AMINOGLYCOSIDE ANTIBIOTICS

7. DIHYDROSTREPTOMYCIN ANALOGUES†

DANA L. DELAWARE, MAHENDRA S. SHARMA, BHASHYAM S. IYENGAR and WILLIAM A. REMERS*

Department of Pharmaceutical Sciences, College of Pharmacy, University of Arizona, Tucson, AZ 85721, U.S.A.

TERESA A. PURSIANO

Bristol-Myers Company, Syracuse, NY 13221-4755, U.S.A.

(Received for publication September 6, 1985)

Dihydrostreptomycin analogues with structural variations in their guanidino groups were prepared. The analogue with a methyl group on the guanidine at C-1 was nearly as active as dihydrostreptomycin against bacteria. However, the 2-imidazolin-2-ylamino substituent at C-1 eliminated activity. No analogue with a substituent on the C-3 guanidino group was active.

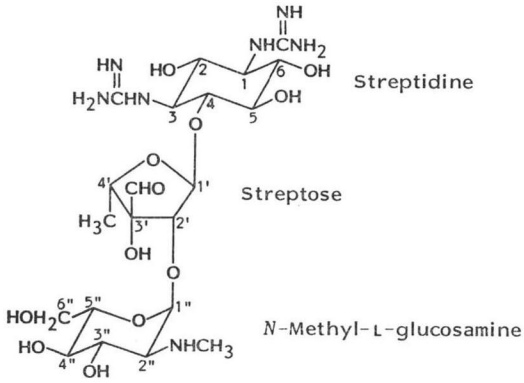
Since its discovery by WAKSMAN in 1944,[1] streptomycin (**1**) has maintained a significant place in the therapy of tuberculosis and certain other bacterial infections. However, a number of species, particularly *Staphylococcus* and Gram-negative enteric bacteria, have developed resistance to streptomycin. This resistance has taken the form of decreased cell permeability, decreased ribosomal affinity, and enzymatic transformation.[2]

Numerous streptomycin analogs have been prepared in attempts to obtain superior therapeutic agents and to define structure-activity relationships. Most of these analogs involved changes in the streptose unit, whose carboxaldehyde group underwent a variety of chemical transformations including reduction to dihydrostreptomycin (**2**),[3] alkylation,[4] reductive amination,[5] and hydrazone formation.[6] The *N*-methyl-L-glucosamine unit also has afforded a number of derivatives, especially involving acylation or other substitution of the nitrogen atom.[7] And recently, 3″-deoxydihydrostreptomycin and 3″-epidihydrostreptomycin were synthesized and found to be active against bacterial strains that phosphorylate or adenylylate the 3″-hydroxyl group of streptomycins.[8~10]

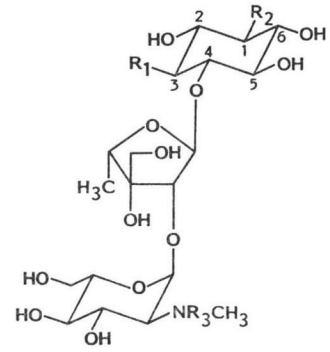
In contrast, only a few streptomycin analogs have been prepared in which changes were limited to the streptidine unit. The natural product bluensomycin (glebomycin), which has a carbamoyl group instead of a guanidino group at C-1, is active against bacteria.[11] However, analogs without both guanidino groups,[12,13] or in which these groups have been replaced by pyrimidinyl groups,[13] are inactive. 6-Deoxydihydrostreptomycin was prepared and showed substantial activity.[14]

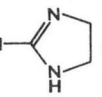
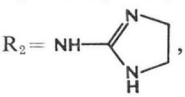
Because of the very limited scope of analog preparation involving the streptidine unit, we thought that additional variants should be made and tested, from the standpoint of defining more closely the

† Taken in part from the Ph.D. dissertations of D. DELAWARE (Purdue University) and M. SHARMA (University of Arizona). This research was started at Purdue University in 1975, moved to the University of Arizona in 1976 and completed in 1985.



