MUTATIONAL BIOSYNTHESIS BY IDIOTROPHS OF *MICROMONOSPORA PURPUREA*

I. CONVERSION OF AMINOCYCLITOLS TO NEW AMINOGLYCOSIDE ANTIBIOTICS

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By mutation and strain improvement techniques idiotrophs of *Micromonospora purpurea*, the gentamicin-producing organism, were obtained which require an exogenous source of 2-deoxystreptamine in order to produce gentamicin. Streptamine incorporation afforded a mixture of 2-hydroxygentamicin C as a complex of essentially the C_1 and C_2 components whereas 2-deoxystreptamine when incorporated by the same idiotroph afforded the same mixture of C_1, C_2 and C_{1a} gentamicins as the parent (m_1) organism. The 2-hydroxygentamicin C complex exhibited broad-spectrum antibiotic activity with an *in vitro* potency less than that for the gentamicin C complex, but with greater activity against selected gentamicin C resistant organisms. The LD_{50} (i.v.) in mice of the 2-hydroxygentamicin C complex indicated that it had approximately half the toxicity of the gentamicin C complex. 2, 5-Dideoxystreptamine afforded a C_1, C_2, and C_{1a} mixture of 5-deoxygentamicins, which also had broad spectrum activity, and exhibited improved activity against several gentamicin-acetylating strains of resistant bacteria. The LD_{50} (i.v.) in mice of the 5-deoxygentamicin C complex indicated that it was about 2.5 times more toxic than the gentamicin C complex. Two derivatives of 2, 5-dideoxystreptamine afforded the same mixture of 5-deoxygentamicins. 2-Epistreptamine upon supplementation to a broth containing growing cultures of these idiotrophs also produced antibiotic.

The technique devised by SHIER *et al.*[1] for producing semi-synthetic aminoglycoside antibiotics by isolating mutants of aminoglycoside-producing organisms which can produce antibiotic only when supplied with an exogenous souce of 2-deoxystreptamine or other suitable aminocyclitol has been used by several groups[2~6] to prepare new aminocyclitol aminoglycoside antibiotics. NAGAOKA and DEMAIN proposed the name "mutational biosynthesis" for this technique and coined the term "idiotroph" for the type of mutant described.[6]

We have isolated a 2-deoxystreptamine-requiring idiotroph from *Micromonospora purpurea*, the organism that produces the gentamicin complex[7] of antibiotics, and by the technique of mutational biosynthesis have produced new gentamicin analogs. We have also by strain improvement techniques produced a more efficient idiotroph.

Materials and Methods

Organism and Culture Conditions

Micromonospora purpurea (NRRL 2953) was obtained from Dr. T. G. PRIDHAM of the U.S. Department of Agriculture and was maintained on N-Z-amine slants containing a medium (medium 1) consisting of (per liter, distilled H_2O) yeast extract, 5 g; glucose, 10 g; soluble starch,

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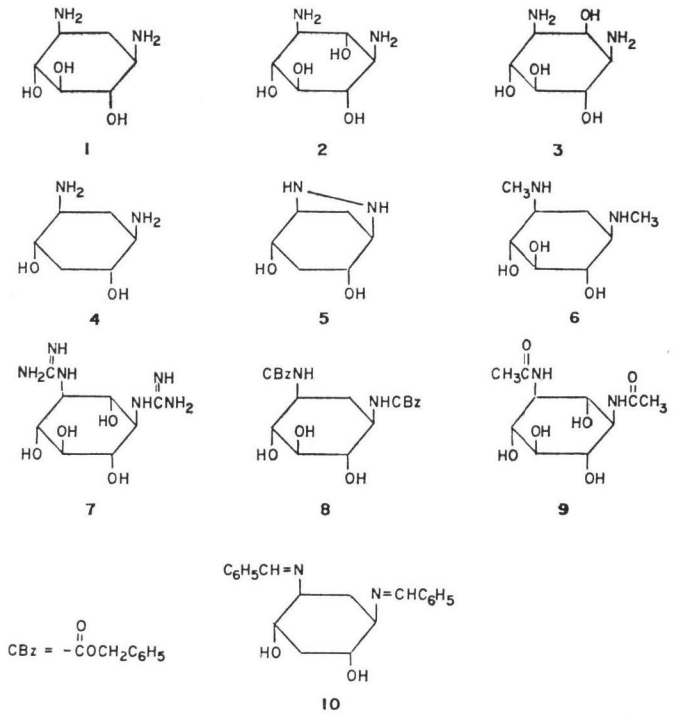
Fig. 1. Compounds tested for incorporation by deoxystreptamine-requiring mutants of *M. purpurea*



