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New Active Streptomycin Derivatives

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The condensation of streptomycin (SM) with nitromethane was investigated and it was found to take place without elimination of water. The nitro derivative was hydrogenated to the corresponding amine. Oxidative deamination of the amine gave an *N*-nitroso dihydrostreptomycin (DHS) analogue which was subsequently hydrogenated to a DHS analogue. This compound, the amine, and the primary condensation product showed antibacterial activities four to ten times lower than the activity of DHS both *in vitro* and *in vivo*.

Streptomycin (SM) is an antibiotic produced by the filamentous bacterium *Streptomyces griseus* and it consists of the three moieties: Streptidine, streptose and *N*-methyl-2-deoxy-2-amino-L-glucose linked together by α-glucosidic bonds as shown in Fig. 1.

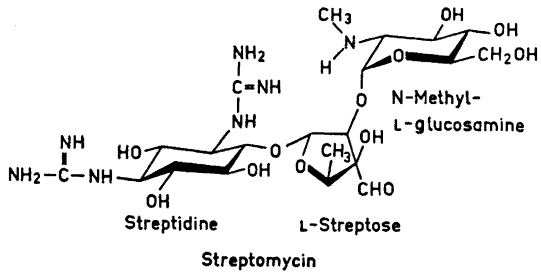

Fig. 1. Streptomycin.

Numerous attempts have been made to improve the pharmacological and bacteriological properties of SM. Most of the derivatives have been made on the L-streptose moiety which contains a reactive carbonyl group.

Oxidation of this group gives SM-acid which is biologically inactive.[1] Reduction of the carbonyl group with hydrogen over palladium gives the valuable antibiotic DHS.[2,3] Reduction with amalgamated aluminium gives

dihydro-desoxy-SM which has the same activity as SM and DHS and is claimed to be less toxic.[4] The fully reduced derivative, the methyl analog to SM has been obtained *via* the diethyl thioacetal, but it has only 10 % activity compared to DHS and SM.[5]

Winsten [6] has described a condensation product between SM and nitromethane. The derivative had antibacterial activity and could be reduced to the corresponding amine which was also an antibiotic. The possible elimination of one molecule of water during the reactions was, however, not discussed and no quantitative measurement of the activities of the derivatives were given. Consequently we decided to reinvestigate this problem. Furthermore the oxidative deamination of the amino derivative was examined.

EXPERIMENTAL

Methods. The purity of the derivatives was examined by high voltage electrophoresis in 0.5 % formic acid. The electrophograms were developed as described by Halliday [7] or bioorthographed. Thin layer chromatography (TLC) was performed according to Heding.[8] NMR spectra were recorded on a VARIAN A-60 spectrometer. Antibacterial activity was estimated by the conventional agar diffusion method. The *in vitro* antibacterial spectra were investigated using selected strains of bacteria. Inoccula of 10^6cells per ml were used and the minimum inhibitory concentration (MIC) for the derivatives was recorded. SM and DHS served as reference antibiotics. Mycin Assay Broth (Difco) served as medium for all organisms except the mycobacteria. For these organisms a modified Proskauer-Beck synthetic medium was employed. Limited *in vivo* studies of the efficacy of the SM derivatives compared to DHS were made by evaluating the ED^{50} values of these antibiotics in mice infected intraperitonally with *Escherichia coli.*

All derivatives were crystallized as reineckates and heliantates as described in the literature.[4] They all showed poorly defined destruction points.

Derivatives. 1. DHS-*N*-nitrosamine. DHS sulfate (80 g) was dissolved in water (200 ml) and the pH adjusted to 4 with sulfuric acid. Sodium nitrite (30 g) was slowly added to this solution and the pH maintained at 4 for 1 h at room temperature. The solution was then neutralized with sodium hydroxide and TLC and electrophoresis showed only one spot corresponding to one new compound. The solution was evaporated to dryness and the residue extracted twice with methanol (40 ml). The derivative was precipitated with acetone (800 ml) after addition of triethylammonium sulfate. Yield 89 g.

2. DHS from DHS-*N*-nitrosamine. DHS-*N*-nitrosamine-sulfate (2 g) was dissolved in water (20 ml) and zinc (1 g) was added. The pH was maintained at 2 with hydrochloric acid for 15 min under stirring. The pH was then adjusted to 8.2 with triethylamine. After filtration the filtrate was added a surplus of triethylammonium sulfate and the product isolated by precipitation with methanol (300 ml). Yield 1.2 g. TLC, electrophoresis and estimates of antibacterial activity showed identity with DHS.

3. SM-nitromethane condensation product. SM-trihydrochloride (90 g) was dissolved in methanol (1500 ml). This solution was added to sodium methoxide (2000 ml, 0.3 N) in methanol followed by the addition of nitromethane (225 ml). The condensation product was isolated by filtration after 2 h of incubation at 4°C. Yield 80 g. The acid salt was converted to the nitro form by dissolving it in a solution of anhydrous acetic acid (60 ml) in methanol (500 ml). The product was obtained by precipitation with acetone (2000 ml). Yield 70 g. (Found: C 23.71; H 4.40; N 21.05. Calc. for $C_{22}H_{42}N_8O_{14}3H(Cr(SCN)_4(NH_3)_2).6H_2O$: C 23.93; H 4.43; N 21.30.)

