.6%) of **24** as colorless needles, m.p. 164–167°, $[\alpha]_D^{22.5}$ −9.8 ±0.5° (*c* 1.07, ethanol).

Anal. Calc. for $C_{16}H_{24}N_2O_8 \cdot 0.5\ H_2O$: C, 50.38; H, 6.61; N, 7.35. Found: C, 50.07; H, 6.56; N, 7.37.

Catalytic reduction of **4** *with Raney nickel.* — A solution of 300 mg of **4** in 10 mL of 50% aqueous ethanol was hydrogenated over 0.65 mL of Raney nickel at a pressure of 5 kg/cm² for 9 h at 41°. After removal of the catalyst by filtration, the filtrate was evaporated and the residue dissolved in 6 mL of water. The solution was adsorbed onto a column of 100 mL of Amberlite CG-50 (NH_4^+) resin, which was eluted with water, collecting 4-mL fractions. Fractions 15–17 gave 102 mg (34.8%) of **6**, $[\alpha]_D^{21}$ +141.3 ±1.9° (*c* 1.02) as a colorless foam, identical with authentic **6** by comparative t.l.c., and i.r. and ^{1}H-n.m.r. spectra. Fractions 20–27 gave 37 mg (12.6%) of **1**, $[\alpha]_D^{22.5}$ +125.3 ±1.6° (*c* 1.03) as a colorless foam, identical with authentic **1** by comparative t.l.c., and i.r. and ^{1}H-n.m.r. spectra.

Catalytic reduction of **17** *with Raney nickel.* — (*A*). A solution of 190 mg of **17** in 50% aqueous ethanol was hydrogenated as already described. The product was dissolved in 3 mL of water, adsorbed onto a column of 100 mL of CM-Sephadex C-25 (NH_4^+) resin, and the column eluted successively with water and 0.2M ammonium hydroxide, collecting 5-mL fractions. Fractions 22–25 gave 4 mg (1.7%) of **18**, $[\alpha]_D^{22}$ +130.0 ±4.3° (*c* 0.34) as a colorless foam, identical with authentic **18** by comparative t.l.c. Fractions eluted by 0.2M ammonium hydroxide gave 53 mg (22.6%) of **15**, $[\alpha]_D^{22}$ +130.2 ±1.7° (*c* 1.03), as a colorless foam, identical with authentic **15** by comparative t.l.c. and i.r. and ^{1}H-n.m.r. spectra.

(*B*). A solution of 200 mg of **17** in 20 mL of 50% aqueous ethanol was hydrogenated over 0.6 mL of Raney nickel for 2 h in an autoclave at 55° and at a pressure of 80 kg/cm^2. The catalyst was filtered off and the filtrate evaporated. The residue was chromatographed on 80 mL of CM-Sephadex C-25 (NH_4^+) resin as already described to give 10 mg (5%) of **18** and 28 mg (14%) of **15**, together with 48 mg (24.6%) of a mixture of **15** and **18**.

*3,2',6',3"-Tetra-*N*-formyl-1-*N*-hydroxytobramycin* (**3**) *and 1-*epi*-3,2',6'3"-tetra-*N*-formyl-1-*N*-hydroxytobramycin* (**8**). — Compound **4** (2.0 g) was dissolved in 40 mL of acetic acid under nitrogen and 310 mg of sodium cyanoborohydride was added with stirring. Stirring was continued for 1.5 h and then 200 mL of ether was added. The resultant precipitate was filtered off and washed with ether. The precipitate was dissolved in 7 mL of water, adsorbed on a column of 200 mL of Amberlite CG-50 (H^+) resin, and the column eluted with water. Fractions 7–21 gave 1.14 g (57.7%) of **8** as a colorless foam, $[\alpha]_D^{24}$ +167.6 ±2.1° (*c* 1); δ_H 5.63 (d, 1 H, $J_{1',2'}$ or $J_{1'',2''}$ 3 Hz, H-1' or H-1") and 5.75 (d, 1 H, $J_{1'',2''}$ or $J_{1',2'}$ 3 Hz, H-1" or H-1').

Anal. Calc. for $C_{22}H_{37}N_5O_{14} \cdot 1.25\ H_2O$: C, 42.75; H, 6.44; N, 11.33. Found: C, 42.71; H, 6.28; N, 11.49.

