5″-AMINO-3′,4′,5″-TRIDEOXYBUTIROSIN A, A NEW SEMISYNTHETIC AMINOGLYCOSIDE ANTIBIOTIC

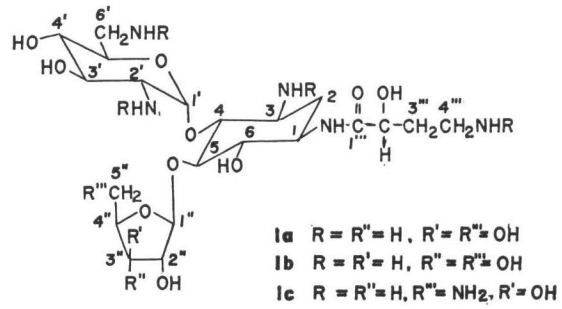
PETER W. K. WOO

Chemistry Department, Research and Development Division,
Parke, Davis and Company, Ann Arbor, Michigan 48106, U. S. A.

(Received for publication March 31, 1975)

5″-Amino-3′,4′,5″-trideoxybutirosin A (**IX**) was synthesized through a reaction series starting from 5″-amino-5″-deoxybutirosin A (**Ic**), the key step being the treatment of its tetra-O-acetylpentakis-N-[(phenylmethoxy)carbonyl]-3′,4′-bis-O-(methylsulfonyl) derivative (**VI**) with zinc-sodium iodide. Compound **IX** exhibits enhanced antibacterial activities, including strains of *Pseudomonas aeruginosa* and *Escherichia coli* which are highly resistant to **Ic**, butirosin or gentamicin.

Butirosin, an aminoglycoside antibiotic complex consisting of butirosin A (**Ia**) and butirosin B (**Ib**), exhibits broad inhibitory activity against gram-positive and gram-negative bacteria, notably opportunist bacterial species within *Pseudomonas*, *Klebsiella*, *Enterobacter*, *Serratia* and *Proteus*.[1,2]

