2. The proportions of ester anion and amide formed from certain esters and sodium amide have been determined. The influence of structure of esters on these courses of reaction has been discussed.

DURHAM, N. C. RECEIVED SEPTEMBER 26, 1945

[CONTRIBUTION FROM THE RESEARCH LABORATORIES, MERCK & CO., INC.]

Streptomyces Antibiotics. IV. Hydrolytic Cleavage of Streptomycin to Streptidine

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Streptomycin has been hydrolyzed in aqueous acid solution to give a basic degradation product, streptidine. Analytical data of several salts and derivatives of streptidine show that the molecular formula of streptidine is $C_8H_{18}N_6O_4$.

One of the first degradation reactions tried on streptomycin was hydrolytic cleavage and examination for basic cleavage products. Cleavage in acid solution was preferred in view of the strongly basic, nitrogenous nature of streptomycin. When acid hydrolysis was tried on a concentrate (500 units/mg.) of streptomycin hydrochloride or on pure streptomycin hydrochloride[1] regenerated from the helianthate, a strongly basic degradation product was formed which was readily isolated and purified as a picrate. Since the picrate of this base was obtained as readily from reaction at 120° as at 25°, the base has considerable stability in acid solution over this temperature range. This base, designated streptidine for convenience, formed an acetyl derivative. Analytical and molecular weight data showed that this derivative was an octaacetate of the molecular formula $C_{24}H_{34}N_6O_{12}$.

Streptidine was also obtained readily as a crystalline sulfate by cleavage of streptomycin in dilute aqueous or methanolic sulfuric acid solution at 25°. The practically pure streptidine sulfate crystallized directly from the reaction solution.

Streptidine was characterized as the following additional crystalline salts: dihydrochloride, carbonate, chloroplatinate, dihelianthate, di-*d*-camphorsulfonate and dihydroiodide. Analytical data on all of these salts are in agreement with the molecular formula, $C_8H_{18}N_6O_4$, for the free base.

Streptidine contains two basic groups as shown by the formation of salts containing two univalent acid ions, such as the dihydrochloride and dihydroiodide. No primary amino groups appear to be present, since streptidine failed to give nitrogen in the Van Slyke amino-nitrogen determination during a five-minute period. The presence of —OH and/or >NH groups is indicated by the infrared absorption as discussed below, and by the formation of octaacetylstreptidine. Although streptidine contains six nitrogen atoms, the absence of typical primary amino groups makes it doubtful that any of the nitrogen atoms are diacetylated. Since streptidine shows no sign of reaction with hydroxylamine, 2,4-dinitrophenylhydrazine or other carbonyl reagents, there are no free aldehydic or ketonic carbonyl groups present. There is no evidence for a carboxyl group in the molecule, and methoxyl groups are absent. Therefore, one or more of the four oxygen atoms in streptidine appears to exist as hydroxyl. Reduction of octaacetylstreptidine was attempted by a reaction with hydriodic acid and red phosphorus in a sealed tube at 160–190°; however, only streptidine dihydroiodide was isolated as a result of deacetylation.

Streptidine is optically inactive and shows only end absorption in the ultraviolet. The octaacetate, however, showed maxima $E_{1\%}$ 400 at 2200 Å. and $E_{1\%}$ 630 at 2525 Å. The infrared absorption spectrum of streptidine dihydrochloride was determined on a solid layer deposited from a methyl cellosolve suspension. Bands appeared at 2.97, 3.38 and 6.10 μ. The band at 2.97 μ is probably due to —OH and >NH groups, while the band at 6.10 μ may be due to a >C=N— bond. In the infrared, octaacetyl streptidine (solution in tetrachlorethane) showed no band at 2.97 μ but exhibited definite strong bands at 5.75 μ and 6.27 μ and a weak band at 6.48 μ. The 6.27 μ band may be due to a >C=N—bond, while the 5.75 μ band is evidently due to the ester carbonyl groups.

In the regeneration of streptomycin hydrochloride or sulfate from the helianthate,[1] there is occasionally observed some inactivation which appears to be due to the presence of excess mineral acid. Thus, a preparation of pure streptomycin helianthate treated with a small excess of hydrogen chloride in absolute methanol gave the expected yield of product, but the specific rotation of the product was −70° instead of −86° and the activity was 600 instead of 800 units/mg. From this material there was isolated streptidine picrate, m. p. 282–284° (dec.), by treating an aqueous solution with sodium picrate. Also, regeneration of streptomycin sulfate from streptomycin helianthate using methanolic sulfuric acid gave on one occasion a considerable yield of crystalline streptidine sulfate. It is therefore important that only the necessary amount of mineral acid be used in regeneration of streptomycin salts from the helianthate. The ease of hydrolysis of streptomycin

(1) Kuehl, Peck, Walti and Folkers, *Science*, **102**, 34 (1945).

is illustrated again by the above-described formation of streptidine sulfate.

