Purification of Streptothricin by Precipitation of a Crystalline Reineckate.—A 400-mg. quantity of streptothricin hydrobromide, 600 units/mg. activity, was dissolved in 10 cc. of water and a saturated solution of Reinecke acid added until no further precipitate was obtained. The precipitate was partly crystalline. It was removed by centrifuging and washed with three 2-cc. portions of cold water. After extracting with 15 cc. of water at 45° for one-half hour, the insoluble portion was removed by centrifuging. As the supernatant liquid cooled slowly, fine needles of streptothricin reineckate deposited; 300 units/mg. activity. A second extraction of the insoluble reineckate with 12 cc. of water at 45°, followed by centrifuging and cooling the supernatant liquid yielded another crop of the crystalline reineckate.

The crystalline streptothricin reineckate was transformed into the hydrobromide by dissolving it in 15 cc. of warm water and adding an aqueous 2.5 *N* pyridine hydrobromide solution until no further precipitate of pyridine reineckate occurred. The pyridine reineckate was removed by filtration and the filtrate was concentrated to dryness at reduced pressure. The residue was dissolved in 2 cc. of methyl alcohol, and an excess of acetone added. The resulting precipitate was removed by centrifuging and dried to yield 150 mg. of streptothricin hydrobromide, 650 units/mg. activity.

The original solution and mother liquors from which the streptothricin reineckate precipitated were combined and treated with an aqueous 2.5 *N* pyridine hydrobromide solution to yield an additional 110 mg. of hydrobromide, assaying 470 units/mg. activity.

Pyridine hydrochloride has been used also to convert the reineckate to streptothricin hydrochloride.

Summary

Streptothricin hydrochloride which has shown 830 units/mg. activity and $[\alpha]^{25}D$ −51.3° has been isolated from culture broths of *Streptomyces lavendulae*. The following sequence of steps was used to produce this product from the broth: charcoal adsorption, elution with formic acid, precipitation with picric acid and direct conversion to hydrochlorides, chromatography of hydrochlorides, helianthate, hydrochloride.

The following additional precipitants have been used to effect purification of streptothricin concentrates: picrolonic acid, flavianic acid, Reinecke salt and phosphotungstic acid.

RAHWAY, NEW JERSEY RECEIVED JANUARY 22, 1946

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

Streptomyces Antibiotics. VII. The Structure of Streptidine

BY ROBERT L. PECK, CHARLES E. HOFFHINE, JR., ELIZABETH W. PEEL, ROBERT P. GRABER, FREDERICK W. HOLLY, RALPH MOZINGO AND KARL FOLKERS

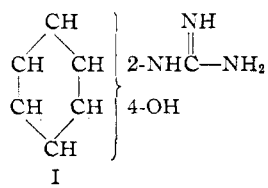
Streptidine is one of the meso forms of 1,3-diguanido-2,4,5,6-tetrahydroxycyclohexane according to present experimental evidence.

Streptidine[1,2] was characterized as an optically inactive, hydroxylated, strongly basic substance having the molecular formula $C_8H_{18}N_6O_4$. No carboxyl, carbonyl or typical primary amino groups were present. Further tests showed the absence of O-, N- and C-methyl groups. Streptidine was found to have eight acetylatable –OH and >NH groups and two basic groups.

The two basic groups of streptidine were shown by potentiometric titration to be of an order of basic strength equivalent to guanidine. The presence of guanido groups in streptidine was proved by degradation of streptidine to guanidine. Streptidine was oxidized in aqueous solution with potassium permanganate, and the liberated guanidine was isolated as the picrate. The yield of the picrate corresponded to 1.3 moles of guanidine per mole of streptidine. Thus, there are two guanido groups in streptidine. These two guanido groups account for all six nitrogen atoms and the strongly basic character of streptidine.

Four of the eight acetyl groups in octaacetylstreptidine[1] can now be considered as N-acetyl groups on the basis of the formation of diacetyl derivatives of monosubstituted guanidines under the experimental conditions used. The remaining four acetyl groups of octaacetylstreptidine would then appear to be O-acetyl groups. Thus, all four oxygen atoms of streptidine are apparently present as hydroxyl groups.

The analytical data[1] on several salts showed that streptidine has two hydrogen atoms less than that number required for a saturated acyclic compound. The infrared and ultraviolet absorption spectra[1] of streptidine did not show bands suggestive of a C=C group. Furthermore, there was no evidence of unsaturation when bromine water was added to an aqueous solution of streptidine dihydrochloride. Thus, streptidine appears to have the carbocyclic structure (I) in which the two guanido groups are in the 1,2-, 1, 3- or 1,4-positions.



I

Each of the remaining four carbon atoms of the ring appears to have one hydroxyl group. Further information on the structure was obtained by a study of the reactions of the products formed by alkaline hydrolysis of streptidine.

Alkaline hydrolysis of streptidine involved two

(1) Peck, Graber, Walti, Peel, Hoffhine and Folkers, THIS JOURNAL, **68**, 29 (1946).