Fractions 22–28 gave 415 mg (21%) of **8** as a colorless foam, $[\alpha]_D^{24}$ +118.2 ±1.7° (*c* 1); δ_H 5.62 (d, 1 H, $J_{1',2'}$ or $J_{1'',2''}$ 3 Hz, H-1' or H-1") and 5.78 (d, 1 H, $J_{1'',2''}$ or $J_{1',2'}$ 3 Hz, H-1" or H-1').

Anal. Calc. for $C_{22}H_{37}N_5O_{14} \cdot 1.25\ H_2O$: C, 42.75; H, 6.44; N, 11.33. Found: C, 42.75; H, 6.28; N, 11.67.

*1,2',6',3"-Tetra-*N*-formyl-3-*N*-hydroxytobramycin* (**16**) *and 3-*epi*-1,2',6',3"-tetra-*N*-formyl-3-*N*-hydroxytobramycin* (**19**). — Compound **17** (700 mg) was reduced with 106.8 mg of sodium cyanoborohydride as already described. Chromatography of the product on a column of Amberlite CG-50 (H^+) resin gave 466 mg (66.4%) of **19** and 107 mg of **16** as colorless foams. Compound **19** had $[\alpha]_D^{22.5}$ +113.0 ±1.5° (*c* 1); δ_H 5.58 (d, 1 H, $J_{1',2'}$ or $J_{1'',2''}$ 4 Hz, H-1' or H-1") and 5.67 (d, 1 H, $J_{1'',2''}$ or $J_{1',2'}$ 4 Hz, H-1" or H-1').

Anal. Calc. for $C_{22}H_{37}N_5O_{14} \cdot 2.5\ H_2O$: C, 41.25; H, 6.29; N, 10.93. Found: C, 41.34; H, 6.49; N, 10.91.

Compound **16** had $[\alpha]_D^{23}$ +129.9 ±1.7° (*c* 1); δ_H 5.70 (d, 2 H, $J_{1',2'}$ and $J_{1'',2''}$ 3 Hz, H-1' and H-1").

Anal. Calc. for $C_{22}H_{37}N_5O_{14} \cdot 3\ H_2O$: C, 40.67; H, 6.67; N, 10.78. Found: C, 40.95; H, 6.70; N, 11.06.

3,2',6',3''-Tetra-N-*formyltobramycin* (**1**) *from* **3**. — A solution of 40 mg of **3** in 2 mL of ethanol was hydrogenated over 0.1 mL of Raney nickel at a pressure of 5 kg/cm^2 for 16 h at room temperature. After removal of the catalyst, the solution was evaporated. The residue was dissolved in a small amount of water, treated with activated charcoal, and lyophilized to give 33 mg of **1**, $[\alpha]_D^{22.5}$ +129.4 ±1.6° (*c* 1) as a colorless foam, identical with authentic **1** by comparative t.l.c., and i.r. and ^{1}H-n.m.r. spectra.

1-epi-*3,2',6',3''-Tetra*-N-*formyltobramycin* (**6**) *from* **8**. — A solution of 800 mg of **8** in a mixture of 9 mL of ethanol and 9 mL of water was hydrogenated over 0.5 mL of ethanol and 9 mL of water was hydrogenated over 0.5 mL of Raney nickel as already described. The product was dissolved in 15 mL of water and adsorbed onto a column of 250 mL of Amberlite CG-50 (NH_4^+) resin, which was eluted with water, collecting 5-mL fractions. Fractions 28–35 gave 703 mg (87.5%) of pure **6**, $[\alpha]_D^{22.5}$ +143.5 ±1.2° (*c* 1), identical with authentic **6** by comparative t.l.c., and i.r. and ^{1}H-n.m.r. spectra.

1,2',6',3''-Tetra-N-*formyltobramycin* (**15**) *from* **16**. — Compound **16** was hydrogenated and **15** obtained in 71% yield; $[\alpha]_D^{23}$ +126.9 ±1.7° (*c* 1). It was a colorless foam, identical with authentic **15** by comparative t.l.c., and i.r. and ^{1}H-n.m.r. spectra.