4. Hydrogenation product of the nitro derivative. The nitromethane condensation product (70 g) was dissolved in water (300 ml). The mixture was hydrogenated at room temperature overnight at a pressure of 1500 psi in the presence of Raney nickel. The catalyst was removed by filtration and the filtrate decolorized with carbon. The pH was adjusted to 5.5 with dilute sulfuric acid and the solution was concentrated under vacuum at 60°C to half volume. The product was precipitated in methanol (2000 ml) containing

a surplus of triethylammonium sulfate. Yield 48.5 g. (Found: C 22.85; H 4,54; N 22.42. Calc. for $C_{22}H_{44}N_8O_{12}.4H(Cr(SCN)_4(NH_3)_2).6H_2O$: C 22.81; H 4.25; N 22.25.) (Found: C 48.51; H 6.11; N 14.68. Calc. for the tetra-heliantate $C_{22}H_{44}N_8O_{12}.4H(C_{14}H_{14}N_3O_3S).6H_2O$: C 48.14; H 6.05; N 14.38.)

5. Preparation of the DHS analog from the amine. The amine disulfate (8 g) was dissolved in water (100 ml) at 0°C. The pH was adjusted to 4 with acetic acid and sodium nitrite (6.9 g) (10 equiv.) was added. The pH was maintained at 4 until TLC and electrophoresis showed only one product. Usually this product was not isolated. The solution was neutralized with ammonia and amalgamated aluminium (8 g) was added and the pH maintained at 7 with dilute sulfuric acid at a temperature below 25°C. The aluminium hydroxide was removed by filtration and the product precipitated with methanol. Yield 2.5 g. (Found: C 23.67; H 4.57; N 22.20. Calc. for $C_{22}H_{43}N_7O_{13}.3H(Cr(SCN)_4(NH_3)_2).6H_2O$: C 24.34; H 4.56; N 20.84.) (Found: C 46.61; H 6.06; N 14.18. Calc. for the tri-heliantate $C_{22}H_{43}N_7O_{13}.3H(C_{14}H_{14}N_3O_3S).6H_2O$: C 46.36; H 6.16; N 13.64.)

RESULTS AND DISCUSSION

DHS serving as a model was found to react with nitrous acid. The reaction product, DHS-*N*-nitrosamine, had no antibacterial activity (Fig. 2).

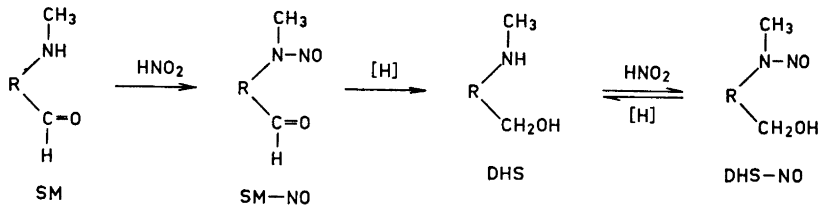

Fig. 2. Formation of SM and DHS-*N*-nitrosamine (SM-NO, DHS-NO); reconversion of SM-NO, DHS-NO to DHS.

The reaction can be followed by TLC and electrophoresis (Table 1). With a surplus of nitrous acid the antibiotics, DHS and SM, are quantitatively transformed to the derivatives. Starting with the commercially available sulfates the derivatives are conveniently obtained in pure form as the dihydrochlorides by metathesis with barium chloride. After removal of barium sulfate and evaporation of the filtrate to dryness the dihydrochlorides of the derivatives can be extracted from the residue leaving inorganic salts undissolved. The *N*-nitrosamine dihydrochlorides can be precipitated from the methanolic solution with acetone.

The *N*-nitroso group is a protecting group which is readily eliminated. Reduction with amalgamated aluminium in neutral solution or with zinc at pH 2 gave fully active DHS in quantitative yields.

TLC and electrophoresis of the nitromethane product (Fig. 3, 1 or 1a) showed that only one product had been formed and that all SM had reacted. The NMR spectrum was in agreement with Winsten [6] (*i.e.* structure 1). The condensation took place without the elimination of water. A new center of asymmetry had been introduced, but no attempts were made to separate the epimers.