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

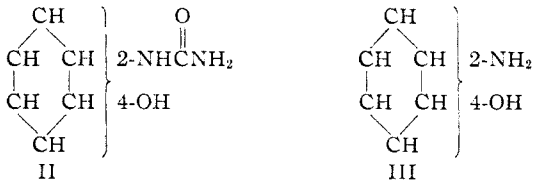
steps. When streptidine was added to an aqueous barium hydroxide solution and the solution was refluxed for about one hour, two moles of ammonia per mole of streptidine was liberated. A crystalline neutral substance, m. p. 290–300° (dec.), was also obtained which had the composition $C_8H_{16}N_4O_6$. This reaction is expressed by the equation

$$C_8H_{18}N_6O_4 + 2H_2O \longrightarrow 2NH_3 + C_8H_{16}N_4O_6$$

When a barium hydroxide solution containing streptidine was refluxed for about twenty-four hours, four moles of ammonia and two moles of carbon dioxide per mole of streptidine were liberated. A new base which had the composition $C_6H_{14}N_2O_4$ was obtained as a crystalline sulfate. This reaction is expressed by the equation

$$C_8H_{18}N_6O_4 + 4H_2O \longrightarrow 4NH_3 + 2CO_2 + C_6H_{14}N_2O_4$$

The neutral urea derivative and the amine (which we call strepturea and streptamine) appear to have structures II and III, respectively.

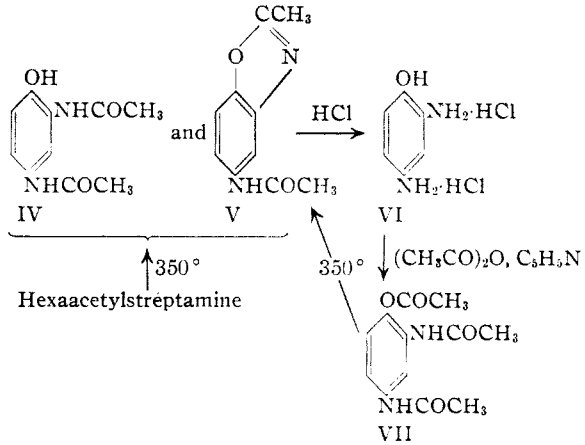


Streptamine was also characterized as the crystalline free base, the dipicrate, the di-*p*-(*p*-hydroxyphenylazo)-benzenesulfonate and the dihydriodide. Streptamine reacted with two equivalents of benzaldehyde to form a crystalline Schiff base. Optical rotation was zero for streptamine. All of its nitrogen was liberated in the Van Slyke amino-nitrogen determination. Hexaacetylstreptamine and hexabenzoylstreptamine were obtained as crystalline derivatives and from these compounds, the crystalline N,N′-diacetylstreptamine and N,N′-dibenzoylstreptamine were prepared by partial deacylation.

In the oxidation of streptamine with periodic acid, six atoms of oxygen per mole of streptamine was consumed. No formaldehyde was formed in this oxidation. Under comparable conditions, inositol consumed six atoms of oxygen and gave no formaldehyde while mannitol consumed five atoms of oxygen and gave two moles of formaldehyde. These data show the absence of —CH_2OH groups in streptamine and support partial structure III for streptamine and, of course, partial structure I for streptidine. In the periodic acid oxidation of N,N′-diacetylstreptamine, two atoms of oxygen were consumed. This indicates that the amino groups do not occupy the 1,2-position, since a 1,2-diacetamido-3,4,5,6-tetrahydroxycyclohexane would be expected to consume three atoms of oxygen. Furthermore, streptamine did not yield a cyclic ureide when treated with phosgene. The 1,3- and 1,4-positions remain for the orientation of the two guanido groups in partial structure I for streptidine.

The conclusion that streptidine had either the 1,3- or 1,4-diguanido- structure was recently reported by other investigators.[3] They observed positive Sakaguchi tests for guanido groups. They also characterized streptidine and hydrolyzed it with 6 *N* alkali to the base $C_6H_{14}N_2O_4$, for which they proposed the name streptamine. The results of their periodate oxidation studies on streptamine, N,N′-dibenzoylstreptamine and streptidine led to the conclusion as to the 1,3- or 1,4-location of the guanido groups. Thus, the results of these two investigations are in agreement.[3a]

It was believed that direct evidence for both the carbon skeleton and the orientation of the nitrogen atoms could be obtained as the result of one degradation, if aromatization of streptamine without elimination of the nitrogen atoms could be achieved. In other words, aromatization of streptamine by the removal of three molecules of water or their equivalent would leave a diaminophenol. The identity of the diaminophenol would supply the desired evidence on the carbon skeleton and on the location of the amino groups. Various thermal dehydration reactions of streptamine in the presence of inorganic reagents were tried without satisfactory results. It was found, however, that when hexaacetylstreptamine was heated in a sealed tube at 350° for one hour, 3.5 moles of acetic acid was produced, as evidenced by titration with standard alkali, and two crystalline products were obtained. One of the products melted at 222–225° and had the composition $C_{10}H_{12}N_2O_3$. This product and 2,4-diacetamidophenol (IV), prepared by catalytic hydrogenation of 2,4-dinitrophenol in a mixture of acetic anhydride and acetic acid, were identical. The second product melted at 210–211.5° and had the composition $C_{10}H_{10}N_2O_2$. Acid hydrolysis of this