This ease of cleavage of streptomycin is compatible with the interpretation that streptomycin is a hydroxylated base, streptidine, attached by glycosidic linkage to streptobiosamine.[2] Using the formula of streptidine, $C_8H_{18}N_6O_4$, with the present formula of streptomycin, $C_{21}H_{37-39}N_7O_{12}$,[3] in an equation with one molecule of water, it is seen that a product of composition $C_{13}H_{21-23}NO_9$ remains. Studies on the characterization of the product, $C_{13}H_{21-23}NO_9$ (streptobiosamine), have been described.[2]

We are indebted to Miss D. G. Smith, Mr. O. Graessle, and Dr. Hans Molitor of the Merck Institute for Therapeutic Research for the following pharmacological results. Streptidine dihydrochloride showed no antimicrobial activity *in vitro* at a concentration of 1 mg./cc. against *E. coli* "N," *S. schottmülleri*, *E. typhosa*, *Staph. aureus* SM or *Staph. albus* 156, or against *E. inguinale*, *M. jelenenum*, *T. gypseum* or *S. schenki*. Streptidine given at the maximum tolerated level failed to protect mice injected intraperitoneally with 1000 lethal doses of *S. schottmülleri* or *Staph. aureus* SM. Acute toxicity studies showed that mice receiving 2.5 to 5.0 g./kg. of streptidine dihydrochloride subcutaneously exhibited dyspnea and died within five to ten minutes. Lower doses produced a slight effect upon the respiration from which the animals recovered. Dr. H. B. Woodruff, of the Microbiological Department, found that streptidine dihydrochloride showed some inhibiting action against *B. subtilis* at concentrations of about 10 mg./cc.

Experimental

Stability of Streptomycin.—Streptomycin is relatively stable at 25° in aqueous solution between about *p*H 2 and 9. About 35% of the activity is lost in six hours and about 80% in twenty-four hours at 25° in 1 *N* hydrochloric acid, and about 90% in three hours in 5 *N* hydrochloric acid. In 0.1 *N* sodium hydroxide at 25°, streptomycin is about 50% inactivated in three hours. Stronger alkali causes more rapid inactivation. Dry streptomycin hydrochloride is stable for six hours at 110° *in vacuo*.

Hydrolysis of Streptomycin Hydrochloride. Isolation of Streptidine Dipicrate and Sulfate.—In an early experiment, 320 mg. of streptomycin hydrochloride (about 500 units/mg.) was dissolved in 2 cc. of water and 1 cc. of concd. hydrochloric acid and hydrolyzed by heating the solution in a sealed tube at 120° for two hours. The solution was then concentrated to dryness *in vacuo*, and the residue was dissolved in methanol and filtered through Darco G-60 to remove dark-colored material. The filtrate was concentrated to dryness *in vacuo*, giving 178 mg. of a light buff powder. Treatment with warm ethanol left 89 mg. of insoluble material. A 76-mg. portion of the ethanol-soluble fraction and 118 mg. of picric acid were dissolved in 10 cc. of hot water. Needle crystals of streptidine dipicrate separated when the solution cooled. The crystals, dried at 25° *in vacuo*, weighed 104 mg., m. p. 278–282° (micro-block). Recrystallization from water raised the melting point to 283–284° (dec.) (micro-block). These crystals are streptidine dipicrate dihydrate.

Anal. Calcd. for $C_{20}H_{24}N_{12}O_{18}\cdot 2H_2O$: C, 31.75; H, 3.73; N, 22.22. Found: C, 31.65, 31.96; H, 3.47, 3.61; N, 22.01.

Heating the dihydrate at 56° *in vacuo* yields anhydrous streptidine dipicrate, m. p. 284–285° (dec.) (micro-block).

Anal. Calcd. for $C_{20}H_{24}N_{12}O_{18}$: C, 33.34; H, 3.36; N, 23.33. Found: C, 33.47; H, 3.71; N, 23.35.

Hydrolysis of streptomycin was also carried out by dissolving 10.7 g. of streptomycin hydrochloride ($[\alpha]^{25}D$ −83° (*c*, 1.0% in water); activity about 750 units/mg.) in 80 cc. of absolute methanol containing 2.5 cc. of concentrated sulfuric acid and allowing the solution to stand at 25°. Rosets of needle crystals slowly separated from the solution. After two days, a first crop of 2.94 g. of crystals was removed by filtration. A further crop of 1.74 g. was obtained from the mother liquors after a few days of standing at 25°. These crystals were practically pure streptidine sulfate containing methanol of crystallization.

Anal. Calcd. for $C_8H_{18}N_6O_4\cdot H_2SO_4\cdot CH_3OH$: C, 27.55; H, 6.17; N, 21.42. Found: C, 27.78; H, 5.47; N, 21.49.

