ACTA CHEMICA SCANDINAVICA 23 (1969) 1275–1278

N-Demethylstreptomycin

II. Degradation Studies

HENRIK HEDING

Novo Terapeutisk Laboratorium A/S, Copenhagen, Denmark

N-Demethylstreptomycin (NDMS) has been subjected to hydrolytic degradation. Mild acid hydrolysis yielded streptidine. Treatment with alkali liberated maltol and penta-acetyl-L-α-glucosamine could be isolated after drastic acid hydrolysis followed by acetylation. Under similar conditions streptomycin yields streptidine, maltol and penta-acetyl-*N*-methyl-L-α-glucosamine. These findings show that *N*-demethylstreptomycin differs only from streptomycin in that it contains unsubstituted L-glucosamine whereas streptomycin contains *N*-methyl-L-glucosamine.

In a recent paper [1] we have described the microbiological formation of a new antibiotic in submerged cultures of *Streptomyces griseus* with added DL-ethionine as the methylation inhibitor. The NMR spectrum of a crude sample of the antibiotic led us to give the new antibiotic the name of *N*-demethylstreptomycin. The degradation studies presented in this paper support this assumption.

RESULTS

Acid hydrolysis of streptomycin under mild conditions specifically cleaves the glucosidic bond between the streptidine and the streptobiosamine moiety of the molecule. If the hydrolysis is carried out in dilute sulfuric acid, the almost insoluble streptidine sulphate precipitates from the solution in high yield.[2] The crystalline precipitate was removed by filtration. Neutralization of the filtrate with an anion exchange resin in the OH^- form, followed by evaporation of the solvent yielded crude streptobiosamine. We have found that a solution of crude NDMS that contained about 10 % streptomycin in sulfuric acid deposited a crystalline precipitate in good yield after standing at room temperature for five days. After several recrystallizations from boiling water the substance was identified as streptidine sulphate monohydrate.

Treatment of streptobiosamine by refluxing with 6 N hydrochloric acid causes complete destruction of the streptose moiety and the liberation of *N*-methyl-L-glucosamine. The *N*-methyl-L-glucosamine can be isolated from the hydrolysis mixture as the pentaacetate after treatment with acetic anhydride in pyridine.[3] Similar treatment of a crude sample of NDMS, shown by NMR analysis to contain about 10 % streptomycin, yielded L-α-glucosamine penta-acetate contaminated with 10 % *N*-methyl-L-α-glucosamine penta-acetate.

Treatment of streptomycin with weak alkali results in transformation of the streptose part of the molecule to the γ-pyrone maltol.[4] The maltol can be isolated by solvent extraction at a low pH and purified by recrystallization from boiling water. A solution of NDMS in 1/10 N sodium hydroxide was heated on a boiling water bath for 10 min, cooled to room temperature, acidified with hydrochloric acid, and extracted twice with chloroform. After evaporation of the solvent the residue was taken up in a small amount of boiling water. Slow cooling yielded a crop of crystalline maltol identical with an authentic sample.

Fig. 1 summarizes the degradation reactions mentioned.

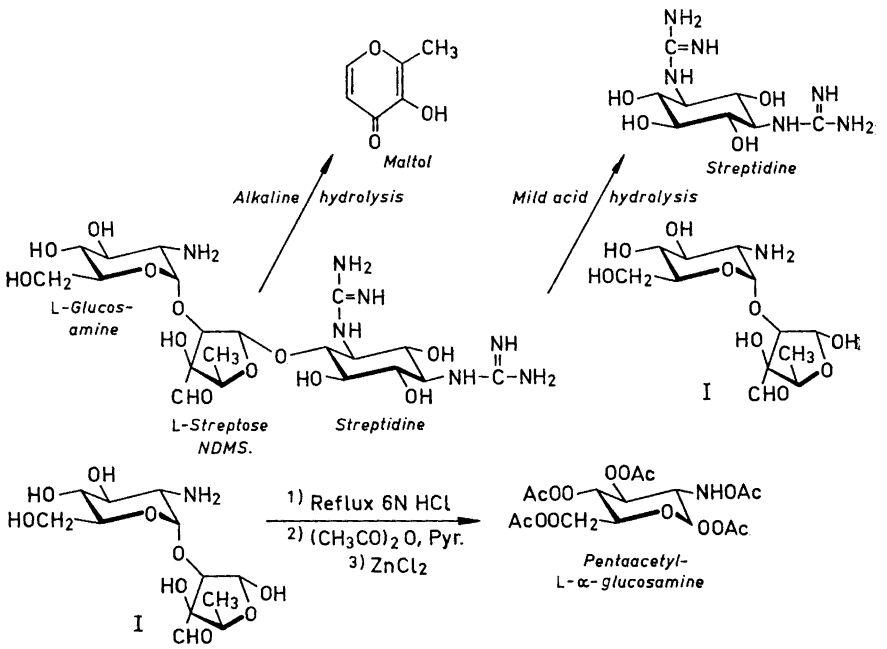

Fig. 1. Summary of the chemical degradation of NDMS.