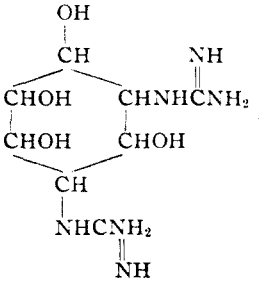
(3) Carter, Clark, Dickman, Loo, Meek, Skell, Strong, Alberi, Bartz, Binkley, Crooks, Hooper and Rebstock, *Science*, **103**, 53 (1946).

(3a) Another paper, which appeared while this article was in press, has also reported the conclusion that a diguanidotetrahydroxycyclohexane structure for streptidine is consistent with degradation results (Fried, Boyack and Winternsteiner, *J. Biol. Chem.*, **162**, 393, (1946)).

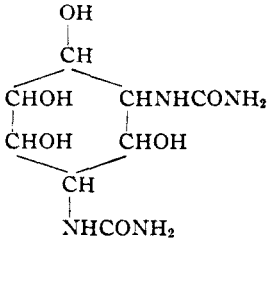
compound yielded a dihydrochloride which was identical with an authentic specimen of 2,4-diaminophenol dihydrochloride (VI). Acetylation of the dihydrochloride from the hydrolysis yielded a triacetyl derivative which was identical with an authentic specimen of 2,4-diacetamidophenyl acetate (VII). 2,4-Diacetamidophenyl acetate was converted to 5-acetamido-2-methylbenzoxazole (V) by heating it at 350° for one hour. This benzoxazole (V) and the degradation product melting at 210–211.5° were identical.

Since these two diaminophenol derivatives were obtained by pyrolysis, the yields of these products are of interest. The yield of the crystalline mixture of the diacetyl derivative (IV) and the benzoxazole (V) was about 95% of the theoretical amount. After chromatographic separation, 0.35 mole of the benzoxazole and 0.2 mole of the diacetyl derivative per mole of hexaacetylstreptamine were obtained in substantially pure form. In another experiment, the presence of 0.65 mole of the benzoxazole per mole of hexaacetylstreptamine in the crude pyrolysis product was determined by ultraviolet absorption spectra analyses. The quantitative separation of these two products was rather difficult by the procedure employed.

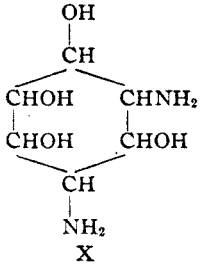
On the basis of the accumulated evidence, streptidine, strepturea, and streptamine have structures VIII, IX and X, respectively, and



VIII



IX



X

streptidine is one of the stereoisomeric forms of 1,3 - diguanido - 2,4,5,6 - tetrahydroxycyclohexane. Since no optical activity has been observed for streptidine or any of its derivatives, it appears to be a meso form.

Experimental

Degradation of Streptidine to Guanidine by Oxidation.—A solution of 200 mg. (0.545 millimole) of streptidine dihydrochloride ($C_8H_{18}N_6O_4 \cdot 2HCl \cdot CH_3OH$) in 10 cc. of water was maintained at 70° on a water-bath and potassium permanganate was added in small portions until the permanganate color persisted for thirty minutes after the last addition. Oxidation was rapid, a copious precipitate of manganese dioxide being deposited. The mixture was cooled and sulfur dioxide was bubbled through it until the manganese dioxide disappeared. A solution of 250 mg. of picric acid in 10 cc. of hot water was added. A crystalline precipitate of guanidine picrate separated. After drying, the picrate weighed 202 mg. (0.70 millimole). The product was recrystallized once from 10 cc. of water; m. p. 332–337° (dec.). No depression of the melting point was observed when this picrate was mixed with authentic guanidine picrate.

Anal. Calcd. for $C_7H_8N_6O_7$: C, 29.17; H, 2.80; N, 29.17. Found: C, 29.21; H, 2.70; N, 29.52.

The yield of guanidine picrate obtained corresponded to the isolation of about 1.3 moles of guanidine per mole of streptidine dihydrochloride oxidized. About 20 mg. of the picrate (0.07 millimole) might have remained in solution as estimated from the solubility of guanidine picrate in water. This estimated amount together with that actually isolated corresponded to a total yield of about 1.41 moles of guanidine per mole of streptidine.

Alkaline Hydrolysis of Streptidine.—In preliminary studies, it was found that ammonia was formed when an aqueous solution of streptidine and barium hydroxide was refluxed. Two moles of ammonia per mole of streptidine was liberated within approximately one hour, and two additional moles of ammonia were gradually produced during the next twenty-four hours. After the first two moles of ammonia were liberated, a crystalline deposit of barium carbonate began to form.