Streptomycin (**1**)



2: $R_1=R_2=NHC(=NH)NH_2$, $R_3=H$ (dihydrostreptomycin)
3: $R_1=R_2=NH_2$, $R_3=H$ (dideamidinodihydrostreptomycin)
4: $R_1=R_2=N=CHC_6H_4Cl$-*p*, $R_3=H$
5: $R_1=R_2=NHCH_2C_6H_4Cl$-*p*, $R_3=H$
6: $R_1=R_2=NHC_2H_5$, $R_3=H$
7: $R_1=R_2=NHC(=NH)CH_3$, $R_3=H$
8: $R_1=R_2=NHC(=S)NHCH_3$, $R_3=C(=S)NHCH_3$
9: $R_1=R_2=NHC(=NH)NHCH_3$, $R_3=H$
10: $R_1=R_2=NH_2$, $R_3=CH_2C_6H_5$
11: $R_1=R_2=NHCH_3$, $R_3=CH_2C_6H_5$
12: $R_1=R_2=N(CH_3)C(=NH)NH_2$, $R_3=H$
13: $R_1=NH_2$, $R_2=$  , $R_3=H$
14: $R_1=NHC(=NH)NH_2$, $R_2=NH_2$, $R_3=H$
15: $R_1=NHC(=NH)NH_2$, $R_2=NHC(=NH)NHCH_3$, $R_3=H$
16: $R_1=NHC(=NH)NH_2$, $R_2=$  , $R_3=H$

structure-activity relationships, and in the hope that more active compounds might be found. Our approach was to degrade dihydrostreptomycin (**2**) (which is equally active to streptomycin),[15] to dideamidinodihydrostreptomycin (**3**), and reconstruct functional groups related to guanidine. Because HEDING and LUTZEN had proposed that dihydrostreptomycin competes with magnesium for binding sites on ribosomes,[13] we felt that some of these functional groups should be relatively basic to ensure protonation under physiological conditions. Furthermore, we hoped that certain analogs such as **4** and **5** with large lipophilic groups might be superior in penetrating the cell membranes of bacterial species resistant because of impermeability to streptomycins.

The first analogs prepared were **4** and **5**. Since Schiff's base formation between *p*-chlorobenzaldehyde and either kanamycin A[16] or gentamicin C_2[17] led to active derivatives, we thought that this might be the best type of derivative for the streptomycin series. Thus, dihydrostreptomycin sulfate was hydrolyzed to dideamidinodihydrostreptomycin (**3**) by WOLFROM's method.[12] Material prepared in this manner did not give a satisfactory combustion analysis. However, after chromatography on Amberlite CG-50 resin the pure sesquihydrate was obtained. Analogs **4** and **5** then were prepared from **3** by treatment with *p*-chlorobenzaldehyde in methanol and reduction of **4** with sodium borohydride. Since neither of these analogs showed any antibacterial activity, it was apparent that structure-activity relationships for kanamycin and gentamicin were not pertinent to streptomycin.

In view of the results just described, we concentrated on analogs that resembled dihydrostreptomycin more closely in structure and basicity. The isosteric diethylamino and diacetamidino analogs (**6** and **7**, respectively) were chosen along with the homologous di-*N*-methylguanidino analog **9**. Syn-

thesis of **6** by treatment of **3** with acetaldehyde and sodium borohydride was straightforward. The PINNER synthesis,[18] involving condensation of **3** with ethyl acetimidate hydrochloride in *N*,*N*-dimethylformamide, afforded **7** in 90% yield. We were unable to prepare di-*N*-methylguanidino analog **9** by treating **3** with methylisothiocyanate. This reaction gave the tri-*N*-methylthiourea derivative **8**. However, **9** was prepared conveniently from **3** and *N*,*S*-dimethylisothiouronium iodide. Synthesis of **12**, in which the two methyl groups are on the nitrogens attached directly to the ring, required an indirect route because of the methylamino group in the *N*-methyl-L-glucosamine moiety. Direct methylation of dideamidinodihydrostreptomycin would give three methylamino groups among which specificity in guanidine formation was unlikely. Therefore, the methylamino group was first protected as its *N*-benzyl derivative (**10**). Methylation of the two amino groups in the streptamine ring was accomplished by treatment with benzyloxycarbonyl chloride followed by lithium aluminum hydride reduction. The product **11** was treated with *N*,*S*-dimethylthiouronium iodide and then the *N*-benzyl group was removed by hydrogenolysis to furnish **12**. An attempt to prepare the diimidazolinyl derivative from **3** and 2-methylthio-2-imidazoline hydroiodide gave only the monosubstituted product, which is assigned structure **13** because it gave a large positive rotation difference, Δ[M]TACu = +1,980.6°, when treated with TACu according to UMEZAWA's procedure. This value is close to that found by UMEZAWA for 3-deamidinodihydrostreptomycin (+1,570°).[19]