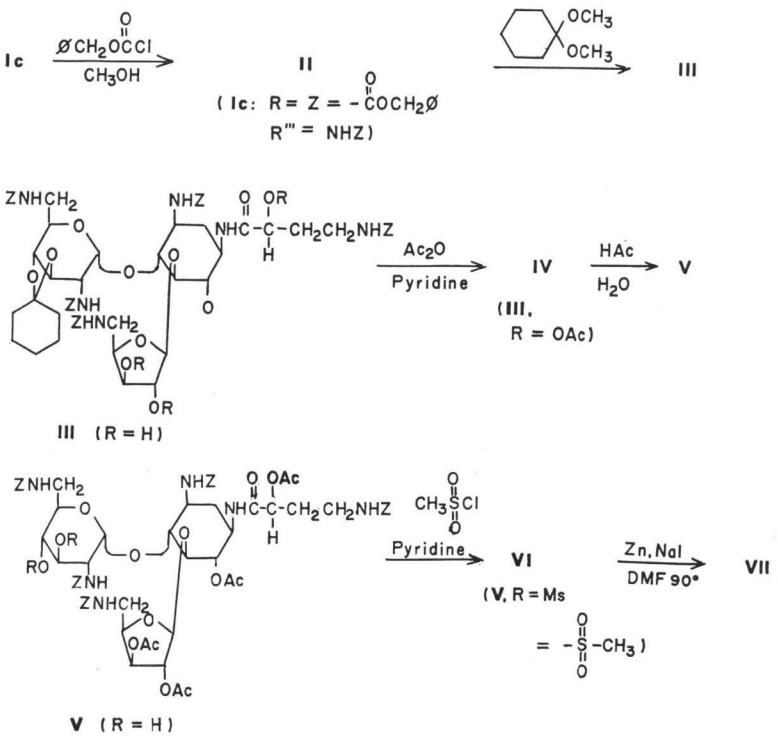


The synthesis of 5″-amino-3′, 4′,5″-trideoxybutirosin A (**IX**)* was undertaken with the objective of incorporating into the butirosin A molecule two different structural features which together might conceivably result in both enhanced potency and widened antibacterial spectrum. CULBERTSON, WATSON, and HASKELL[3] have shown that replacement of the 5″-hydroxyl group of butirosin A by an amino group results in enhanced antibiotic activities, especially against strains of *Pseudomonas aeruginosa* and *Serratia marcescens*. UMEZAWA *et al.*[4,5,6] and DAVIES *et al.*[7,8] have shown that a number of aminoglycoside antibiotics are enzymatically inactivated by certain resistant strains of *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*, through the mechanism of phosphorylation of the 3′-hydroxyl group. In particular, one of the enzymes, kanamycin-neomycin phosphotransferase II, isolated from certain *E. coli* carrying R factors, has been shown to inactivate butirosin through this mechanism. Inactivation by 3′-phosphorylation reportedly could by prevented by conversion of the 3′-hydroxyl group, or both the 3′- and 4′-hydroxyl groups, to the corresponding deoxy functions, as exemplified by 3′-deoxykanamycin,[9] 3′,4′-dideoxykanamycin B,[10] 3′,4′-dideoxybutirosin A,[11] and others.[12~17] Recently, observations with 4′-deoxykanamycin led to the suggestion that the 4′-hydroxyl group is involved in the binding to phosphotransferase II,[18] thus raising a question as to the possible utility of a 4′-deoxy function in preventing certain types of 3′-phosphorylative

* O-2,6-Diamino-2,3,4,6-tetradeoxy-α-D-*erythro*-hexopyranosyl-(1→4)-O-[5-amino-5-deoxy-β-D-xylofuranosyl-(1→5)]-N[1]-[(S)-4-amino-2-hydroxy-1-oxobutyl]-2-deoxystreptamine.

inactivation. It was our hope that **IX**, the target of our synthesis, while possibly retaining the generally enhanced potency of 5''-amino-5''-deoxybutirosin (**Ic**), could acquire a widened spectrum to include organisms which might inactivate **Ic** through reactions requiring the intermediacy of the 3'-, the 4'-, or both the 3'- and 4'-hydroxyl groups.

The synthesis of **IX** was based on the reaction of the 3',4'-bis-O-methanesulfonate of a suitably protected butirosin A derivative (**VI**) with zinc and sodium iodide, a reaction developed by TIPSON and COHEN[10] and recently applied to aminoglycoside antibiotics. The starting material, **Ic**, was converted in 90 % yield to the pentakis-N-[(phenylmethoxy)carbonyl] derivative (**II**) by reaction with benzyl chloroformate in the presence of methanol and sodium bicarbonate. Compound **II** was then treated with 1,1-dimethoxycyclohexane in N,N-dimethylformamide in the presence of (4-methylphenyl)sulfonic acid *in vacuo* to give a mixture of products, from which the 3', 4'-cyclohexylidene (**III**) was isolated in 24 % yield after chromatography over silica gel. The other products of the cyclohexylidenation mixture can be easily reconverted to **II** by treatment with aqueous acetic acid and used again for the preparation of **III**. The four hydroxyl groups of **III** were acetylated to give the tetra-O-acetate (**IV**). The cyclohexylidene group was then removed by treatment with aqueous acetic acid to give **V**, in which free hydroxyl groups are present only at the 3' and 4' position. Treatment of **V** with methanesulfonyl chloride in pyridine gave, after chromatography, a 74 % yield of the bis-O-methanesulfonate (**VI**). Treatment of **VI** with zinc and sodium iodide in N,N-dimethylformamide at 90°C gave, after silica gel chromatography, a 47 % yield of homogeneous 3',4'-unsaturated compound (**VII**), in addition to an estimated 10 % as mixture with unreacted starting material. The O-acetyl groups in **VII**