Alkaline Hydrolysis of Streptidine to Strepturea.—A solution of 371 mg. (1.01 millimoles) of streptidine dihydrochloride in 10 cc. of 0.372 *N* barium hydroxide was refluxed for one hour in a nitrogen atmosphere. The volatile base was collected in traps containing standard hydrochloric acid. Titration of the acid solution in the traps with alkali showed that 0.81 millimole of ammonia had been produced during the refluxing period. To the hot clear hydrolysis solution, 4.83 cc. of 0.771 *N* sulfuric acid was added, and the precipitated barium sulfate was removed. The filtrate was evaporated and the residue was extracted with methanol. The insoluble material, which weighed 210 mg., was dissolved in 5 cc. of hot water and 8 cc. of methanol was added. As the solution cooled, clusters of needle crystals of strepturea separated. The crystals darkened at 270° and decomposed about 290–300° (micro-block). An aqueous solution of the crystals was neutral to litmus.

Anal. Calcd. for $C_8H_{16}N_4O_6$: C, 36.36; H, 6.10; N, 21.20. Found: C, 35.85; H, 6.43; N, 20.60.

Alkaline Hydrolysis of Streptidine to Streptamine. Streptamine Sulfate.—One gram (3 millimoles) of streptidine dihydrochloride was dissolved in 15 cc. of carbon dioxide-free water in a monel metal flask. The flask was equipped with a reflux condenser, a nitrogen inlet and traps containing standard hydrochloric acid to collect evolved ammonia. After introducing 100 cc. of saturated aqueous barium hydroxide, the solution was refluxed for about twenty-three hours. The ammonia was collected in the traps by passing nitrogen through the apparatus. Titration of the acid solution in the traps with alkali showed that 3.7 moles of ammonia per mole of streptidine had been collected during the refluxing period. The volatile base was converted to ammonium chloride and to ammonium chloroplatinate.

A crystalline precipitate of barium carbonate had formed in the hydrolysis flask. The supernatant solution was siphoned from the precipitate through a sintered filter, and the precipitate was washed with carbon dioxide-free water. Treatment of the crystalline residue of barium carbonate with sulfuric acid gave 1.144 g. of barium sulfate and liberated carbon dioxide. The yield of carbon dioxide was equivalent to the formation of 1.82 moles of carbon dioxide per mole of streptidine during the hydrolysis. The hydrolysis solution was heated on the steam-bath and 41.17 ml. of 0.771 *N* sulfuric acid was added slowly. After re-

moval of barium sulfate by filtration, the filtrate was freeze-dried. The faintly buff-colored amorphous residue of streptamine sulfate was dried at 56° *in vacuo;* yield, 710 mg. A portion of this material was crystallized from water. The crystals showed no melting point up to 300° (micro-block) though some darkening was observed from about 280°.

Anal. Calcd. for $C_6H_{14}N_2O_4 \cdot H_2SO_4$: C, 26.12; H, 5.84; N, 10.14. Found: C, 26.13; 26.41; H, 6.18, 5.93; N, 10.29; NH_2-N (Van Slyke), 10.20.

From 310 mg. of the amorphous product described above, there was obtained 215 mg. of the crystalline streptamine sulfate. The mother liquors yielded further amounts after addition of a small amount of aqueous sulfuric acid.

Streptamine.—To a stirred suspension of 1.240 g. of streptamine sulfate in 50 cc. of boiling water, 19.36 cc. of 0.465 *N* barium hydroxide solution was added slowly. The mixture was refluxed for ten minutes, cooled and filtered to remove the barium sulfate. These operations were carried out under a nitrogen atmosphere. The barium sulfate weighed 1.052 g. The colorless filtrate was concentrated under reduced pressure (nitrogen atmosphere) to a volume of about 5 cc. when crystals began to separate. Carbon dioxide-free water was added to dissolve the crystals, and the solution was diluted with about five volumes of ethanol causing the separation of needles. The first crop was recrystallized once giving 375 mg. of a white product. These crystals began to sinter at 205°, darkened from 210–250° but showed no apparent melting up to 290° (micro-block). They were dried at 25° *in vacuo* for analyses.

Anal. Calcd. for $C_6H_{14}N_2O_4 \cdot {}^1/_2H_2O$: C, 38.49; H, 8.08; N, 14.97. Found: C, 38.65; H, 7.62; N, 15.43.

Streptamine Di-*p*-(*p*-hydroxyphenylazo)-benzenesulfonate.—An aqueous solution containing 72 mg. of streptamine in 0.5 cc. of water was mixed with a solution of 300 mg. of *p*-(*p*-hydroxyphenylazo)-benzenesulfonic acid in 3 cc. of water. Long yellow-orange needles separated. This salt was recrystallized once from hot water and dried at 100° *in vacuo*. The crystals decomposed between 270–300° (micro-block).

Anal. Calcd. for $C_{30}H_{34}N_6O_{12}S_2$: C, 49.04; H, 4.66; N, 11.44. Found: C, 48.82; H, 4.67; N, 11.61.