Antibacterial screening of compounds **6** through **13** revealed no activity at concentrations of 63 μg/ml or lower. This result was surprising, particularly for compound **9** which differs from dihydrostreptomycin only by the methyl group on each guanidine residue. It was now apparent that the streptidine ring of dihydrostreptomycin is highly sensitive to structural changes in its ability to bind to ribosomes. Our approach was modified to making changes in only one of the guanidine residues

Table 1. Antibacterial activity of dihydrostreptomycin derivatives by serial dilution.[a]

Organism[b]	MIC (μg/ml)			Organism[b]	MIC (μg/ml)		
	2	**15**	**16**		**2**	**15**	**16**
S.a. A-9537	2	8	>63	*E.c.* A20520	>63	>63	>63
A22210	4	8	>63	*K.p.* A20468	>63	>63	>63
A21978	>63	>63	>63	*P.m.* A-9900	4	16	>63
A20240	>63	>63	>63	*P.r.* A-9637	2	1	32
A22058	1	2	63	*P.s.* A21210	32	32	>63
A22231	2	4	>63	A20894	>63	>63	>63
E.c. A22356	4	4	>63	A21207	>63	>63	>63
A22480	>63	>63	>63	*P.a.* A21508	4	8	>63
A-9632	2	2	>63	A-9843A	4	8	>63
A20665	>63	>63	>63	A20601	>63	>63	>63
A20683	>63	>63	>63	A20897	16	16	>63
A20895	>63	>63	>63	A20653	>63	>63	>63
A22045	>63	>63	>63	A20741	>63	>63	>63
A21218	2	4	>63	A22233	>63	>63	>63
A20732	>63	>63	>63	A21509	16	16	>63

[a] All assays were determined at Bristol-Myers Company, Syracuse, N.Y. For a complete description of the antibacterial assay protocol see MISIEK, M.; T. A. PURSIANO, L. B. CRAST, F. LEITNER & K. E. PRICE: Antimicrob. Agents Chemother. 1: 54, 1972

[b] Abbreviations for bacteria: *S.a.*, *Staphylococcus aureus*; *E.c.*, *Escherichia coli*; *K.p.*, *Klebsiella pneumoniae*; *P.m.*, *Proteus mirabilis*; *P.r.*, *Providencia rettgeri*; *P.s.*, *Providencia stuartii*; *P.a.*, *Pseudomonas aeruginosa*. Numbers refer to Bristol-Myers culture numbers.

at a time. The residue on C-1 appeared the better one to modify because the structural change in bluensomycin (urea for guanidine) occurs at this position.[11] At about this time a publication from UMEZAWA's laboratory appeared.[19] It revealed that partial hydrolysis of dihydrostreptomycin followed by chromatography could furnish the isomeric monodeamidino derivatives and that the one retaining the guanidine residue at C-3 (**14**) retained some antibacterial activity, whereas the other did not. Furthermore the 1-*N*-(4-amino-2-hydroxybutyryl) derivative of **11** had antibacterial activity, although it was less than that of dihydrostreptomycin. These results made it clear that the guanidine residue at C-1 was the one to vary.

By modifying slightly the procedure for dihydrostreptomycin hydrolysis we obtained a product mixture that consisted mostly of the 1-deamidino derivative **14**. This compound could be purified by crystallization from water - acetone, which made the chromatographic separation unnecessary. Treatment of **11** with *N,S*-dimethylisothiouronium iodide or with 2-methylthio-2-imidazoline hydroiodide gave the expected *N*-methylamidino derivative **15** or imidazolino derivative **16** as their hydroiodide salts. Antibacterial assay of **15** and **16** (Table 1) showed that the former was nearly as active as dihydrostreptomycin against the various species, whereas the latter was inactive.

Conclusion

From UMEZAWA's study[19] and ours it is clear that no variations are permitted in the guanidine residue at C-3. Even the addition of a methyl group destroys antibacterial activity, as shown by the difference between **9** and **15**. Modification of the guanidine residue at C-1 can be done with retention of activity, which is nearly complete when only a methyl group is added. However, no increase in activity has been found for any derivative at this position. The aminohydroxybutyryl group did not protect dihydrostreptomycin against inactivation by bacterial strains that phosphorylate it,[19] in contrast to the significant protection that this group provides for other aminoglycosides such as kanamycin and ribostamycin. As noted above, there appears to be little correlation between substituent effects in streptomycin derivatives and other types of aminoglycosides.