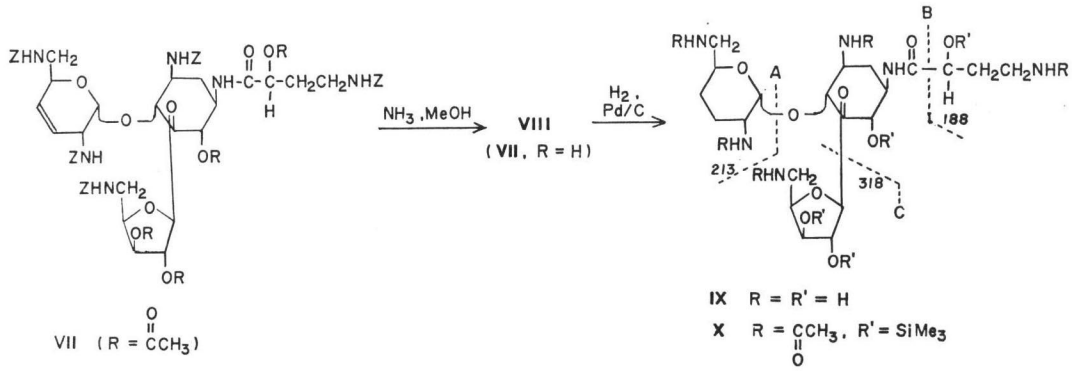
were removed by treatment with methanolic ammonia. The double bond in the resulting compound **VIII** was hydrogenated and the N-[(phenylmethoxy)carbonyl] groups were simultaneously hydrogenolyzed in aqueous acetic acid using palladium on charcoal as catalyst. The product, **IX**, was adsorbed on Amberlite IRC 50 in the ammonium form and isolated by elution with 1 M aqueous ammonia. The main fraction represents a yield of 43 % based on the amount of **VII** used and was homogeneous by thin-layer chromatography with a mobility which was different from butirosin and **Ic**.

The structure of **IX** is consistent with the mass spectrum of its penta-N-acetyl-tetrakis-O-(trimethylsilyl) derivative (**X**). Electron impact fragmentation of **X** gave strong peaks at *m/e* 213 (33 %), 188 (20 %), and 318 (32 %) ($SiMe_3$ peak at *m/e* 73 as 100 %), as expected from

Table 1. Comparative *in vitro* antibacterial activities of 5″-amino-3′,4′,5″-trideoxybutirosin A (**IX**), butirosin (mostly **Ia**), 5″-amino-5″-deoxybutirosin A (**Ic**), and gentamicin.

Test organisms		MIC (minimal inhibitory concentration, mcg/ml)[a]			
		IX	Butirosin sulfate[b]	**Ic**	Gentamicin sulfate[c]
Streptococcus faecalis	MGH-2	>200	>200	>200	100
Staphylococcus aureus	S18713	3.1	50	25	3.1
Klebsiella pneumoniae	MGH-1	6.3	6.3	6.3	6.3
Serratia marcescens	IMM-5	12.5	25	6.3	6.3
Enterobacter cloacae	IMM-50	6.3	6.3	6.3	3.1
Pseudomonas aeruginosa	#28	1.6	12.5	3.1	3.1
	VAD-12-7-7	1.6	6.3	3.1	1.6
	74C-1	3.1	12.5	6.3	3.1
	733	3.1	12.5	3.1	3.1
	LA3399	6.3	50	12.5	12.5
	UI-18	3.1	12.5	3.1	1.6
	Aquilar[d]	6.3	>200	>200	12.5
	G76[e]	6.3	50	12.5	100
	58.38	1.6	12.5	3.1	3.1
	130[f]	3.1	25	6.3	100
	PST-1[g]	3.1	25	12.5	>200
Escherichia coli	CW[f]	3.1	6.3	6.3	100
	JR 76.2[h]	12.5	>200	>200	200
	R5W677[i]	1.6	3.1	3.1	0.8
	JR35W677[j]	3.1	6.3	12.5	3.1
Providence	164[k]	100	>200	100	100

a Microtitration dilution in Tryptic Soy broth; standard procedure, overnight 37°C incubation, approximately 100 viable units in inoculum.
b Containing 710 μg base/mg, mostly component A.
c Potency of 561 μg/mg.
d Butirosin and gentamicin-resistant clinical isolates.
e Gentamicin-resistant clinical isolate.
f Containing gentamicin acetyl transferase I.
g Containing gentamicin acetyl transferase III.
h Containing gentamicin adenylyl transferase, streptomycin phosphotransferase, and neomycin-kanamycin phosphotransferase II.
i Containing kanamycin acetyl transferase.
j Containing neomycin-kanamycin phosphotransferase I and streptomycin phosphotransferase.
k Containing gentamicin acetyl transferase II.



fragmentation pathways A, B, and C, respectively.[20] The location of the 3′,4′-dideoxyfunction is thus firmly established.