Streptamine Dipicrate.—A solution of 149 mg. of streptamine dihydrochloride (m. p. 245–255° (dec.), prepared by metathetical reaction from streptamine sulfate and barium chloride) in 10 cc. of water was mixed with 270 mg. of picric acid and warmed until the solution was clear. After cooling, needles of streptamine dipicrate separated; yield, 250 mg. The crystals decomposed partially between 260–285° (micro-block) but did not definitely melt up to 300°.

Anal. Calcd. for $C_6H_{14}N_2O_4 \cdot 2C_6H_3N_3O_7$: C, 33.97; H, 3.17; N, 17.61. Found: C, 33.57; H, 3.42; N, 17.52.

Streptamine Dihydriodide.—A mixture of 139 mg. of streptamine sulfate, 150 mg. of red phosphorus and 5 cc. of hydriodic acid (sp. g. 1.7) was heated in a bomb tube at 160° for fifteen hours. The mixture was diluted with water and filtered, and the colorless filtrate was evaporated to dryness on the steam-bath. The crystalline residue was dissolved in about 6 cc. of methanol and carefully diluted with ether whereupon a fine white crystalline precipitate of streptamine dihydriodide was deposited. The crystals were washed with ether and dried; weight 203 mg. The streptamine dihydriodide decomposed gradually above 280° (micro-block).

Anal. Calcd. for $C_6H_{14}N_2O_4 \cdot 2HI$: C, 16.60; H, 3.72; N, 6.46; I, 58.48. Found: C, 16.40; H, 3.89; N, 6.68; I, 57.17.

Reaction of Streptamine with Benzaldehyde.—A mixture of 25 mg. of streptamine and 0.1 cc. of benzaldehyde was warmed gently. Solution occurred readily, but within a few minutes a crystalline product separated. The crystals were isolated by centrifuging, and were recrystallized from a mixture of ethanol, benzene and petroleum ether. The product melted at 222–228° (dec.) (micro-block). This sample showed a maximum, $E_{1\%}$ 956 at 2475 Å., in the ultraviolet.

Anal. Calcd. for $C_{20}H_{22}N_2O_4 \cdot H_2O$: C, 64.50; H, 6.50; N, 7.52. Found: C, 64.96; H, 6.89; N, 8.09.

Hexaacetylstreptamine.—A mixture of 5 cc. of acetic anhydride, 81 mg. of streptamine sulfate and 50 mg. of sodium acetate was refluxed for one hour. The mixture was then evaporated and the residue was extracted with chloroform. The chloroform extract yielded 40 mg. of a crystalline product which was recrystallized from chloroform–ether. The crystals showed partial melting with transition to longer needles at about 250° but did not melt up to 300° (micro-block).

Anal. Calcd. for $C_{18}H_{26}N_2O_{10}$: C, 50.20: H, 6.09; N, 6.50; CH_3CO, 60.0. Found: C, 51.00; H, 6.05; N, 6.03; CH_3CO, 61.1.

The chloroform-insoluble residue left after separation of the chloroform extract consisted of two types of crystals. These were readily separated on the basis of their different specific gravities. After suspending in chloroform, the sodium sulfate settled rapidly leaving a suspension of long needles which was removed with a pipet. The needle crystals were collected on a filter; weight 90 mg. When heated on the micro-block, these crystals showed partial melting and transition to longer needles at 240–247° as observed for the chloroform-soluble acetylation product.

Anal. Calcd. for $C_{18}H_{26}N_2O_{10}$: C, 50.20; H, 6.09; N, 6.50; CH_3CO, 60.0. Found: C, 50.08; H, 6.10; N, 6.17; CH_3CO, 56.9.

When either form of hexaacetylstreptamine was heated in a sealed capillary, the melting point was about 342–345° (cor.).

N,N′-Diacetylstreptamine.—A solution of 38 mg. of chloroform-insoluble hexaacetylstreptamine in 10 cc. of absolute methanol at 0° was saturated with gaseous ammonia. This solution was allowed to warm to room temperature and stand for about three hours. The solvent and excess ammonia were then removed under reduced pressure leaving a crystalline residue. The product was recrystallized from methanol–ethanol; yield 16 mg. The crystals became opaque at about 220° and melted at 283–284° (micro-block). The crystals were dried *in vacuo* at 56° for analyses.

Anal. Calcd. for $C_{10}H_{18}N_2O_6$: C, 45.79; H, 6.92; N, 10.68. Found: C, 45.70; H, 6.95; N, 10.39.

Hexabenzoylstreptamine.—A mixture of 744 mg. of streptamine dihydrochloride, 3.5 cc. of benzoyl chloride and 5 cc. of pyridine was heated just to boiling, then cooled and diluted with 15 cc. of ice water. The suspension of crystals was made acid with dilute hydrochloric acid and extracted with chloroform. After washing with dilute sodium bicarbonate solution and with water, the chloroform extract was dried, evaporated to about 5 cc. and diluted with 10 cc. of ether, causing the separation of a copious crystalline precipitate. The crystalline product, 780 mg., was recrystallized from chloroform–ether. The crystals showed no melting point up to 300°.

Anal. Calcd. for $C_{48}H_{38}N_2O_{10}$: C, 71.81; H, 4.77; N, 3.49. Found: C, 72.06; H, 5.03; N, 3.35.