3-epi-*1,2',6'3''-Tetra*-N-*formyltobramycin* (**18**) *from* **19**. — Compound **19** was hydrogenated and **18** obtained in 88.5% yield, $[\alpha]_D^{22}$ +131.8 ±1.7° (*c* 1), as a colorless foam, identical with authentic **18** by comparative t.l.c., and i.r. and ^{1}H-n.m.r. spectra.

Conversion of **3** *and* **8** *into 1-deamino-3,2',6',3''-tetra*-N-*formyl-1-hydroxyiminotobramycin* (**4**). — *A*. A solution of 55 mg of **3** in 1 mL of water was stirred for 52 h at room temperature. The solution was evaporated and the residue dissolved in a small amount of acetonitrile–water, and adsorbed onto a column of 10.8 g of silica gel that was eluted (8-mL fractions) with 10:1:1 (v/v) acetonitrile–ethanol–water. Fractions 11–14 gave 16 mg (30.2%) of **4**, $[\alpha]_D^{25}$ +174.9 ±2.1° (*c* 1).

Anal. Calc. for $C_{22}H_{35}N_5O_{14} \cdot 2\ H_2O$: C, 41.97; H, 6.24; N, 11.12. Found: C, 41.81; H, 6.77; N, 10.85.

This product was identical with authentic **4** by comparative t.l.c., and ^{1}H-n.m.r. spectra.

B. A solution of 100 mg of **8** in 2 mL of water was stirred for 207 h at room temperature. The solution was evaporated and the residue chromatographed on a column of silica gel as just described to give 37 mg (46.8%) of **4**, $[\alpha]_D^{25}$ +178.8 ±2.0° (*c* 1).

Conversion of **16** *and* **19** *into 3-deamino-1,2',6',3''-tetra*-N-*formyl-3-hydroxyiminotobramycin* (**17**). — *A*. A solution of 81 mg of **16** in 1.6 mL of water was stirred for 337 h at room temperature. The solution was evaporated and the residue dissolved in a small amount of water–acetonitrile, adsorbed onto a column of 19 g of silica

gel, and the column eluted with 10:1:1 (v/v) acetonitrile–ethanol–water, collecting 8-mL fractions. Fractions 35–65 gave 43 mg (53.3%) of **17**, $[\alpha]_D^{23.5}$ +93.2 ±1.2° (*c* 1).

Anal. Calc. for $C_{22}H_{35}N_5O_{14} \cdot 3\ H_2O$: C, 40.80; H, 6.39; N, 10.82. Found: C, 40.87; H, 6.09; N, 10.80.

This product was identical with authentic **17** by comparative t.l.c., and ^{1}H-n.m.r. spectra.

From fractions 108–116, 8 mg of **16** was recovered.

B. A solution of 100 mg of **19** in 2 mL of water was stirred for 337 h at room temperature. The solution was evaporated and the residue chromatographed on a column of silica gel to give 49 mg (49.2%) of **17**, $[\alpha]_D^{23.5}$ +92.5 ±1.3° (*c* 1); 15 mg of **19** was recovered.

1-N-*Hydroxytobramycin* (**11**). — A solution of 100 mg of **3** in a mixture of 0.25 mL of water, 1.51 mL of methanol, and 0.31 mL of concentrated hydrochloric acid was stirred for 24 h at 37.5°. Water (4 mL) was added, the solution was made neutral with Amberlite IR-45 (OH^-) resin, and the resin was filtered off and washed with water. The combined filtrate and washings were evaporated, and a solution of the residue in 1 mL of water was passed through a column of 6 mL of Amberlite IRA-400 ($CH_3CO_2^-$) resin, which was washed with 25 mL of water. The combined eluate and washings were concentrated to ~2 mL and the pH of the solution was adjusted to 2.4 by the addition of 8.4 mL of 0.05M sulfuric acid. The solution was concentrated and 13 mL of ethanol was added. The resulting precipitate was filtered off and washed with ethanol. The precipitate was dissolved in 2 mL of water, treated with activated charcoal, and lyophilized to give 122 mg (88.4%) of the sulfate of **11** as a colorless foam, $[\alpha]_D^{24}$ +82.5 ±1.1° (*c* 1); δ_H 5.73 (d, 1 H, $J_{1'',2''}$ 3 Hz, H-1″) and 6.40 (d, 1 H, $J_{1',2'}$ 3 Hz, H-1′).