The *in vitro* antibacterial activities of 5″-amino-3′,4′,5″-trideoxybutirosin A (**IX**), compared with butirosin (mostly component A, **Ia**), 5″-amino-5″-deoxybutirosin A (**Ic**), and gentamicin, are shown in Table 1. Noteworthy are the activities of **IX** against: (1) *E. coli* JR76.2, which inactivates **Ia** and **Ic** with neomycin-kanamycin phosphotransferase II through phosphorylation at the 3′-hydroxyl group, and which also inactivates gentamicin with adenylyl transferase; (2) *Ps. aeruginosa* Aquilar, against which **Ia** and **Ic** are both inactive; and (3) *Staph. aureus* S18713, against which **Ia** and **Ic** are active only at more than eight-fold higher concentrations. Against other strains of *Ps. aeruginosa*, including those which carry gentamicin inactivating enzymes, **IX** is generally more active than **Ia**, as indicated by two- to eight-fold differences in minimal inhibitory concentrations.

Experimental*

5″-Amino-5″-deoxypentakis-N-[(phenylmethoxy)carbonyl] butirosin A (**II**).

To a stirred solution of 5.006 g of 5″-amino-5″-deoxybutirosin A (**Ia**) (mol wt, 554.6; 9.01 mmol or 45 meq) in 18.75 ml of water at 0°C was added 35 ml of methanol, followed by 10.111 g (120 meq) of sodium bicarbonate. The mixture was stirred in an ice-water bath, and 12.0 ml of benzyl chloroformate (95 %) (*ca.* 82 meq) was added dropwise during 30 minutes in 1-ml portions, each portion being followed by a 5-ml portion of cold methanol. Tlc showed that the product, **II**, formed rapidly. After stirring for 26.5 hours at about 5°C, 6.0 ml (4.54 g, 52 meq) of 1-pentanamine was added dropwise to react with excess benzyl chloroformate. After 16.5 hours of stirring at *ca.* 5°C, 3.0 ml of acetic acid diluted to 16.5 ml with methanol was gradually added to neutralize the 1-pentanamine. The mixture was filtered, and the filtered salt was washed with methanol. Filtrate and washings were concentrated *in vacuo* to 34.5 g of oil. The oil was triturated with three 100-ml portions and then one 300-ml portion of ether, then dissolved in about 150 ml of chloroform and washed eight times with 5-ml portions of water. The chloroform solution was dried with sodium sulfate and evaporated to dryness yielding 10.052 g (91 % yield) of solid **II**. Tlc in 10 % M in C showed one major component with Rf 0.5.

* Unless otherwise specified, tlc was performed using glass plate precoated with silica gel (Quanta QIF, 10-cm length, Quantum Industries, Fairfield, New Jersey) and aqueous solution of ammonium molybdate containing phosphoric and sulfuric acids as spray. Column chromatography was performed using silica gel (E. Merck 60, 0.013~0.20 mm) packed by gravity as slurries in solvents. Compositions of solvent mixtures are expressed in volume %; *e.g.*, 12%M in C means 12 ml of methanol (M) diluted to 100 ml with chloroform (C) (which contains *ca.* 0.75% ethanol).

Anal. Calcd. for $C_{81}H_{72}N_6O_{21}$ (1225.25): C, 59.79; H, 5.92; N, 6.86.
Found (after drying *in vacuo* at 60°C for 2 hours): C, 59.72; H, 5.84; N, 6.99.

5''-Amino-3',4'-O-cyclohexylidene-5''-deoxypentakis-N-[(phenylmethoxy)carbonyl]butirosin A (**III**).

To a solution of 2.6919 g (2.2 mmol) of **II** in 27 ml of dried N,N-dimethylformamide in a r.b. flask with a side-arm were added 160 mg of *p*-toluensulfonic acid monohydrate and 2.0 ml of 1,1-dimethoxycyclohexane. The flask was fitted with a coil condenser the top of which was connected through a stopcock to a vacuum of 15 mm mercury. The side-arm was fitted with a very fine capillary through which air previously dried with concentrated sulfuric acid was allowed to bubble into the solution *in vacuo*. With the solution *in vacuo*, the flask was placed in a water bath at 50°C for 34 minutes and then removed from the water bath for 10 minutes. The vacuum was then removed. After 40 minutes 0.5 ml of N,N-diethylethanamine was added, and the solution was evaporated *in vacuo* (bath temperature below 50°C) to 3.492 g of a cyclohexylidenation mixture containing more than seven components.