N,N′-Dibenzoylstreptamine.—A solution of 750 mg. of hexabenzoylstreptamine in 60 cc. of methanol containing a catalytic amount of sodium methoxide was refluxed for three hours. Then 5 cc. of dry pyridine was added and refluxing was continued for fifteen minutes. The clear solution was then evaporated to dryness under reduced pressure. The residue was dissolved in water and the solution was acidified with dilute hydrochloric acid. The crystalline precipitate was collected on a filter and recrystallized from aqueous methanol. The yield of recrystallized N,N′-dibenzoylstreptamine from this experiment was 160 mg.; m. p. 293–295° (micro-block).

Anal. Calcd. for $C_{20}H_{22}N_2O_6$: C, 62.16; H, 5.74; N, 7.25. Found: C, 62.26; H, 5.64; N, 7.52.

Periodate Oxidation of Streptamine.—To an aqueous solution containing 53.6 mg. of streptamine sulfate (0.194

millimole) and 0.5 g. of sodium bicarbonate, 6.0 cc. of 0.217 *M* aqueous periodic acid (1.306 millimoles) was added, and the solution was immediately diluted to a total volume of 50 cc. with water. Aliquots of 3 cc. each were withdrawn at intervals for titration with standard alkaline arsenite solution. Excess potassium iodide was added to each aliquot before titration, and a few drops of 1% starch indicator were added just before the end-point. Five atoms of oxygen per mole of streptamine were consumed within about three minutes, and the reaction was essentially complete after one hour when a total of six atoms of oxygen was used. Under the same conditions, glucose consumed five atoms of oxygen (three hours); inositol six atoms (forty-five minutes); mannitol, five atoms (one hour); 2-aminopropanediol-1,3 two atoms (five minutes); and serine two atoms (seventeen hours).

A check on the possible presence of hydroxymethylene groups was made. A 97.7-mg. sample of streptamine sulfate was oxidized with excess periodate in 150 cc. of water containing 0.5 g. of sodium bicarbonate for sixteen hours. After this period, 21 cc. of 1 *N* alkaline arsenite was added and the solution was immediately neutralized with about 3 cc. of concentrated hydrochloric acid. It was then buffered to *p*H 4.5 by the addition of 10 cc. of 5 *N* sodium acetate solution and 5 cc. of 5 *N* hydrochloric acid. After adding 82 cc. of aqueous dimedon solution (328 mg. of dimedon), the mixture was diluted to a total volume of 300 cc. After standing for twenty-four hours, there was no precipitation and the solution remained clear. In an exactly comparable experiment, 75.3 mg. of mannitol gave 241.4 mg. of the formaldehyde-dimedon condensation product which corresponds to a yield of 1.996 moles of formaldehyde per mole of mannitol. Inositol under the same conditions gave no formaldehyde derivative. It was concluded from these results that a —CH_2OH group was not present in streptamine.

Periodate Oxidation of N,N′-Diacetylstreptamine.—A sample of N,N′-diacetylstreptamine weighing 12.9 mg. (0.049 millimole) was dissolved in 10 cc. of a 0.0213 *M* aqueous solution of potassium periodate (0.213 millimole). One cubic centimeter aliquots were withdrawn at intervals for titration with standard alkaline arsenite solution. An excess of potassium iodide and about 50 mg. of sodium bicarbonate were added to each aliquot prior to the titration, and starch indicator was used. The periodate consumed at the end of the reaction, or after about thirty hours, corresponded to two atoms of oxygen per mole of N,N′-diacetylstreptamine.

Estimation of Acetic Acid Produced by Pyrolysis of Hexaacetylstreptamine.—A sample of hexaacetylstreptamine weighing 1.287 g. was heated in a bomb tube at 350° for one hour. The resulting product, a dark semi-solid mixture, was extracted with about 80 cc. of warm ether. The cooled ether extract deposited some crystalline material. The crystals were removed and combined with the ether-insoluble portion. The ether filtrate was mixed with 15 cc. of methanol and titrated with standard sodium hydroxide using phenolphthalein as the indicator. The neutralization required 10.25 cc. of 1.022 *N* sodium hydroxide, corresponding to about 10.5 millimoles of acetic acid. About 3.5 moles of acetic acid were therefore produced per mole of hexaacetylstreptamine.

5-Acetamido-2-methylbenzoxazole from Hexaacetylstreptamine.—The titration solution described in the preceding section was mixed with 100 cc. of ethyl acetate, washed several times with water to remove methanol and sodium acetate, and dried over anhydrous magnesium sulfate. The ether-insoluble material was dissolved in ethyl acetate and filtered to remove an insoluble charred residue, and the filtrate was combined with the ether-ethyl acetate solution recovered from the titration. Evaporation of this extract *in vacuo* yielded 635 mg. of a crystalline residue. Since the acetic acid produced was equivalent to 630 mg., the total recovered products corresponded to 98.5% of the starting weight.