Experimental

Dideamidinodihydrostreptomycin (**3**)[12,13]

A solution of dihydrostreptomycin sulfate (2.5 g) in water (10 ml) was treated with saturated barium hydroxide solution (125 ml). The mixture was filtered and the filtrate was heated at reflux until ammonia evolution ceased (27 hours). It was cooled, filtered, and neutralized with carbon dioxide. After filtration, the resulting solution was concentrated to a syrup, which was dissolved in hot methanol, cooled, and filtered. Concentration of the filtrate to 20% of its original volume and cooling gave 0.89 g (99%) of **3** as a white solid with mp 165~171°C (dec). This product was purified further by chromatography on a column of Amberlite IRC-50 (NH_4^+) with elution by 0.1 N ammonium hydroxide. Ninhydrin positive fractions were combined and concentrated to give 115 mg of **3** as its crystalline sesquihydrate, mp 170~171°C.

Anal Calcd for $C_{19}H_{37}N_3O_{12}\cdot 1.5H_2O$: C 43.34, H 7.65, N 7.98.
Found: C 43.68, H 7.75, N 8.00.

1,3-Bis(*N*-4-chlorobenzylidene)dideamidinodihydrostreptomycin (**4**)

A solution of **3** (100 mg) and 4-chlorobenzaldehyde (84 mg) in methanol (10 ml) was heated at reflux for 4.5 hours, cooled, and concentrated. The oily residue was dissolved in hot chloroform and cooled in an ice bath, whereupon the product crystallized. This procedure gave 89 mg (62%) of **4** as the dihydrate, mp 175~180°C: IR (KBr) 1640 cm^{-1} (C=N); NMR (DMSO-d_6) δ 7.4 and 7.8 (8, phenyl), 8.2 (s, 2, HC=N). The analytical sample, prepared by a further crystallization from $CHCl_3$ -

hexane, had mp 179~180°C.

Anal Calcd for $C_{33}H_{43}Cl_2N_3O_{12}\cdot 2H_2O$: C 50.71, H 6.08, N 5.39, Cl 9.07.
Found: C 50.55, H 5.68, N 5.95, Cl 8.55.

1,3-Bis(4-chlorobenzylamino)dideguanidinodihydrostreptomycin (**5**)

A solution of **3** (100 mg) and 4-chlorobenzaldehyde (84 mg) in methanol (10 ml) was heated at reflux for 1 hour. Excess sodium borohydride was added and the mixture was heated 3 hours further. It was cooled, acidified to pH 2.0 with concentrated methanolic HCl, and charged onto a column of Amberlite IRC-50 (NH_4^+). This column was eluted with 0.05 N ammonium hydroxide and fractions with pH 8.0 to 10 were combined and concentrated. Treatment of the syrupy residue with *n*-hexane gave 53.3 mg (36%) of **5** as the dihydrate, white crystals with mp 184~185°C: IR (KBr) 810 cm^{-1} (1,4-benzenoid); NMR (DMSO-d_6) δ 7.35 (s, 8, phenyl). The analytical sample was recrystallized from $CHCl_3$ - hexane to give mp 184~185°C.

Anal Calcd for $C_{33}H_{47}Cl_2N_3O_{12}\cdot 2H_2O$: C 50.54, H 6.50, N 5.37, Cl 9.04.
Found: C 50.39, H 6.50, N 5.95, Cl 9.10.

1,3-Di(ethylamino)dideguanidinodihydrostreptomycin (**6**)

A mixture of **3** (100 mg), acetaldehyde (2 ml, excess) and methanol (3 ml) was stirred for 6 hours, concentrated to one-half volume, and treated with sodium borohydride (50 mg, excess). The pH was reduced to 2.0 with methanolic HCl and then readjusted to 10.0 with 5% sodium hydroxide. The resulting solution was directly chromatographed on Amberlite CG-50 (NH_4^+) with 0.05 N ammonium hydroxide as solvent. Fractions with pH of 8~10 were combined and concentrated under reduced pressure. The white crystalline residue (30 mg, 29%) had mp 206~208°C (dec): NMR (D_2O) δ 1.06 (s, t, 9, CH_3), 2.9 (q, 4, CH_2CH_3).

Anal Calcd for $C_{23}H_{45}N_3O_{12}$: C 49.70, H 8.18, N 7.56.
Found: C 49.90, H 8.02, N 7.50.