A similar cyclohexylidenation mixture, 219 mg, was similarly prepared from 154 mg of **II**.

The two crops of cyclohexylidenation mixture (3.711 g starting from a total of 2.846 g or 2.33 mmol of **II**) in 9 ml of chloroform were added to a column of silica gel packed in chloroform (54 g, 1.9 cm×44 cm). The column was then developed with 190 ml of chloroform (fr 1~13, 5-minute fractions), then with 200 ml of 1% M in C (fr 14~24), 200 ml of 2% M in C (fr 25~36), 200 ml of 4% M in C (fr 37~49), 200 ml of 6% M in C (fr 50~61), 250 ml 8% M in C (fr 62~77), and 250 ml 12% M in C (fr 72~93). Fractions were combined as follows: fr 34~54, 1,481 mg, polycyclohexylidenated material; fr 55, 135 mg, poly- and monocyclohexylidene; fr 56~64, 741 mg (24.3%), monocyclohexylidene; fr 65~89, 638 mg, mixture of monocyclohexylidene and unreacted starting material, Rf 0.65 and 0.42, respectively, in 10% M in C.

Anal. Calcd. for $C_{87}H_{80}N_6O_{21}$ (1305.36): C, 61.64; H, 6.18; N, 6.44.
Found (fr 57~61): C, 60.72; H, 5.98; N, 6.12.

The polycyclohexylidenated material can be readily converted back to **II** by treatment with acetic acid-water (8 : 2~9 : 1).

Tetra-O-acetyl-5''-amino-3',4'-O-cyclohexylidene-5''-deoxypentakis-N-[(phenylmethoxy)carbonyl] butirosin A (**IV**) and 2'', 2''', 3'', 6-tetra-O-acetyl-5''-amino-5''-deoxypentakis-N-[(phenylmethoxy)carbonyl] butirosin A (**V**) ($C_{69}H_{80}N_6O_{25}$, 1396.59).

To compound **III**, 417 mg (fr 57~61 above), in 3.2 ml of pyridine was added 0.8 ml of acetic anhydride. After 23 hours at room temperature the reaction mixture was evaporated to dryness and further dried *in vacuo* over potassium hydroxide overnight to give 491 mg of **IV**. To a solution of **IV** in 3.2 ml of glacial acetic acid was added 0.8 ml of water with swirling. After 4.7 hours the reaction mixture was evaporated to a syrup *in vacuo* and further dried *in vacuo* over potassium hydroxide. Tlc in 3% M in C showed one major spot, Rf 0.28. Repeated treatment with 3.2 ml of glacial acetic acid and 0.8 ml of water for 6 hours did not change the tlc pattern. The yield of **V** was 433 mg after the second treatment.

In similar runs, compound **III**, 190 mg (fr 56 above) and 83 mg (fr 61~64 above), yielded 200 and 92 mg of **V**, respectively.

Tetra-O-acetyl-5''-amino-5''-deoxy-3',4'-bis-O-(methylsulfonyl)pentakis-N-[(phenylmethoxy) carbonyl] butirosin A (**VI**) $C_{71}H_{84}N_6O_{29}S_2$, 1552.76).

To a solution of 427 mg of compound **V** (0.306 mmol, 0.612 meq) in 5.3 ml of dry pyridine, cooled in an ice-water both, was added 0.18 ml (265 mg, 2.3 mmol) of methanesulfonyl chloride with swirling. The ice bath was allowed to melt, and after 48 hours at room temperature 0.2 ml of water was added. The reaction mixture was evaporated *in vacuo* to 1.45 g, dissolved in 40 ml of chloroform and extracted four times with 4-ml portions of water. After drying with sodium sulfate, the chloroform extract was evaporated to 478 mg of brown foamy solid containing mainly compound **VI**, Rf 0.48 (3% M in C) (Rf of starting material 0.21) and small

amount of two slower moving spots, presumably the monomethanesulfonate derivatives of **V**, Rf 0.35 and 0.28.