The 635 mg. of crystalline material was dissolved in about 5 cc. of ethyl acetate giving a yellow solution which exhibited a green fluorescence. The solution was warmed with Darco G-60 for decolorization and filtered. The filtrate was passed through a column (1 × 15 cm.) of activated aluminum oxide. The chromatographic column was developed first with ethyl acetate and then with acetone. The ethyl acetate eluate yielded 250 mg. of crystals in plate-form. These crystals showed a transition to a different size at 190° and melted at 205–209°. After recrystallization from acetone–ether, the crystals showed the same transition effect and melted at 210–211.5° (micro-block). This substance, 5-acetamido-2-methylbenzoxazole, showed an absorption maximum, $E_{1\%}$ 228 at 2885 Å., in the ultraviolet.

Anal. Calcd. for $C_{10}H_{10}N_2O_2$: C, 63.14; H, 5.30; N, 14.73; CH_3CO, 45.2; mol. wt., 190.2. Found: C, 63.57; H, 5.26; N, 14.40; CH_3CO, 48.3; mol. wt. (ebullioscopic in acetonitrile), 161.

To a sample of the pyrolysis product melting at 210–211.5° weighing 68.5 mg. was added 1.0 cc. of concentrated hydrochloric acid. The compact plates were immediately converted to rosets of stout needles. The mixture was refluxed in nitrogen atmosphere, all the crystals going into solution on heating. After five minutes, long needles began to separate from the refluxing solution. One cc. of water was added to redissolve the crystals, and refluxing was continued for three-quarters of an hour. The solution was then evaporated to dryness *in vacuo*. The residue was washed with acetone to remove a small amount of reddish sirup. The colorless crystals which remained weighed 66.1 mg. The crystals sintered at 227°, decomposed between 230–240° with the formation of a reddish-purple pigment, but did not melt up to 260° (micro-block).

Anal. Calcd. for $C_6H_{10}N_2OCl_2$: C, 36.57; H, 5.12; N, 14.22; Cl, 35.98. Found: C, 36.60; H, 5.17; N, 13.50; Cl, 35.49.

The melting point behavior was not changed when the material was mixed with authentic 2,4-diaminophenol dihydrochloride.

A 50-mg. sample of the 2,4-diaminophenol dihydrochloride obtained as described above was mixed with 0.5 cc. of pyridine and 0.5 cc. of acetic anhydride. After ten minutes the excess reagent was removed *in vacuo*. The product was crystallized from acetone–ether; yield 31 mg. of needles melting at 187–189° (micro-block).

Anal. Calcd. for $C_{12}H_{14}N_2O_4$: C, 57.59; H, 5.64; N, 11.20. Found: C, 57.93; H, 5.58; N, 11.25.

There was no depression of this melting point when the sample was mixed with authentic 2,4-diacetamidophenyl acetate which melted at 187–190° (micro-block). The ultraviolet absorption spectra of the "natural" and synthetic products were identical. Maxima, $E_{1\%}$ 885–899 at 2350 Å. and $E_{1\%}$ 46–52 at 2850 Å. were observed.

2,4-Diacetamidophenol from Hexaacetylstreptamine.—From the acetone eluates of the chromatographic column, there was obtained after evaporation a product in the form of needles which weighed 150 mg. and melted at 218–225° (micro-block). After recrystallization from acetone–ether, the crystals sintered at 218° and melted at 222–225° (micro-block).

Anal. Calcd. for $C_{10}H_{12}N_2O_3$: C, 57.68; H, 5.81; N, 13.46. Found: C, 57.90; H, 5.86; N, 13.65.

When this product was mixed with authentic 2,4-diacetamidophenol, there was no depression of the melting point.

The ultraviolet absorption spectra of the "natural" and synthetic products were identical. Two maxima, $E_{1\%}$ 203–208 at 2350 Å. and $E_{1\%}$ 1046–1061 at 3000 Å., were observed.

2,4-Diacetamidophenol.—Five and five-tenths grams of 2,4-dinitrophenol was hydrogenated at 15–40 lb. pressure over 0.1 g. of platinum oxide[4] in 75 ml. of acetic anhydride and 220 ml. of acetic acid. Hydrogenation ceased after three molar equivalents had been absorbed. The catalyst

(4) Adams, Voorhees and Shriner, "Organic Syntheses," Coll. Vol. I, 463 (1941).

was removed. The resulting solution was concentrated under reduced pressure. During the concentration, crystals separated and were collected. After recrystallization from water the 2,4-diacetamidophenol[5] melted at 222–225° (micro-block).

2,4-Diacetamidophenyl Acetate.—After the crystals, which collected during concentration of the solution from the reduction, had been removed the remaining solution was concentrated to dryness under reduced pressure at 60–80°. The resulting material was recrystallized first from methanol, then to constant melting point from water. The 2,4-diacetamidophenyl acetate[5] melted at 187–190° (micro-block).

Anal. Calcd. for $C_{12}H_{14}N_2O_2$: C, 57.59; H, 5.64; N, 11.20. Found: C, 57.63; H, 5.52; N, 11.05.