20 g; N-Z-amine-Type A (Difco), 5 g; calcium carbonate, 1 g; and agar, 15 g. Vegetative growth from these slants was transferred to flasks containing medium 2 consisting of (per liter, distilled H_2O) beef extract, 3 g; tryptone, 5 g; dextrose, 1 g; soluble starch, 24 g; yeast extract, 5 g; and calcium carbonate, 4 g and incubated for 4 days at 37°C on a rotary shaker. From this first stage seed a 10 % inoculum was transferred to medium 2 and fermentation was conducted as above at 28°C for 7~8 days.

Mutation Methods and Selection of Deoxystreptamine-Requiring Mutants

The organism was grown in medium 2 for 3 days at 37°C and the resultant cells were harvested by centrifugation, washed and resuspended in a phosphate-buffered saline solution (pH 7.1) resulting in a final cell volume of 7 %. This suspension was treated with the mutagenic agent N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) at 500 μg/ml for 30 minutes. The mutagenized culture was then centrifuged, decanted and washed with buffered saline. The resulting cell mass was resuspended in buffered saline solution and was vigorously shaken with 12 mesh silicon carbide granules (Hengar Co., Phil., Pa). Ten-fold serial dilutions were made in buffered saline solution and aliquots were plated in a medium (medium 3) containing (per liter, distilled H_2O) trypticase glucose extract, 5 g; trypticase peptone, 3 g; glucose, 1 g; agar, 15 g and incubated at 37°C until colonies were evident (about 7 days). Well isolated colonies were picked to duplicate plates (medium 3), one set of which was overlaid with a spore suspension of *Bacillus subtilis*. After incubation at 37°C for 18~24 hours the patches which showed no zone of inhibition on the *B. subtilis* plate were transferred from the master plate to medium 1 slants and incubated until full growth was evident. Vegetative growth from these slants was transferred to medium 2 and incubated at 37°C on a rotary shaker for 3 days after which portions were streaked as bands about 1-cm wide on the surface of medium 3 plates and incubated at 37°C until growth was evident (3~4 days). Filter paper discs were then saturated with a solution of deoxystreptamine (500 μg/ml) and placed on top of the culture streak. After in-

cubation for 24 hours, the discs were removed and placed on plates containing streptomycin assay agar (medium 4) containing (per liter, distilled H_2O) beef extract, 1.5 g; yeast extract, 3.0 g; peptone, 6.0 g and agar, 15.0 g. These plates were overlaid with a spore suspension of *B. subtilis* and incubated at 37°C for 18~24 hours to allow for growth of the challenge organism. Isolates showing zones of inhibition surrounding the disc were designated as deoxystreptamine idiotrophs. One such mutant which had lost its capacity to produce antibiotic without an exogenous source of deoxystreptamine was designated VIb. This procedure is amenable to screening "non-producing" mutants against a large number of aminocyclitol derivatives.

Strain Improvement Procedures

Micromonspora purpurea mutant VIb was cultivated in medium 2 at 37°C for 3 days and the resultant cells were harvested by centrifugation, washed and resuspended in buffered saline. This suspension was treated with MNNG as above. Samples of the mutagenized culture were plated in medium 3 at 37°C until colonies were evident (7 days). Colonies were picked to duplicate plates containing medium 4.

One set served as a master plate for later recovery whereas the second set contained 25 μg/ml of streptamine sulfate and an overlay spore suspension of *B. subtilis* as a challenge test organism. These plates were incubated at 37°C for 24 hours and examined for zones of inhibition. Those mutants showing the largest zones were transferred from the master plate onto slants of medium 1 and incubated for one week at 37°C. These mutants were compared with *M. purpurea* mutant VIb.