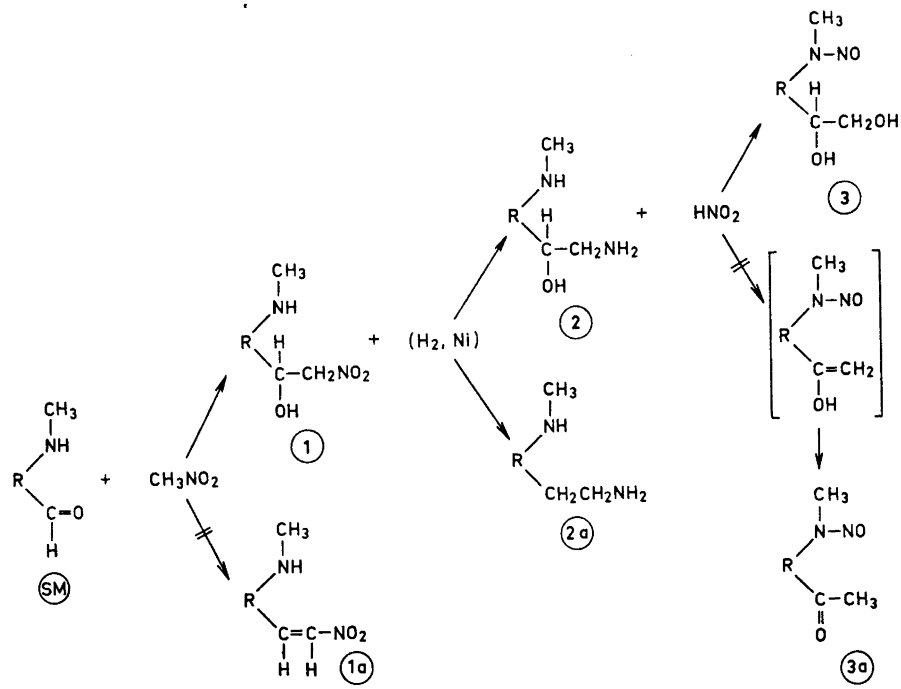

Fig. 3. Condensation of SM with CH_3NO_2 yields (1), hydrogenation (H_2,Ni) of (1) yields (2), treatment of (2) with HNO_2 leads to the DHS-NO-analogue (3).

Hydrogenation of the nitro derivative gave the corresponding amine (structure 2). The possible elimination of water at this step leading to structure 2a did not – according to the NMR spectrum – take place.

Oxidative deamination of the amine gave an *N*-nitrosamine (structure 3 or 3a). The NMR spectrum showed a characteristic change of the chemical shift of the *N*-CH_3 singlet from 2.87 to 3.28 also recognized in the spectrum of DHS-*N*-nitrosamine. Furthermore the NMR spectra excluded structure 3a. The diazotation of the amine resulted in the same change in relative R_F-value (TLC), as seen when DHS was converted to *N*-nitroso-derivative (Table 1). A similar change in the relative electrophoretic mobility could be observed.

Table 1. R_F-values and electrophoretic mobilities of SM-derivatives relative to SM. The abbreviations are explained in Figs. 2 and 3. R-OH is the hydrogenation product of (3) in Fig. 3.

	SM	SM-NO	DHS	DHS-NO	R-NO_2	R-NH_2	R-OH~NO	R-OH
TLC Relative R_F	1.00	1.43	0.88	1.42	0.90	0.71	1.42	0.86
El-phoresis Relative mob.	1.00	0.75	1.00	0.72	0.95	1.09	0.72	1.00

Hydrogenation of the *N*-nitrosamine (structure 3) with amalgamated aluminium gave the DHS analog. Reduction with zinc in dilute acid gave several unidentified compounds.

The antimicrobial activity of the derivatives towards a number of Gram-negative and Gram-positive bacteria was estimated and compared to the activity of DHS.

Table 2. Estimates of antibacterial activities of SM-derivatives. 1 is (1) in Fig. 3. 2 is (2) in Fig. 3 and 3 is the hydrogenation product of (3) in Fig. 3.

In vitro activity:

	Minimum inhibitory concentration (μg/ml)			
Bacteria	DHS	1	2	3
Gram positive				
S. aureus 209P	0.8	0.7	2.4	5.0
S. aureus 13709	0.8	2.7	2.4	2.5
S. pyogenes 8306	3.1	–	–	10.1
S. pyogenes 12384	3.1	–	–	10.1
S. faecalis	12.5	–	–	>100
Gram negative				
K. pneumoniae K8	0.8	2.7	2.4	2.5
E. coli 01	0.8	5.3	4.7	10.1
E. coli 04	1.6	5.3	4.7	10.1
P. vulgaris 5209	3.1	10.8	19	41
P. vulgaris 16868	6.2	–	–	41
Ps. aeruginosa 769	12.5	–	–	>100
Ps. aeruginosa 1999	12.5	–	–	>100

In vivo activity in mice:

	Effective doses -50, mg/kg			
Infecting agent	DHS	1	2	3
S. aureus-Smith	abt. 5	abt. 35	abt. 11	–
E. coli 01	2.5	35	abt. 11	20

Table 2 shows that the three derivatives had significantly lower activity than DHS both *in vitro* and *in vivo*. Furthermore, the amino derivative had the same or lower activity *in vitro* when it was compared to the nitromethane condensation product, whereas *in vivo* the opposite was the case. The DHS analogue was, unexpectedly, the least active of the derivatives, the activity was only 10 % compared to DHS in both test systems.

Because of the low activities the toxicity of the derivatives was not investigated.

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