In similar runs, 200 mg (0.143 mmol) and 87 mg (0.062 mmol) of compound **V** yielded 224 and 92 mg, respectively, of crude compound **VI**.

Crude compound **VI**, 784 mg, in 5 ml of chloroform was added to a column of 14.9 g of silica gel in chloroform (1.4 cm×27 cm). The column was developed with 70 ml of chloroform, 70 ml of 0.5% M in C, then with 165 ml of 1% M in C (fr 35~93). Fractions were combined as follows: fr 47~57, 403.8 mg (50.9%), OMs_2; fr 58~62, 180.4 mg (22.7%), OMs_2 and trace of OMs_1; fr 63~68, 91.9 mg, OMs_2 and OMs_1.

Tetra-O-acetyl-5''-amino-3',4',5''-trideoxypentakis-N-[(phenylmethoxy)carbonyl]butirosin-3',4'-ene A (**VII**) $C_{69}H_{78}N_6O_{23}$, 1235.17).

To a solution of 180.4 mg (0.116 mmol) of compound **VI** in 3.6 ml of dried N,N-dimethylformamide were added 228 mg of Linde Molecular Sieve Type 3A, 1.836 g (12.25 mmol) of dried sodium iodide and 922.8 mg (14.11 mmol) of zinc dust. Mixture became hot and lumpy and was stirred magnetically at 91°C (oil bath temperature) for 1.5 hours. The mixture was stirred with 25 ml of chloroform, filtered through Celite and washed with 10- and 5-ml portions of chloroform. The combined chloroform solutions were washed with 20 ml of water, twice with 5-ml portions of saturated sodium thiosulfate solution, and then three times with 10-ml portions of water. After drying with sodium sulfate the chloroform solution was evaporated to 193 mg of yellow oil, Rf in 2.5% M in C, 0.42 (major component, 3',4'-ene product) and 0.50 (minor component, starting material).

Similarly, 373 mg (0.240 mmol) of compound **VI** was converted to 382 mg of crude 3',4'-ene product **VII**.

The two crops of crude product **VII**, totaling 570 mg, in 7.5 ml of chloroform were added to a column of 17.12 g of silica gel in chloroform (1.5 cm×29 cm). The column was developed with 50 ml of chloroform, 50 ml each of 0.5% M in C, 0.75% M in C, and 0.85% M in C, 130 ml of 1% M in C (fr 30~57, fr 37~57 being half-size fractions), 100 ml 1.5% M in C (fr 58~73), and finally 100 ml of 2.5% M in C (fr 74~91). Fractions were combined as follows: fr 33~34, 20 mg, starting material; fr 35~44, 167 mg, starting material and 3',4'-ene; fr 45~66, 207 mg (47.0%), 3',4'-ene.

5''-Amino-3',4',5''-trideoxypentakis-N-[(phenylmethoxy)carbonyl]butirosin-3',4'-ene A (**VIII**).

Ammonia gas was bubbled into a solution of 140 mg (0.113 mmol) of compound **VII** in 5 ml of methanol at 0°C for 4 minutes. The resulting solution, 6.5 ml, was kept at 4°C for 7 hours. The solution was aerated with nitrogen for 5 minutes, then evaporated to dryness *in vacuo* giving 117 mg of product **VII**, Rf 0.30 (8% M in C). Tlc indicated a high degree of purity.

Similarly 65.8 mg (0.053 mmol) of compound **VII** was deacylated to 58.4 mg of compound **VIII**.

5''-Amino-3',4',5''-trideoxybutirosin A (**IX**).