5-Acetamido-2-methylbenzoxazole.—A 342-mg. sample of 2,4-diacetamidophenyl acetate was heated in a bomb tube at 350° for one hour. The product was a semicrystalline dark mass possessing the odor of acetic acid. This material was dissolved in warm ethyl acetate, decolorized with Darco G-60 and evaporated to dryness. The faintly buff-colored crystalline product weighed 250 mg. After recrystallization from acetone, the crystals showed the typical transition to larger thin plates at 190° and melted at 210–212° (micro-block).

Anal. Calcd. for $C_{10}H_{10}N_2O_2$: C, 63.14; H, 5.30; N, 14.73. Found: C, 63.42; H, 5.12; N, 14.95.

When this synthetic product was mixed with the "natural" pyrolysis product melting at 210–211.5°, the melting point of the mixture was 210–212° with the usual transition at about 190°.

The ultraviolet absorption spectra of the "natural" and synthetic products were identical. A maximum, $E_{1\%}$ 220–228 at 2885 Å., was observed.

(5) Kehrmann and Bahatrian, *Ber.*, **31**, 2399 (1898).

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Summary

Streptidine was degraded to guanidine by oxidation with potassium permanganate, and the yield of guanidine showed that streptidine contains two guanido groups. On treatment with barium hydroxide solution, streptidine was converted stepwise into a neutral substance, strepturea, and a basic substance, streptamine. These alkali degradation products have the composition $C_8H_{16}N_4O_6$ and $C_6H_{14}N_2O_4$, respectively. Periodate oxidation studies and other information showed that streptidine is a 1,3- or 1,4-diaminotetrahydroxycyclohexane. Pyrolysis of hexaacetylstreptamine gave acetic acid, and an excellent yield of 2,4-diacetamidophenol and 5-acetamido-2-methylbenzoxazole.

On the basis of the accumulated evidence, streptidine is one of the meso forms of 1,3-diguanido-2,4,5,6-tetrahydroxycyclohexane. Strepturea and streptamine are the corresponding urea- and amine-like structures.

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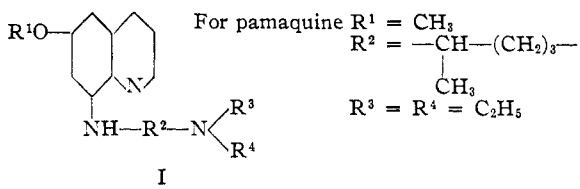
[CONTRIBUTION FROM THE DEPARTMENT OF RESEARCH IN PURE CHEMISTRY, MELLON INSTITUTE OF INDUSTRIAL RESEARCH]

Two 6-β-Hydroxyethoxy-8-diethylaminoalkylaminoquinolines

BY MARCUS S. MORGAN AND LEONARD H. CRETCHER

Although pamaquine is a potent antimalarial, the Army[1] no longer advises its routine use for malaria therapy because the margin of safety between therapeutic and toxic doses is too small. In spite of recognized limitations, pamaquine possesses unique plasmodicidal action, namely, (a) gametocidal action on *Plasmodium falciparum*,[2] (b) reduction of the relapse rate in benign tertian malaria,[2] and (c) causal prophylactic activity at the toxic dose level as reported by James, *et al.*[3]

The present investigation was undertaken with the object of synthesizing analogs which would be less toxic to the host and yet retain the therapeutic activity of compounds of type I. In work previously published from this Laboratory, Cretcher and Pittenger propounded the principle of hydroxyalkylation[4] as a means of detoxification of some pharmacologically active compounds.



I

This principle was applied by Cretcher and associates[5] to a number of drugs and resulted in the discovery of the anti-pneumococcic drug, hydroxyethylapocupreine.[6] We therefore deemed it desirable to employ this approach in the synthesis of new pamaquine analogs.

Although the hydroxyl group has been introduced into the aliphatic side-chain (R^2) by several investigators[7] and recently 8-γ-hydroxypropyl-6-

(1) Surgeon General's Office, Circular Letter No. 153; *J. Am. Med. Assoc.*, **123**, 205 (1943).

(2) League of Nations, Fourth General Report of the Malaria Commission, Geneva, 1937, p. 120.

(3) James, Nicol and Shute, *Lancet*, [2], 341 (1931).

(4) Cretcher and Pittenger, THIS JOURNAL, **47**, 2560 (1925).

(5) For previous papers on hydroxyalkylation from this Laboratory, *cf.* THIS JOURNAL, **47**, 2560, 3083 (1925); **50**, 2758 (1928); **57**, 575 (1935); **59**, 227 (1937); **60**, 1473, 1582 (1938); **61**, 1783 (1939); **65**, 1092 (1943); *Chem. Rev.*, **30**, 49 (1942); *Chem. Eng. News*, **23**, 527 (1945).

(6) U. S. Patents 2,033,679 (March 10, 1936); 2,172,607 (Sept. 12, 1939).

(7) (a) Rohrmann and Shonle, THIS JOURNAL, **66**, 1643 (1944); (b) Magidson and Strukov, *Arch. Pharm.*, **271**, 569 (1933).