First stage seeds of *M. purpurea* VIb and the new isolates were prepared by transferring a loopful of vegetative growth from slants into flasks containing medium 2. These were incubated for 4 days at 28°C on a rotary shaker. A 5% inoculum was then transferred to sets of medium 2 with and without deoxystreptamine (200 μg/ml). These flasks were incubated and assayed daily by the disc diffusion method for antibiotic production. One isolate was selected for comparison with *M. purpurea* mutant VIb using streptamine. This mutant was designated VIb-3P.

Screening of Idiotrophs with Potential Precursors

Preliminary detection of antibiotic activity was determined by disc testing of broth which had been supplemented with 500 μg/ml of potential precursor, against *B. subtilis*. In addition, paper and silica gel chromatography were used. The chromatography systems were (1) Whatman No. 1 paper saturated with 0.95 M sulfate-bisulfate and developed in descending fashion in 90% aqueous ethanol +1.5% NaCl followed by bioautography using *B. subtilis* and (2) Silica gel 60 F 254 thin-layer plates (TLC) developed in the lower phase of $CHCl_3$-MeOH-conc. NH_3 (28%) (1:1:1) followed by bioautography using *B. subtilis*.

Isolation of New Antibiotics

The antibiotics were obtained from the fermentation broth by adjustment of the pH to 2 with 10 N H_2SO_4, removal of the cells on filter aid, adjustment of the filtrate to pH 6, followed by ion-exchange chromatography (Bio-Rex 70, Na^+ form 20~50 mesh). The column was eluted with acid to afford the mixture of antibiotics. The purified antibiotic mixture was then obtained by the isolation procedures described for gentamicin.[8] The individual components were obtained

by preparative thick-layer chromatography (Silica gel PF 254, 1.0 mm×40 cm×20 cm plates) using the lower phase of a $CHCl_3$-MeOH-conc. NH_3 (28 %) (1:1:1) system. The bands were visualized by spraying the edges and middle of the plate with ninhydrin indicator or by iodine vapor.

Preparation of Aminocyclitols and Characterization of New Antibiotics

All melting points are uncorrected. The mass spectra reported were measured with a Joelco JMS-1-OCS mass spectrograph. Infrared spectra were determined on a Model-21 Perkin Elmer infrared spectrophotometer. ^{1}H-NMR spectral measurements were made on a Varian HA-100 spectrophotometer using $(Me)_4Si$ as an internal standard. Ultra-violet spectra were recorded on a Cary spectrophotometer Model 15.

Aminocyclitols

2-Deoxystreptamine·2HBr (**1**) was obtained from neomycin sulfate by the procedure of KUEHL *et al.*[9] Streptamine·H_2SO_4 (**2**) and streptidine·H_2SO_4 (**7**) were obtained from streptomycin by the procedure of PECK *et al.*[10] 2-Epistreptamine·2HCl (**3**) was prepared by the procedure of SUAMI *et al.*[11] 4, 6-Hydrazino-1, 3-cyclohexanediol (**5**) and 2, 5-dideoxystreptamine ·2HCl (**4**) were prepared by the procedure of SUAMI *et al.*[12] N, N′-Diacetylstreptamine (**9**) and N, N′-dimethyl-2-deoxystreptamine·2HCl (**6**) were prepared by procedures described by SHIER *et al.*[2] 1, 3-Di-N-carbobenzyloxy-2-deoxystreptamine (**8**) was prepared by the method of UMEZAWA *et al.*[13]

1, 3-Di-N-benzylidine-2, 5-dideoxystreptamine (**10**). A solution of 4.5 g (0.031 mol) of compound **4** in 20 ml of benzaldehyde was heated under reflux in a N_2 atmosphere overnight. Benzene (150 ml) was added and the solution was cooled. The precipitated SCHIFF base was collected to afford 8.5 g of crude **10**. Several recrystallizations from acetonitrile afforded 4.2 g of the analytical sample, mp 151~154°C; Mass spectrum $(M^+-1)=321$; UV_{max} (95 % EtOH) 249.5 nm (ε 37,000).

Anal. Calcd. for $C_{20}H_{22}N_2O_2$: C, 74.51; H, 6.88, N, 8.69.
Found: C, 74.5; H, 7.0; N, 8.6.

These structures are shown in Fig. 1.