Anal. Calc. for $C_{18}H_{37}N_5O_{10} \cdot 2.5\ H_2SO_4 \cdot 7\ H_2O$: C, 25.29; H, 6.60; N, 8.19; S, 9.38. Found: C, 25.04; H, 6.49; N, 8.10; S, 9.48.

1-epi-*1*-N-*Hydroxytobramycin* (**12**). — Compound **8** (200 mg) was deformylated with a mixture of water, methanol, and concentrated hydrochloric acid and treated as already described to give 243 mg (89.7%) of the sulfate of **12** as a colorless foam, $[\alpha]_D^{24}$ +117.7 ±1.5° (*c* 1); δ_H 5.77 (d, 1 H, $J_{1'',2''}$ 3 Hz, H-1″) and 6.43 (d, 1 H, $J_{1',2'}$ 3 Hz, H-1′).

Anal. Calc. for $C_{18}H_{37}N_5O_{10} \cdot 2.5\ H_2SO_4 \cdot 6\ H_2O$: C, 25.84; H, 6.50; N, 8.37; S, 9.58. Found: C, 25.71; H, 6.60; N, 8.18; S, 9.38.

3-N-*Hydroxytobramycin* (**13**). — Compound **16** (157 mg) was deformylated with a mixture of water, methanol, and concentrated hydrochloric acid and treated as already described to give 206 mg (99.2%) of the sulfate of **13** as a colorless foam, $[\alpha]_D^{24}$ +89.4 ±1.3° (*c* 1); δ_H 5.72 (d, 1 H, $J_{1'',2''}$ 3 Hz, H-1″) and 6.43 (d, 1 H, $J_{1',2'}$ 3 Hz, H-1′).

Anal. Calc. for $C_{18}H_{37}N_5O_{10} \cdot 2.5\ H_2SO_4 \cdot 8\ H_2O$: C, 24.77; H, 6.70; N, 8.02; S, 9.19. Found: C, 24.52; H, 6.58; N, 8.02; S, 9.59.

3-epi-*3*-N-*Hydroxytobramycin* (**14**). — Compound **19** (205 mg) was de-

formylated with a mixture of water, methanol, and concentrated hydrochloric acid to give 258 mg (90.4%) of the sulfate of **14** as a colorless foam, $[\alpha]_D^{22.5}$ +59.6 ±0.9° (*c* 1), δ_H 5.70 (d, 1 H, $J_{1'',2''}$ 3 Hz, H-1″) and 6.43 (d, 1 H, $J_{1',2'}$ 3 Hz, H-1′).

Anal. Calc. for $C_{18}H_{37}H_5O_{10} \cdot 2.5\ H_2SO_4 \cdot 7\ H_2O$: C, 25.29; H, 6.60; N, 8.19; S, 9.38. Found: C, 25.16; H, 6.33; N, 7.89; S, 9.71.

1-epi-*Nebramine* (**25**). — A solution of 100 mg of **9** in 4 mL of 4M hydrochloric acid was heated for 4.5 h under reflux at 110°. The solution was evaporated, and the residue, dissolved in 10 mL of water, adsorbed onto a column of 60 mL of Amberlite CG-50 (NH_4^+) resin, which was gradient-eluted with 0.5 L of water (10-mL fractions) and 0.5 L of 0.5M ammonium hydroxide. Fractions 99–110 gave 50 mg of **25** as a colorless foam. The sulfate of **25** had $[\alpha]_D^{22}$ +71.7 ±1.1° (*c* 1); δ_H 6.18 (d, 1 H, $J_{1',2'}$ 4 Hz, H-1′).

Anal. Calc. for $C_{12}H_{26}N_4O_5 \cdot 2\ H_2SO_4 \cdot 5\ H_2O$: C, 24.32; H, 6.80; N, 9.46; S, 10.82. Found: C, 24.24; H, 6.56; N, 9.36; S, 10.68.

ACKNOWLEDGMENTS

The authors thank Dr. J. G. Whitney of Eli Lilly & Co. for kindly supplying tobramycin, and Messrs. Toshihiko Horii and Hiroshi Hashizume for technical assistance.

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