1,3-Diacetamidinodideguanidinodihydrostreptomycin (**7**)

A solution of **3** (140 mg) in *N*,*N*-dimethylformamide (4 ml) was treated with ethylacetimidate hydrochloride[18] (81 mg) and the mixture was heated 6 hours at 60°C. Water (1 ml) was added and the mixture was heated 1 hour further, cooled, and concentrated. Trituration of the oily residue with acetone gave 165 mg (90%) of **7** as the 2.5 hydrochloride monohydrate, mp 186~189°C: IR (KBr) 1655 cm^{-1} (C=N).

Anal Calcd for $C_{23}H_{43}N_5O_{12}\cdot 2.5HCl\cdot H_2O$: C 39.98, H 6.94, N 10.14, Cl 12.81.
Found: C 39.80, H 6.61, N 10.47, Cl 13.10.

1,3,2″-Tris(*N*-methylaminothiocarbonyl)dideamidinodihydrostreptomycin (**8**)

A mixture of **3** (100 mg), *N*-methylisothiocyanate (43 mg) and methanol (5 ml) was heated at reflux for 2 hours, cooled, diluted with water (15 ml), washed two times with $CHCl_3$, and concentrated. Addition of acetone to the residual syrup gave 75 mg (56%) of **8** as the 2.5 hydrate, a white powder with mp 198~204°C (dec): IR (KBr) 1520 cm^{-1} (NC=S); NMR (D_2O) δ 3.0 (m, 12H, NCH_3). Recrystallization from methanol - acetone gave the analytical sample, mp 198~204°C (dec).

Anal Calcd for $C_{25}H_{46}N_6O_{12}S_3\cdot 2.5H_2O$: C 39.33, H 6.68, N 11.01, S 12.60.
Found: C 39.77, H 6.29, N 10.44, S 12.26.

1,3-Bis(*N*-dimethylamidino)dideamidinodihydrostreptomycin (**9**)

A solution of **3** (100 mg) and *N*,*S*-dimethylthiouronium iodide (200 mg) in dry *N*,*N*-dimethylformamide (5 ml) was heated at 100~110°C for two days. The resulting solution was concentrated under reduced pressure and the residue was purified by chromatography on Amberlite IRC-50 resin which had been neutralized by treatment with 1.0 N ammonium hydroxide and then with water. For the chromatography, a water solution of the residue was added to the Amberlite column and was eluted with water, followed by ammonium hydroxide of increasing strength (0.1~0.5 N). Fractions were examined by TLC on silica gel using 1-butanol - acetic acid - water (4: 1: 2) and those giving a single spot were combined and concentrated to a small volume. Addition of acetone gave a pale yellow solid that weighed 67 mg (50%) after drying under vacuum at 50°C for 4 hours: mp 270°C

(dec): IR (KBr) 3450~3360, 1640 (C=N), 1540 cm^{-1}; NMR (D_2O) δ 1.1 (d, 3H, 4′-CH_3), 2.1 (s, 3H, 2″-NCH_3), 2.7 (s, 6H, amidino CH_3).

Anal Calcd for $C_{23}H_{45}N_7O_{12}\cdot H_2CO_3\cdot 2H_2O$: C 40.62, H 7.20, N 13.82.
Found: C 40.28, H 6.63, N 13.78.

2″-*N*-Benzyldideamidinodihydrostreptomycin (**10**)

A suspension of **2** (free base, 1.0 g) in water (10 ml) and acetonitrile (10 ml) was stirred with benzaldehyde (1.06 g, 10 mmol). After 6 hours, sodium cyanoborohydride (1.26 g) was added. The mixture was stirred overnight, concentrated under reduced pressure, and treated with saturated barium hydroxide solution (150 ml). A white precipitate that formed was removed by filtration and the filtrate was heated at reflux temp until the evolution of ammonia ceased (30 hours). The mixture was cooled, filtered through diatomaceous earth, and the filtrate was treated with carbon dioxide. Precipitated barium carbonate was removed by filtration and the filtrate was concentrated to a small volume. This concentrate was chromatographed on neutralized Amberlite IRC-50 exchange resin with elution first by water and then by ammonium hydroxide in increasing concentration (0.1~0.5 N). Fractions showing a single spot on TLC (silica gel with 1-butanol - acetic acid - water, 4: 1: 2) were combined and concentrated to give 0.6 g (60%) of **10** as the carbonate salt with mp 235~237°C (dec): IR (KBr) 3340, 1660, 1550 cm^{-1}; NMR (D_2O) δ 1.2 (d, 3H, 4′-CH_3), 2.5 (s, 3H, 2″-NCH_3), 7.4 (s, 5H, phenyl).