Recrystallization was effected by dissolving the sulfate in hot water containing some sulfuric acid and adding acetone to the hot solution to the point of turbidity. On cooling, streptidine sulfate crystallized. The crystals showed no melting point up to 300° (micro-block). They were dried at 100°.

Anal. Calcd. for $C_8H_{18}N_6O_4\cdot H_2SO_4$: C, 26.66; H, 5.60; N, 23.33. Found: C, 26.91; H, 5.66; N, 23.20.

Streptidine Dihydrochloride.—A sample of 234 mg. of streptidine dipicrate was suspended in 3 cc. of methanol and 0.1 cc. of concentrated hydrochloric acid was added. The solution was mixed with 30 cc. of acetone, causing separation of white flocculent precipitate. The precipitate of streptidine dihydrochloride was isolated by centrifugation, washed with acetone, and dried *in vacuo;* yield, 112 mg. The amorphous product was hygroscopic and melted gradually (dec.) from 170–210° (micro-block). It was dried at 100° *in vacuo*.

Anal. Calcd. for $C_8H_{18}N_6O_4\cdot 2HCl$: C, 28.66; H, 6.01; N, 25.07. Found: C, 29.31; H, 6.57; N, 25.16.

The amorphous dihydrochloride crystallized from absolute methanol. Rosets of needles were obtained on recrystallization from the same solvent. The crystals were extremely hygroscopic and appeared to contain methanol of crystallization.

Anal. Calcd. for $C_8H_{18}N_6O_4\cdot 2HCl\cdot CH_3OH$: C, 29.43; H, 6.59. Found: C, 29.44; H, 6.29.

Streptidine Carbonate.—A solution of 2.7 g. of streptidine dihydrochloride and 0.85 g. of sodium carbonate in 100 cc. of water was warmed and treated with 100 cc. of methanol. Crystallization was induced by scratching the walls of the flask. After standing for two days, the crystals were filtered and dried; yield 1.63 g. When heated on the micro-block, the crystals began to darken slightly at 210°, sintered at 240° and gradually decomposed on further heating.

Anal. Calcd. for $C_8H_{18}N_6O_4\cdot H_2CO_3\cdot H_2O$: C, 31.58; H, 6.48; N, 24.55. Found: C, 31.25; H, 6.31; N, 24.54.

Streptidine Chloroplatinate.—A solution of 21 mg. of streptidine dihydrochloride in 0.5 cc. of water was mixed with 1 cc. of a 10% solution of chloroplatinic acid and diluted to 12 cc. with absolute ethanol. On standing in the cold room for several days, crystals were slowly deposited. The crystals, after washing with water and alcohol and drying at 100° *in vacuo*, weighed 31 mg. They decomposed at about 231° (micro-block).

Anal. Calcd. for $C_8H_{18}N_6O_4\cdot H_2PtCl_6$: C, 14.29; H, 3.00; N, 12.50; Pt, 29.04. Found: C, 14.38; H, 3.08; N, 11.97; Pt, 28.82.

Streptidine Dihelianthate.—A solution of 1 g. of methyl orange in 70 cc. of hot water was added with stirring to a solution of 421 mg. of streptidine dihydrochloride in 5 cc. of warm methanol. A voluminous, finely crystalline, orange-brown precipitate appeared and was collected by

(2) Brink, Kuehl and Folkers, *Science*, **102**, 506 (1945).

(3) Peck, Brink, Kuehl, Flynn, Walti and Folkers, THIS JOURNAL, **67**, 1866 (1945).

centrifuging; yield 993 mg. One recrystallization from aqueous methanol, followed by recrystallization from hot water, gave orange-brown needles which sintered at 225° and melted with decomposition at 240–250° (micro-block).

Anal. Calcd. for $C_{28}H_{48}N_{12}O_{16}S_2 \cdot 2H_2O$: C, 47.57; H, 5.77. Found: (sample dried at 56° *in vacuo*) C, 47.25; H, 5.60. Calcd. for $C_{28}H_{48}N_{12}O_{16}S_2$: C, 49.53; H, 5.54. Found: (sample dried at 100° *in vacuo*) C, 49.31; H, 5.30.

Streptidine Di-*d*-camphorsulfonate.—To a solution of 1.05 g. of streptidine dihydrochloride in 3 cc. of methanol was added a solution of 1.6 g. of sodium *d*-camphorsulfonate in 3 cc. of methanol. On standing at 25° overnight, sodium chloride had separated. It was filtered and the filtrate was concentrated to effect more complete separation of sodium chloride. After filtering again, the filtrate containing streptidine di-*d*-camphorsulfonate was diluted to 6.5 cc. with methanol and treated with 10 cc. of absolute ethanol. A voluminous precipitate of fine white needles separated at once. The crystals were filtered and dried; weight, 1.49 g. After recrystallization from methanol and then from 1:1 methanol–ethanol, there was obtained 0.90 g. of needles, melting at about 185–190° (micro-block) with some bubbling, resolidifying at about 220–230°, and finally melting with decomposition at about 285–292°. The rotation was $[\alpha]^{25}D$ +13.5° (*c*, 4.83% in water) after drying at 100° *in vacuo*.