A slurry of 38.8 mg of 20% palladium on charcoal (2.44 mmol hydrogen/g) in 2 ml of methanol was added to a solution of 175 mg of compound **VIII** in 6 ml of methanol and 2 ml of 2 N acetic acid. The mixture was stirred magnetically and hydrogen was bubbled through it for 2.5 hours. An additional 20 mg of the palladium catalyst in 2 ml of methanol and 1 ml of 2 N acetic acid was added, and hydrogen was bubbled through for 3 hours. Finally 15.4 mg of the palladium catalyst in 1 ml of 2 N acetic acid and 1 ml of methanol was added, and hydrogen was bubbled through for 2 hours. (Hydrogenolysis of the (phenylmethoxy)carbonyl group, as indicated by carbon dioxide evolved and detected as barium carbonate, was essentially complete within 1.5 hours of hydrogenation.) The mixture was filtered and the filtrate evaporated to dryness *in vacuo* to give 128 mg of white solid. A solution of the solid in 5 ml of water was percolated through a column of 5 ml IRC 50 (ammonium form), a weak cation-exchange resin. The column was then washed with 60 ml of water, then developed successively with 50 ml of

0.25 M, 50 ml of 0.5 M, 50 ml of 1 M, and 40 ml of 1 M aqueous ammonia. The ammonia eluates were individually evaporated *in vacuo* and lyophilized to give four fractions: A, 4.0 mg; B, 5.4 mg; C, 46.5 mg (46%); D, 13.6 mg. Fraction C, which represents the major portion of the reaction product **IX**, was shown by tlc to consist of one nihydrin-positive component with mobility different from that of **Ia** or **Ic**.

Anal. Calcd. for $C_{21}H_{42}N_6O_9 \cdot H_2CO_3 \cdot 1.5H_2O$ (611.66): C, 43.20; H, 7.75; N, 13.74.
Found: C, 43.65; H, 7.46; N, 13.70.

The Rf values of **IX**, **Ia**, and **Ic**, in various chromatographic systems, are listed in order as follows: upper phase of chloroform - methanol - 17% ammonia (2 : 1 : 1), silica gel (Quanta Q1F, 20 cm), 0.44, 0.48, and 0.38; same solvent, alumina (E. Merck, Alumina G, 20 cm), 0.70, 0.51, and 0.53; chloroform - methanol - 29% ammonia - water (1 : 6 : 4 : 2), cellulose (E. Merck, Avicel microcrystalline celluose F, 20 cm) 0.65, 0.64, and 0.60.

Penta-N-acetyl-5″-amino-3′,4′,5″-trideoxy-tetrakis-O-(trimethylsilyl)butirosin A (**X**) ($C_{43}H_{84}N_6O_{14}Si_4$, 1020.51).

To a solution of 4.7 mg of **IX** in 0.2 ml of water were added, in succession, 0.47 ml methanol and 0.27 ml acetic anhydride. After 5 hours the solution was evaporated to dryness. The residue was dissolved in 1.8 ml pyridine and treated with 0.6 ml 1, 1, 1, 3, 3, 3-hexamethyldisilazane and 0.2 ml chlorotrimethylsilane. After 5 hours the reaction mixture was evaporated to dryness *in vacuo* and extracted with benzene (portions totaling 7.5 ml) to give 5.6 mg of **X**. The mass spectrum of **X** was determined at 70 e.v. using the direct inlet system at 270°C with a Finnigan 1015 quadrupole spectrometer.

Acknowledgment

The author wishes to express his thanks to Dr.J. DAVIES, University of Wisconsin, for supplying most of the organisms containing aminoglycoside-inactivating enzymes, and to Dr.C.L. HEIFETZ and co-workers for the determination of antibacterial activities.