Anal Calcd for $C_{26}H_{43}N_3O_{12}\cdot 2H_2CO_3\cdot 4H_2O$: C 42.80, H 7.00, N 5.30.
Found: C 42.90, H 7.42, N 4.82.

2″-*N*-Benzyl-1,3-di-*N*-methyldideamidinodihydrostreptomycin (**11**)

A suspension of **10** (0.6 g) and sodium carbonate (1.0 g) in water (10 ml) and toluene (5 ml) was cooled at 0°C, stirred, and treated with a solution of benzyl chloroformate (1.0 ml) in toluene (5 ml), added gradually. After 2 hours, the mixture was placed in a refrigerator overnight. The resulting white precipitate was collected, washed with ether and with water, and dried overnight at 50°C under vacuum. It weighed 0.45 g and showed IR (KBr) peaks at 3400, 1700, 1680 (C=O) and 1540 cm^{-1}.

Without further purification, this carbobenzyloxy derivative (0.34 g) was suspended in freshly distilled tetrahydrofuran (60 ml), cooled at 0°C and treated with lithium aluminum hydride (1.0 g) added in portions. After 30 minutes the mixture was warmed to reflux temp and kept there 3 days. Excess hydride was decomposed by adding dilute NaOH at 0°C and the resulting aluminum salts were removed by filtration and washed with tetrahydrofuran. The combined filtrate and wash were concentrated and the residual solid was chromatographed on Amberlite IRC-50 resin in the NH_4^+ form. The Amberlite column was eluted first with water and then with ammonium hydroxide solution, gradually increasing in strength from 0.1 N to 0.5 N. Fractions showing a single spot on TLC (1-butanol - acetic acid - water, 4: 1: 2) were combined and concentrated. Further purification of the solid by dissolution in methanol - water and precipitation with acetone gave 0.17 g (60%) of **11**: mp 156~160°C: IR (KBr) 3400~3300, 1650, 1550 cm^{-1}; NMR (DMSO-d_6) δ 1.1 (d, 3H, 4′-CH_3), 2.5 (s, 9H, 3NCH_3), 7.3 (s, 5H, phenyl).

Anal Calcd for $C_{28}H_{47}N_3O_{12}\cdot H_2CO_3\cdot 3H_2O$: C 47.46, H 7.57, N 5.72.
Found: C 47.43, H 7.38, N 5.90.

1,3-Di-*N*-methyldihydrostreptomycin (**12**)

A mixture of **11** (0.20 g, 0.32 mmol), *N*,*S*-dimethylthiouronium iodide (0.44 g) and *N*,*N*-dimethylformamide (5 ml) was heated at 100~110°C for 48 hours, cooled, and concentrated under reduced pressure. The residue was purified by chromatography on neutralized Amberlite IRC-50 resin. The solvent was water followed by ammonium hydroxide solution, which was increased in concentration from 0.1 N to 0.5 N. Fractions giving a single spot on TLC (1-butanol - acetic acid - water, 4: 1: 2) were combined and concentrated to a small volume. Addition of acetone gave a solid that weighed 0.13 g after drying under vacuum. It showed IR (KBr) peaks at 3420, 1700, 1665 (C=N) and 1420 cm^{-1}.

Without further purification this solid (50 mg) was dissolved in water (10 ml) and dioxane (5 ml) treated with acetic acid (0.5 ml) and 10% palladium-on-charcoal (0.2 g) and shaken with hydrogen at

3.5 kg/cm² in a Parr apparatus for 24 hours at room temp. The mixture was filtered and concentrated under reduced pressure to an oil that solidified on addition of toluene and reconcentration. This solid was chromatographed on neutralized Amberlite IRC-50 resin with water followed by ammonium hydroxide solution (increased gradually from 0.1 N to 0.5 N) as solvent. Fractions giving a positive ninhydrin test were combined and concentrated. Crystallization of the residue from a water - methanol - acetone mixture gave 30 mg (70%) of **12** as white solid with mp 240°C (dec): IR (KBr) 3360, 1665, 1650 (C=N), 1560 cm^{-1}; NMR (D_2O) δ 1.3 (d, 3H, 4′-CH_3), 2.0 (s, 9H, 3NCH_3).

Anal Calcd for $C_{23}H_{45}N_7O_{12}\cdot H_2CO_3\cdot 2H_2O$: C 40.62, H 7.20, N 13.82.
Found: C 40.48, H 7.04, N 14.05.