Anal. Calcd. for $C_{28}H_{58}N_6O_{12}S_2 \cdot H_2O$: C, 45.15; H, 7.04; N, 11.28. Found: (sample dried at 56° *in vacuo*) C, 45.42; H, 7.10; N, 11.41. Calcd. for $C_{28}H_{56}N_6O_{12}S_2$: C, 46.27; H, 6.93. Found: (sample dried at 100° *in vacuo*) C, 46.25; H, 6.52.

Octaacetylstreptidine.—A mixture of 1.01 g. of streptidine dihydrochloride, 494 mg. of fused sodium acetate and 20 cc. of acetic anhydride was refluxed gently for one hour. The solution was then concentrated to dryness *in vacuo*, water was added, and an insoluble crystalline product separated; yield 1.52 g. The product was recrystallized once from chloroform–petroleum ether and once from chloroform–ether; yield 1.22 g.; m. p. 260–262° (micro-block).

Anal. Calcd. for $C_{44}H_{64}N_6O_{12}$: C, 48.15; H, 5.73; N, 14.04; acetyl, 57.52. Found: C, 48.20; H, 5.73; N, 13.75; acetyl, 57.0.

The acetyl determination was carried out by saponification with hot 1 *N* sodium hydroxide in 50% methanol for two hours prior to distillation of acetic acid. A cryoscopic molecular weight determination on octaacetylstreptidine carried out in dioxane as solvent gave a value of 540 (calcd. 598.6). The accuracy of this determination is estimated to be of the order of ±10%. An attempted ebullioscopic determination of the molecular weight using methanol as solvent gave no significant results, since the octaacetylstreptidine underwent methanolysis. The methanolysis involved partial deacetylation, which indicates that a portion of the acetyl groups are represented by O-acetyl.

Streptidine Dihydroiodide.—A mixture of 304 mg. of octaacetylstreptidine, 150 mg. of red phosphorus, 576 mg. of iodine and 5 cc. of hydriodic acid (sp. g., 1.7) was heated in a bomb tube for seven hours at 160–190°. The solution was diluted with water, filtered and concentrated to dryness *in vacuo*. The residue was dissolved in methanol, filtered and mixed with about two volumes of ether whereupon white crystals of streptidine dihydroiodide separated. The salt was recrystallized once from methanol–ether; yield 48 mg. It was converted to a picrate, m. p. 284–285°. The melting point of a mixture of this picrate with streptidine dipicrate was 284–285°.

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Summary

Acid hydrolysis of streptomycin has yielded an optically inactive hydroxylated base, streptidine.

Streptidine has been characterized by the following crystalline salts: dipicrate, sulfate, carbonate, dihydrochloride, dihydroiodide, dihelianthate, di-*d*-camphorsulfonate and chloroplatinate. Streptidine appears to contain one or more hydroxyl groups, but no primary amino, carboxy, methoxy or carbonyl groups. It formed an octaacetyl derivative.

Streptidine has the molecular formula $C_8H_{18}N_6O_4$.

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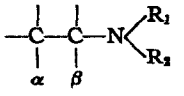
[CONTRIBUTION FROM THE PURDUE RESEARCH FOUNDATION AND THE DEPARTMENT OF CHEMISTRY OF PURDUE UNIVERSITY]

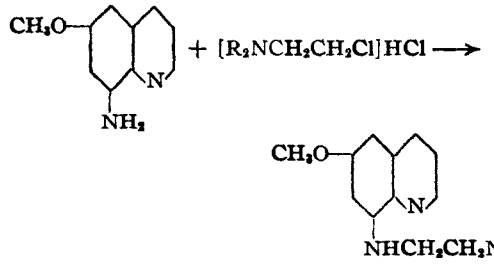
Derivatives of 6-Methoxy-8-aminoquinoline[1,2]

BY G. BRYANT BACHMAN AND H. HARRY SZMANT[3]

A number of derivatives of 6-methoxy-8-aminoquinoline containing the grouping



attached to the 8-amino nitrogen have been prepared by reactions similar to the following



It has been our purpose to study the variations in chemotherapeutic activity associated with changes in the R groups and with changes in substituent alkyl groups on the α and β carbons.

(1) Read before the Organic Section at the New York City meeting of the American Chemical Society, September, 1944.

(2) From the Ph.D. dissertation of H. Harry Szmant, Purdue University, Lafayette, Indiana.

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