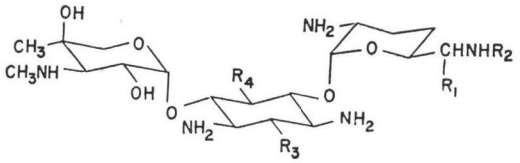
New Antibiotics

2-Hydroxygentamicin C_1 (**14**); mp 119~123°C; pmr $\delta(D_2O)$ 5.87, 5.60 (anomeric H, 2H), 5.22 (exchangeable H, 12H), 3.15, 3.09 (NCH_3, 6H), 2.9~4.8 (CHO, CHN, CH_2O,13 H), 1.9~2.6 (CH_2CH_2, 4H), 1.72 ppm (CH_3C, CH_3CH, 6H). Mass spectrum M^+ 493 fragments. *m/e* 436, 376, 366, 363, 338, 335, 320, 160, 157. $[\alpha]_D^{25°}+128.5$ (0.2 % H_2O). Analysis of **14** was obtained on the H_2SO_4 salt; mp>300°C.

Anal. Calcd. for $C_{21}H_{43}N_5O_3\cdot 2\frac{1}{2}H_2SO_4\cdot 2H_2O$: C, 32.55; H, 6.75; N, 9.04; S, 10.35.
Found: C, 32.5; H, 6.9; N, 9.4; S, 9.8.

2-Hydroxygentamicin C_2 (**15**); mp 115~119°C; pmr $\delta(D_2O)$ 5.82, 5.56 (anomeric H, 2H), 5.20 (exchangeable H, 13H), 3.09 (NCH_3, 3H), 3.0~4.6 (CHO, CHN, CH_2O, 13H) 1.9~2.5 (CH_2CH_2, 4H), 1.73 ppm (CH_3C, CH_3CH, 6H); mass spectrum (M^++1) 480 fragments. *m/e* 436, 366, 362, 349, 338, 321, 320, 160, 143; $[\alpha]_D^{25°}+137.1$ (0.2 % H_2O). Analysis of **15** was obtained on the H_2SO_4 salt; mp 228~230°C.

Fig. 2. Hydroxy- and deoxy- gentamicins



11. Gentamicin C_1: $R_1=R_2=CH_3$, $R_3=H$, $R_4=OH$
12. C_2: $R_1=CH_3$, $R_2=R_3=H$, $R_4=OH$
13. C_{1a}: $R_1=R_2=R_3=H$, $R_4=OH$
14. 2-Hydroxygentamicin C_1: $R_1=R_2=CH_3$, $R_3=R_4=OH$
15. C_2: $R_1=CH_3$, $R_2=H$, $R_3=R_4=OH$
16. C_{1a}: $R_1=R_2=H$, $R_3=R_4=OH$
17. 5-Deoxygentamicin C_1: $R_1=R_2=CH_3$, $R_3=R_4=H$
18. C_2: $R_1=CH_3$ $R_2=R_3=R_4=H$
19. C_{1a}: $R_1=R_2=R_3=R_4=H$

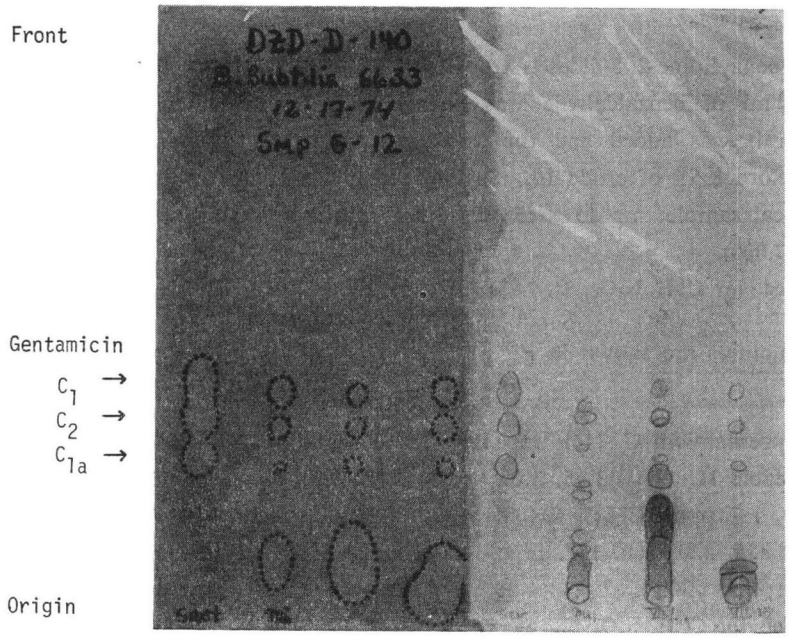
Anal. Calcd. for $C_{20}H_{41}N_5O_8\cdot 2\frac{1}{2}H_2SO_4\cdot 3H_2O$: C, 30.85; H, 6.73; N, 8.99; S, 10.29.
Found: C, 30.5; H, 6.5; N, 9.0; S, 10.1.