1-*N*-(2-Imidazolinyl)dideamidinodihydrostreptomycin (**13**)

A mixture of **3** (100 mg), 2-methylthio-2-imidazoline (266 mg) and *N*,*N*-dimethylformamide (10 ml) was heated at 100~110°C for 40 hours. It was then concentrated under reduced pressure and the residue was chromatographed on neutralized Amberlite IRC-50 resin. The solvent was water followed by ammonium hydroxide solution (increased gradually from 0.1 N to 0.5 N). Recrystallization of the product from water - acetone gave 68 mg (60%) of **13** as light yellow solid with mp 200°C (dec): IR (KBr) 3300, 1660 (C=N), 1360 cm^{-1}; NMR ($CDCl_3$) δ 1.2 (d, 3H, 4′-CH_3), 2.4 (s, 3H 2″-NCH_3), 3.6 (br s, 4H, imidazoline); ^{13}C NMR δ 164 (C=N), 109 (C-1′), 101 (C-1″), 88.5 (C-4′), 83.0 (C-3′), 45 (CH_2), 36 (NCH_3), 15 (4′-CH_3); $[M]^{25}$ −1,287° (*c* 0.6, H_2O), $\Delta[M]_{TACu}$ +1,980.6°.

Anal Calcd for $C_{22}H_{41}N_5O_{12}\cdot 3H_2O$: C 42.51, H 7.56, N 11.27.
Found: C 42.23, H 7.32, N 11.16.

Partial Hydrolysis of Dihydrostreptomycin

A solution of **1** (3.0 g) in water (10 ml) was cooled in an ice bath and treated with saturated barium hydroxide solution (125 ml). The cooled mixture was stirred 30 minutes, filtered, and then kept at 27°C for 7 days. It was neutralized by adding solid carbon dioxide, the resulting precipitate of barium carbonate was removed by filtration, and the filtrate was examined by TLC. It showed Rf values of 0.056 and 0.050 (silica gel with $CHCl_3$ - methanol - 17% ammonium hydroxide, 2: 2: 1). The slower moving spot was much more intense than the other. It corresponded to **14**.[18] The filtrate was concentrated to 5 ml and diluted with 15 ml of methanol, which caused the product to precipitate. Crystallization from water - acetone gave 650 mg of **14** as a pale yellow solid that showed a single spot on TLC.

1-*N*-Deamidino-1-*N*-methylamidinodihydrostreptomycin (**15**)

A mixture of **14** (250 mg), *N*,*S*-dimethylisothiouronium iodide (200 mg) and *N*,*N*-dimethylformamide (50 ml) was heated under nitrogen at 110~120°C for 72 hours, cooled, and concentrated under reduced pressure. Addition of methanol (15 ml) to the brown oily residue gave a precipitate that was washed with methanol and then crystallized from water - acetone. This procedure gave 35 mg (15%) of **15** as its hydroiodide, a pale yellow solid with mp 228~235°C (dec): $[\alpha]_{546}^{22}$ −158.2°; IR (KBr) 3300, 1660 (C=N), 1360 cm^{-1}; NMR (D_2O) δ 1.2 (d, 3H, 4′-CH_3), 2.4 (s, 3H, 2″-CH_3), 2.97 (s, 3H, amidino CH_3).

Anal Calcd for $C_{22}H_{43}N_7O_{12}\cdot 1.3HI$: C 34.59, H 5.85, N 12.83.
Found: C 34.17, H 5.48, N 12.54.

1-*N*-Deamidino-1-*N*-(2-imidazolinyl)dihydrostreptomycin (**16**)

A mixture of **14** (250 mg), 2-methylthio-2-imidazoline hydrochloride (200 mg) and *N*,*N*-dimethylformamide (50 ml) was heated under nitrogen at 110~120°C for 72 hours, cooled, and concentrated under reduced pressure. Treatment of the oily brown residue with 15 ml of methanol gave a precipitate that was washed twice with 5 ml portions of methanol. Crystallization from water - acetone gave 50 mg (18%) of **16** as its hydroiodide, a pale yellow solid with mp 240~245°C (dec): $[\alpha]_{546}^{22}$ −254.0°; IR (KBr) 3300, 1660 (C=N), 1360 cm^{-1}; NMR (D_2O) δ 1.2 (d, 3H, 4′-CH_3), 2.4, (s, 3H, 2″-NCH_3), 3.6 (br s, 4H, imidazoline).

Anal Calcd for $C_{23}H_{43}N_7O_{12}\cdot 1.2HI\cdot 2H_2O$: C 34.56, H 6.04, N 12.27, I 19.08.
Found: C 34.11, H 5.42, N 11.92, I 18.33.