EXPERIMENTAL

Streptidine sulphate monohydrate from NDMS, 3HCl. 10 g of NDMS, 3HCl was dissolved in 50 ml N H_2SO_4 and heated at 37°C for 3 days. It was then cooled to 0°C for 16 h and filtered. The filter cake was washed twice with 25 ml of cold water and then dissolved in a minimal amount of boiling water. Slow cooling yielded crystalline streptidine sulphate monohydrate which was further purified by two recrystallizations from water. Yield 2.0 g; m.p. above 300°C (decomp.). (Found: C 25.54; H 5.95; N 22.50; O 38.11; S 8.60. Calc. for $C_8H_{22}N_6O_9S$: C 25.58; H 5.88; N 22.20; O 38.10; S 8.50.)

L-α-*Glucosamine penta-acetate from NDMS, 3HCl.* 10 g of NDMS, 3HCl was dissolved in 50 ml of 6 N HCl and left at room temperature for 48 h. The solution was then evaporated to dryness *in vacuo* at 50°C on a rotary evaporator. The resulting dry hydrolysis mixture was dissolved in 100 ml of distilled water and added to 100 ml of wet cation exchange resin, IRC-50, in the sodium form to absorb streptidine. The suspension was stirred for 2 h at constant pH, 7.0, and then filtered. After evaporation to dryness the dry material, a mixture of *N*-demethyl-streptobiosamine hydrochloride and sodium chloride, weighed 5 g. The material was dissolved in 25 ml of 6 N HCl and refluxed for 1 h. After evaporation to dryness, 10 ml of acetic anhydride and 250 mg of freshly fused zinc chloride was added, and the resulting mixture heated for 1 h on a boiling water bath. The mixture was again evaporated to dryness and the dry material taken up in 40 ml of water. The aqueous solution was decolourized by stirring with 1 g of active carbon (SU. 18) for 1 h and then filtered. The almost colourless filtrate was extracted twice with one volume of chloroform and the extract dried over anhydrous magnesium sulphate. After evaporation of the solvent the remaining yellow oil was dissolved in 50 ml of boiling ether. After standing overnight, a white crystalline precipitate was deposited from the ether solution. Two recrystallizations from ether yielded 162 mg; m.p. 138–139°C uncorrected. (Found: C 49.69; H 6.04; N 3.50; O 39.88. Calc. for $C_{16}H_{23}NO_{10}$: C 49.20; H 5.98; N 3.61; O 41.2.) $[\alpha]_D^{20} = -90.4°$ (*c* 0.69 in $CHCl_3$). $[\alpha]_D^{20} = +88.7°$ (c 1 in $CHCl_3$) for D-α-glucosamine penta-acetate prepared from commercial D-α-glucosamine hydrochloride. $[\alpha]_D^{20} = -101.6°$ (*c* 1 in $CHCl_3$) for *N*-methyl-L-α-glucosamine penta-acetate prepared from streptomycin.

Thin layer chromatography on silica gel, developed twice with ethyl acetate and treated with iodine vapour showed that the material was a mixture of two compounds with R_F-values of 0.40 and 0.23. The proportion between the amounts was estimated to 1:10. The R_F-value of *N*-methyl-L-α-glucosamine pentaacetate prepared from streptomycin was found to 0.39. D-α-Glucosamine penta-acetate had an R_F-value of 0.22.

The IR-spectrum of the mixture was completely identical with a similar spectrum of a mixture of 90 % *N*-methyl-L-α-glucosamine penta-acetate and 10 % D-α-glucosamine penta-acetate. Similarly a 60 Mc NMR spectrum of the mixture showed a band at 2.82 ppm corresponding to approximately 10 % contamination with the *N*-CH_3 analogue present in the starting material. No further attempts were made to identify this degradation product.

Maltol from NDMS, 3HCl. 4 g of NDMS, 3HCl was dissolved in 20 ml of water and the pH adjusted to 11.5 with sodium hydroxide. The solution was heated to 80°C for 30 min and after cooling to room temperature acidified with dilute sulfuric acid and extracted twice with one volume of chloroform. The chloroform phase was dried over anhydrous magnesium sulphate and then evaporated to dryness. The crystalline residue was recrystallized three times from boiling water. Yield 34 mg; m.p. 166–167°C uncorrected; m.p. for autentic maltol 165–167°C. (Found: C 54.14; H 4.67. Calc. for $C_6H_6O_3$: C 54.14; H 4.80.)

The compund showed strong absorbancy at 274 mμ in acid solution and gave a characteristic violet colour with ferric ions with absorbancy maximum at 520 mμ. This is in good agreement with the literature.[4]

DISCUSSION

The chemical investigation presented in this paper shows that NDMS differs from streptomycin only in the glucosamine moiety. Whereas streptomycin contains *N*-methyl-L-glucosamine, NDMS contains unsubstituted L-glucosamine.

Our results do not reveal the steric configuration of the two glucosidic bonds in the molecule. In streptomycin they are both α-bonds [5,6] and by analogy with this the α-configuration would also be expected in NDMS.

There may exist two or more tautomers of NDMS due to intramolecular reactions between the carbonyl function and other functional groups. Such tautomers are known to exist in case of streptomycin,[7-9] but the assignment of the structure of each tautomer is based on circumstantial evidence. Our results do not reveal information on the possible existence of such isomers of NDMS.

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Received September 28, 1968.