5-Deoxygentamicin C_1 (**17**); pmr $\delta(D_2O)$ 5.52, 5.42 (anomeric H, 2H), 5.20 (exchangeable, 10H), 3.00, 2.92 (CH_3N, 6H), 1.68, 1.62 (CH_3C, CH_3CH, 6H), 1.3~4.5 ppm (all other H, 17H), mass spectrum M^+461 fragments. *m/e* at 404, 344, 334, 331, 306, 303, 288, 160, 157.

5-Deoxygentamicin C_{1a} (**19**); pmr $\delta(D_2O)$ 5.52, 5.44 (anomeric H, 2H) 5.20 (exchangeable H, 11H), 3.00 (CH_3N, 3H), 1.67 ($\underline{CH_3}C$, 3H), 1.3~4.5 (all other H, 19H); mass spectrum M^+433 fragments. *m/e* at 334, 316, 306, 303, 288, 275, 160, 129.

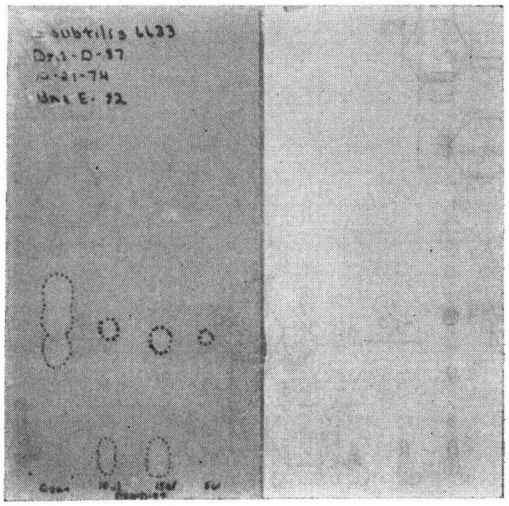
5-Deoxygentamicin C_1+C_2 (**17** and **18**);

Fig. 3. TLC chromatography of gentamicin produced when 2-deoxystreptamine (**1**) is supplemented to *M. purpurea* VIb and VIb-3P
Solvent system: $CHCl_3$–MeOH–conc. NH_4OH (1:1:1, lower phase)



1=Commercial gentamicin U.S.P. 2=*M. purpurea* M_1 production.
3=*M. purpurea* VIb+(**1**) production. 4=*M. purpurea* VIb-3P+(**1**) production

Fig. 4. TLC chromatography of the gentamicin complex compared to the product obtained by supplementation of streptamine (**2**) to the broth of growing *M. purpurea* VIb
Solvent system: $CHCl_3$–MeOH–conc. NH_4OH (1:1:1, lower phase)



1=commercial gentamicin U.S.P. 2, 3 & 4=different concentrations of product obtained from supplementation with (**2**). 4 on bioautograph is isolated 2-hydroxygentamicin C_1 (**14**).

Table 1. *In vitro* antibacterial activity of the 2-hydroxygentamicin complex compared to the gentamicin complex

Test organism	Minimum Inhibitory Concentration (mcg/ml)	
	2-OH Gentamicin (**14** and **15**)	Gentamicin (**11**, **12** and **13**)
Staphylococcus aureus Smith	0.78	0.39
Escherichia coli Vogel	3.13	3.13
Escherichia coli W 677/HJR 66	50	>100
Escherichia coli JR 76.2	6.25	50
Escherichia coli JR 35	1.56	1.56
Escherichia coli JR 89	25	50
Escherichia coli K12ML1629	1.56	1.56
Proteus mirabilis MGH-1	6.25	1.56
Klebsiella pneumoniae A20636	6.25	25
Klebsiella pneumoniae 39645	3.13	1.56
Pseudomonas aeruginosa MGH-2	1.56	0.39
Pseudomonas aeruginosa A20897	50	>100
Enterobacter cloacae A20960	3.13	25
Providencia 164	50	100
Providencia stuartii A20894	100	>100

mass spectrum M^+ 461 and 447 fragments at *m/e* 404, 344, 334, 331, 330, 317, 306, 303, 289, 288, 160, 157, 143.