Acknowledgment

We thank Dr. RONALD L. BUCHANAN of Bristol-Myers Company for his help in obtaining biological testing.

References

1) SCHATZ, A.; E. BUGIE & S. A. WAKSMAN: Streptomycin, a substance exhibiting antibiotic activity against Gram-positive and Gram-negative bacteria. Proc. Soc. Exp. Biol. Med. 55: 66~69, 1944
2) UMEZAWA, H.: Biochemical mechanism of resistance to aminoglycoside antibiotics. *In* Drug Action and Drug Resistance in Bacteria. 2. Aminoglycoside Antibiotics. *Ed.*, S. MITSUHASHI, pp. 211~218, University of Tokyo Press, Tokyo, 1975
3) PECK, R.; R. GRABER, A. WALTI, E. PEEL, C. HOFFHINE, C. & K. FOLKERS: *Streptomyces* antibiotics. IV. Hydrolytic cleavage of streptomycin to streptidine. J. Am. Chem. Soc. 68: 29~31, 1946
4) HEDING, H.; G. N. FREDERICKS & O. LUTZEN: New active streptomycin derivatives. Acta Chem. Scand. 26: 3251~3256, 1972
5) TREFFERS, H. P.; N. O. BESLER & D. C. ALEXANDER: Genetic studies on resistance to streptomycin and a substituted streptomycin (N^1-γ-hydroxypropylstreptomycylamine). Antibiot. Ann. 1953: 595~603, 1953
6) FRIED, J. & O. WINTERSTEINER: Streptomycin. II. Reduction and oxidation products of streptomycin and streptobiosamine. J. Am. Chem. Soc. 69: 79~86, 1947
7) HEDING, H.; G. J. FREDERICKS & O. LUTZEN: New active streptomycin derivatives. Acta Chem. Scand. 26: 3251~3256, 1972
8) SANO, H.; T. TSUCHIYA, S. KOBAYASHI, M. HAMADA, S. UMEZAWA & H. UMEZAWA: Synthesis of 3″-deoxydihydrostreptomycin active against resistant bacteria. J. Antibiotics 29: 978~980, 1976
9) TSUCHIYA, T.; S. SAKAMOTO, T. YAMASAKI, S. UMEZAWA & H. UMEZAWA: Synthesis of 3″-epidihydrostreptomycin active against resistance bacteria. J. Antibiotics 35: 639~641, 1982
10) TSUCHIYA, T. & T. SHITARA: Synthesis of 3″-epidihydrostreptomycin. Carbohydr. Res. 109: 59~72, 1982
11) BANNISTER, B. & A. ARGOUDELIS: The chemistry of bluensomycin. II. The structure of bluensomycin. J. Am. Chem. Soc. 85: 234~235, 1963
12) WOLFROM, M. & W. POLGLASE: Degradative studies on streptomycin. J. Am. Chem. Soc. 70: 2835~2836, 1948
13) HEDING, H. & O. LUTZEN: Streptomycin: Correlation between *in vivo* and *in vitro* activity of ten representative derivatives. J. Antibiotics 25: 287~291, 1972
14) TSUCHIYA, T.; T. KISHI, S. KOBAYASHI, Y. KOBAYASHI, S. UMEZAWA & H. UMEZAWA: Synthesis of 6-deoxydihydrostreptomycin. Carbohydr. Res. 104: 69~77, 1982
15) BARTZ, Q. R.; J. CONTROULIS, H. M. CROOKS, Jr. & M. C. REBSTOCK: Dihydrostreptomycin. J. Am. Chem. Soc. 68: 2163~2166, 1946
16) HOOPER, I.; D. WHITEHEAD, H. TAYLOR, Y. PERRON, F. PALERMITI & D. JOHNSON: Kanamycin. I. Characterization and acid hydrolysis studies. J. Am. Chem. Soc. 80: 752~753, 1958
17) WEINSTEIN, J.; J. COOPER & J. A. WAITZ: Gentamicin antibiotics. 4. Some condensation products of gentamicin C_2 with aromatic and aliphatic aldehydes. J. Med. Chem. 14: 1118~1120, 1971
18) PINNER, A.: Die Imidoather und Ihre Derivative. Robert Oppenheim, Berlin, 1892
19) USUI, T.; T. TSUCHIYA & S. UMEZAWA: 1- And 3-deamidino derivatives of dihydrostreptomycin and some 1-*N*-acyl derivatives. J. Antibiotics 31: 991~996, 1978