References

1) WOO, P. W. K.; H. W. DION & Q. R. BARTZ: Butirosins A and B, aminoglycoside antibiotics. III. Structures. Tetrahedron Letters 1971-26: 2625~2628, 1971
2) HEIFETZ, C. L.; J. A. CHODUBSKI, I. A. PEARSON, C. A. SILVERMAN & M. W. FISHER: Butirosin compared with gentamicin *in vitro* and *in vivo*. Antimicr. Agents & Chemoth. 6: 124~135, 1974
3) CULBERTSON, T. P.; D. R. WATSON & T. H. HASKELL: 5″-Amino-5″-deoxybutirosin, a new semisynthetic aminoglycoside antibiotic. J. Antibiotics 26: 790~793, 1973
4) YAGISAWA, M.; H. YAMAMOTO, H. NAGANAWA, S. KONDO, T. TAKEUCHI & H. UMEZAWA: A new enzyme in *Eschrichia coli* carrying R-factor phosphorylating 3′-hydroxyl of butirosin A, kanamycin, neamine and ribostamycin. J. Antibiotics 25: 748~750, 1972
5) DOI, O.; S. KONDO, N. TANAKA & H. UMEZAWA: Purification and properties of kanamycin phosphorylating enzyme from *Pseudomonas aeruginosa*. J. Antibiotics 22: 273~282, 1969
6) DOI, O.; M. MIYAMOTO, N. TANAKA & H. UMEZAWA: Inactivation and phosphorylation of kanamycin by drug-resistant *Staphylococcus aureus*. Appl. Microbiol. 16: 1282~1284, 1968
7) BRZEZINSKA, M. & J. DAVIES,: Two enzymes which phosphorylate neomycin and kanamycin in *Escherichia coli* strains carrying R factors. Antimicr. Agents & Chemother. 3: 266~269, 1973
8) BENVENISTE, R. & J. DAVIES: Mechanisms of antibiotic resistance in bacteria. Ann. Rev. Biochem. 42: 471~506, 1973
9) UMEZAWA, S.; T. TSUCHIYA, R. MUTO, Y. NISHIMURA & H. UMEZAWA: Synthesis of 3′-deoxykanamycin effective against kanamycin-resistant *Escherichia coli* and *Pseudomonas aeruginosa*. J. Antibiotics 24: 274~275, 1971
10) UMEZAWA, H.; S. UMEZAWA, T. TSUCHIYA & Y. OKAZAKI: 3′, 4′-Dideoxykanamycin B active against kanamycin-resistant *Escherichia coli* and *Pseudomonas aeruginosa*. J. Antibiotics 24: 485~487, 1971

11) SAEKI, H.; Y. SHIMADA, Y. OHASHI, M. TAJIMA, S. SUGAWARA & E. OHKI: Synthesis of 3′,4′-dideoxybutirosin A, active against butirosin resistant bacteria. Chem. Pharm. Bull. Japan 22: 1145～1150, 1974
12) UMEZAWA, S.; T. TSUCHIYA, T. JIKIHARA & H. UMEZAWA: Synthesis of 3′,4′-dideoxyneamine active against kanamycin resistant *E. coli* and *P. aeruginosa*. J. Antibiotics 24: 711～712, 1971
13) UMEZAWA, S.; T. TSUCHIYA, D. IKEDA & H. UMEZAWA: Synthesis of 3′, 4′-dideoxy and 3′, 4′, 5″-trideoxyribostamycin active against kanamycin-resistant *E. coli* and *P. aeruginosa*. J. Antibiotics 25: 613～616, 1972
14) UMEZAWA, S.; D. IKEDA, T. TSUCHIYA & H. UMEZAWA: Synthesis of 1-N-((S)-4-amino-2-hydroxybutyryl)-3′,4′-dideoxneamine. J. Antibiotics 26: 304～306, 1973
15) IKEDA, D.; T. TSUCHIYA, S. UMEZAWA, H. UMEZAWA & M. HAMADA: Synthesis of 3′,4′-dideoxybutirosin B. J. Antibiotics 26: 307～309, 1973
16) TAKAGI, Y.; T. MIYAKE, T. TSUCHIYA, S. UMEZAWA & H. UMEZAWA: Synthesis of 3′-deoxykanamycin B. J. Antibiotics 26: 403～406, 1973
17) IKEDA, D.; T. TSUCHIYA, S. UMEZAWA & H. UMEZAWA: Synthesis of 3′-deoxyribostamycin. J. Antibiotics 26: 799～801, 1973
18) UMEZAWA, S.; Y. NISHIMURA, Y. HATA, T. TSUCHIYA, M. YAGISAWA & H. UMEZAWA: Synthesis of 4′-deoxykanamycin and its resistance to kanamycin phosphotransferase II. J. Antibiotics 27: 722～725, 1974
19) TIPSON, R. S.; A. COHEN: Action of zinc dust and sodium iodide in N,N-dimethylformamide on contiguous, secondary sulfonyloxy groups: a simple method for introducing nonterminal unsaturation. Carbohydrate Res. 1: 338～340, 1965
20) WOO, P. W. K.: Butirosins A and B, aminoglycoside antibiotics. II. Mass spectrometric study. Tetrahedron Letters 1971-26: 2621～2624, 1971