These structures are shown in Fig. 2.

In Vitro Testing

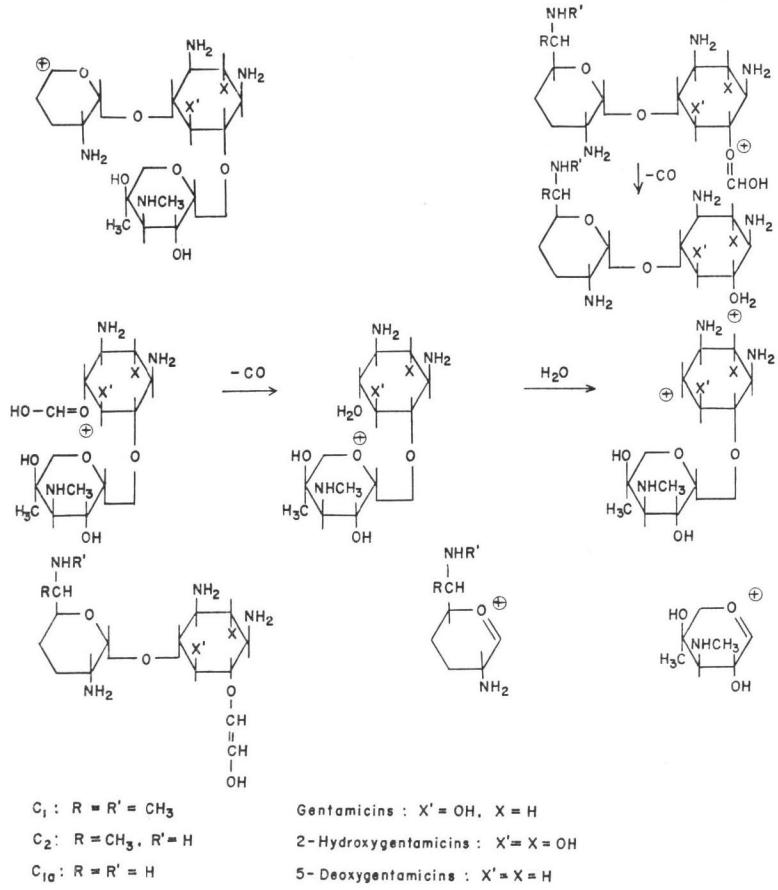
The antibiotics prepared were tested *in vitro* by tube dilution tests in MUELLER-HINTON broth by standard two-fold dilution procedures.[14]

Results and Discussion

After examining approximately 45,000 isolates of *M. purpurea* surviving exposure to the mutagen, (MNNG), for antibiotic activity, one idiotrophic mutant (*M. purpurea* VIb) which was capable of producing antibiotic only when supplemented with 2-deoxystreptamine (**1**), was obtained.

M. purpurea (parent strain, M_1) normally produces a mixture of gentamicin antibiotics consisting mostly of the gentamicin C complex.[7] This complex consists of three anti-

Fig. 5. Structures of major mass fragments



biotics called C_1, C_2 and C_{1a} (**11**, **12**, **13**).[15] The mixture of gentamicin C antibiotics produced by supplementation of the growing idiotroph VIb with 2-deoxystreptamine (**1**) was identical to the gentamicin C mixture produced by the parent strain (M_1) when compared by bioautography, (Fig. 3). The separated components were identical to the three authentic components when compared by mass spectrometry.

Streptamine (**2**), a close analog of deoxystreptamine (**1**) successfully afforded new antibiotics when supplemented to growing cultures of deoxystreptamine-requiring mutants of the organisms that produce neomycin,[2] paromomycin,[2] ribostamycin,[3] butirosin,[4] and sisomicin.[5] A growing culture of *M. purpurea* VIb when supplemented with streptamine produced a mixture of 2-hydroxygentamicins. The C_1 component (**14**) was by far the major component of the mixture with the C_2 component (**15**) not visible by bioautography of an isolated crude antibiotic mixture (Fig. 4). Column chromatography of the crude antibiotic mixture afforded pure 2-hydroxygentamicin C_1 (**14**). The new antibiotic which was more polar than gentamicin C_1 (**11**) when compared by silica gel thin-layer chromatography was characterized by pmr, ms and analysis of its H_2SO_4 salt. The mass spectrum showed a molecular ion (493) and major fragments (Fig. 5) each 16 mass units (*i.e.*, one oxygen) greater than that obtained from gentamicin C_1 (**11**).[16]

Table 2. *In vitro* antibacterial activity of the 5-deoxygentamicin complex and its C_1 component compared to the gentamicin complex

Test organism	Minimum inhibitory concentration (mcg/ml)		
	5-Deoxygentamicin complex (**17**, **18**, **19**)	5-Deoxygentamicin C_1 (**17**)	Gentamicin complex (**11**, **12**, **13**)
Staphylococcus aureus Smith	0.195	0.195	0.39
Escherichia coli Vogel	1.56	1.56	3.13
Proteus mirabilis MGH-1	1.56	0.78	1.56
Klebsiella pneumoniae 39645	0.78	0.78	1.56
Pseudomonas aeruginosa MGH-2	0.78	1.56	0.78
Escherichia coli JR76.2	50	50	50
Escherichia coli JR89	6.25	12.5	50
Enterobacter cloacae A20960	25	12.5	25
Klebsiella pneumoniae A20636	25	25	25
Providencia stuartii A20894	25	50	100
Providencia 164	12.5	25	100
Pseudomonas aeruginosa A20741	100	100	100
Pseudomonas aeruginosa A20897	12.5	6.25	100

Table 3. Comparative acute (i.v.) toxicity of 2-hydroxygentamicin C complex, 5-deoxygentamicin C complex and gentamicin C complex in the mouse

Compound	7-Day LD_{50} mg/kg	Relative toxicity
Gentamicin (C_1, C_2 and C_{1a})	87.0 (74.3~100)	1.0 (defined)
2-Hydroxygentamicin (C_1 and C_2)	150 (134~200)	0.57 (0.44~0.73)
5-Deoxygentamicin (C_1, C_2 and C_{1a})	34.6 (31.1~37.8)	2.6 (2.0~3.3)

It was apparent that the biosynthetic pathway to products (C_1, C_2 and C_{1a}) had been altered when streptamine was supplemented to a culture of mutant VIb and that the shift in ratio of components could be due to the poor antibiotic producing ability of this strain.

By MNNG treatment of mutant VIb and strain selection techniques using streptamine (**2**) supplementation, another idiotroph of *M. purpurea* (VIb-3P) was isolated. This idiotroph afforded the usual mixture of gentamicins (C_1, C_2 and C_{1a}) when a growing culture was supplemented with 2-deoxystreptamine (**1**) (Fig. 3). When streptamine (**2**) was used a mixture of 2-hydroxygentamicins (C_1 and C_2; **14** and **15**) was obtained. The two components were separated by thick-layer chromatography and characterized by pmr, mass spectrometry and analysis of their H_2SO_4 salts. The C_1 (**14**) component was identical to that obtained from mutant VIb. The mass spectrum of the 2-hydroxygentamicin C_2 (**15**) showed an M+1 peak (480) and major fragments (Fig. 5), each 16 mass units greater than that obtained from gentamicin C_2 (**12**).[16] Only a trace amount of a more polar antibiotic was isolated from the 2-hydroxygentamicin complex obtained from either mutant to which was assigned the C_{1a} structure (**16**) on the basis of a mass spectrum that contained fragments at *m/e* 129 (purpurosamine) and 160 (garosamine).

The 2-hydroxygentamicin complex (C_1, C_2, **14**, **15**) was tested against a series of gram-negative and gram-positive organisms, some of which show gentamicin resistance by the tube dilution technique (Table 1). The MIC values indicate that 2-hydroxygentamicin has slightly less *in vitro* activity against gentamicin-sensitive organisms but greater activity against some

gentamicin-resistant strains such as *E. coli* JR 76.2, *E. cloacae* A20960 and *K. pneumoniae* A20636 known to contain adenylating R-factor enzymes.

2, 5-Dideoxystreptamine (**4**), a compound described by SUAMI *et al.*[12] and used by TESTA *et al.*[5] in supplementation studies with an idiotroph of *M. inyoensis* to yield a 5-deoxy analog of sisomicin, showed antibiotic activity when fed to growing idiotrophs. Such a process using a growing culture of VIb-3P afforded a new complex of antibiotics, 5-deoxygentamicin C_1, C_2 and C_{1a} (**17**, **18**, **19**). TLC of this C complex indicated that the corresponding analogs to the gentamicin C complex were less polar when developed with the same solvent system. Thick-layer chromatographic separation (silica gel) afforded the C_1 (**17**) and C_{1a} (**19**) components, which were subjected to mass spectrographic characterization. 5-Deoxygentamicin C_1 (**17**) gave a mass peak at 461, 5-deoxygentamicin C_{1a} (**19**) gave a mass peak at 433. The C_2 component was contaminated with C_1 but showed a mass peak at 447. These masses, as well as the major fragments were 16 mass units less than those for the gentamicin C analogs (Fig. 5). The 5-deoxygentamicin complex (C_1, C_2, C_{1a}, **17**, **18**, **19**) as well as the individual C_1 (**17**) and C_{1a} (**19**) components were tested against a series of gram-negative and gram-positive organisms some of which show gentamicin resistance (Table 2).

The MIC values of the 5-deoxygentamicin complex were similar to the gentamicin complex against most of the organisms screened. The new antibiotic complex was more active against the gentamicin-resistant *E. coli* JR-89, which contains the 3-amino (of deoxystreptamine), acetylating enzyme, and *P. aeruginosa* A20897. It is interesting to note here that the C_1 component is quite active against *P. aeruginosa* A20897, an organism that contains the 6'N-acetylating enzyme.

The acute (i.v.) toxicity (LD_{50}) of the 2-hydroxygentamicin C complex was compared to the 5-deoxygentamicin C complex and to gentamicin C complex (Table 3). The relative toxicity of the 2-hydroxygentamicin C complex was about half that of the gentamicin C complex whereas the 5-deoxygentamicin C complex was about 2.5 times as toxic as the gentamicin C complex.

4, 6-Hydrazino-1, 3-cyclohexandiol (**5**), the synthetic precursor[12] of 2, 5-dideoxystreptamine (**4**), as well as 1, 3-di-N-benzylidine-2, 5-dideoxystreptamine (**10**) afforded the same 5-deoxygentamicin complex (**17**, **18**, **19**) when supplemented to growing cultures of VIb or VIb-3P as shown by silica-gel bioautography.

2-Epistreptamine (**3**),[11] a compound that was successfully bioconverted by the mutant of *Streptomyces fradiae*[1] to hybrimycins B_1 and B_2, also stimulated antibiotic biosynthesis when added to *M. purpurea* VIb or VIb-3P. The activity observed on a test plate (*B. subtilis* overlay) was much less than that observed when an equal amount of streptamine was supplemented. This result is not unexpected in light of the activity difference observed by SHIER *et al.* between hybrimycins A (streptamine analog) and B (epistreptamine analog).[1,17]

Compounds **6**, **7**, **8**, and **9** did not yield antibiotic when fed to growing cultures of these idiotrophs, as was expected.

Deoxystreptamine (**1**) or 2, 5-dideoxystreptamine (**4**) are incorporated by the *M. purpurea* mutant VIb-3P to give the same ratio of C_1, C_2 and C_{1a} antibiotics as the parent (M_1) strain of *M. purpurea*. Incorporation of streptamine, however, is such that the major components of the C complex of antibiotics produced are almost totally C_1 and C_2. If one considers the branched pathway suggested by TESTA and TILLY[18] for the production of gentamicins C_1, C_2

and C_{1a} antibiotics from a common precursor antibiotic, then it would appear that streptamine incorporation interferes almost completely with the branch of the biosynthetic pathway leading to C_{1a}.

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