Degradation of Streptomycin and the Structure of Streptidine and Streptamine

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Brink, *et al.* (*1*) recently described the degradation of streptomycin into two basic fractions which they designated as streptidine and streptobiosamine. Information concerning the composition of streptobiosamine and its derivatives was presented, and the empirical formula of streptidine was given. Similar work has been in progress in our laboratories, the results of which closely parallel those reported. The purpose of this communication is to present certain of these data with especial reference to the structure of the streptidine.

Streptomycin hydrochloride (*2*) is completely inactivated on standing 24 hours in anhydrous 1.0 N methanolic hydrogen chloride without forming a new basic group. The addition of two volumes of ether completely precipitates the guanidine, which we previously reported (*2*) as one of the functional groups of streptomycin. From the supernatant solution there is readily obtained an amorphous, optically active hydrochloride of a nonguanidine base whose properties agree with those of "methyl streptobiosaminide dimethyl acetal hydrochloride" (*1*).

Addition of picric or sulfuric acid to an aqueous solution of the guanidine hydrochloride gives an insoluble crystalline picrate or sulfate. These salts are readily recrystallized from hot water.

Guanidine picrate: m.p. 271–273°. *Anal.* Calcd. for $C_8H_{18}N_6O_4 \cdot 2C_6H_3N_3O_7$: C, 33.34; H, 3.36; N, 23.33. Found: C, 33.33; H, 3.60; N, 23.50.

Guanidine sulfate: Dec. ca. 310°. *Anal.* Calcd. for $C_8H_{18}N_6O_4 \cdot H_2SO_4 \cdot H_2O$: C, 25.40; H, 5.82; N, 22.20; S, 8.45. Found: C, 25.43, 25.75; H, 5.63, 5.86; N, 22.20 (Micro Dumas), 0.0 (Van Slyke amino nitrogen); S, 8.57, 8.30.

Guanidine hydrochloride: Obtained by dissolving the picrate in methanolic hydrogen chloride and adding ether. *Anal.* Calcd. for $C_8H_{18}N_6O_4 \cdot 2HCl$: C, 28.66; H, 5.97; N, 25.08; Cl, 21.19. Found: C, 29.10; H, 6.23; N, 24.20; Cl, 20.80.

These analytical data agree best for salts of a diguanidine base of the composition $C_8H_{18}N_6O_4$. This compound has the same empirical formula as that suggested by Brink, *et al.* (*1*) for streptidine, and, although there are no confirmatory data, we presume they are identical.

Streptidine sulfate is also obtained in excellent yield by allowing a solution of streptomycin chloride in 1 N sulfuric acid to stand at 37° for 45 hours. The sulfate is precipitated in nicely crystalline form by adding three to five volumes of acetone to the reaction mixture.

Streptidine is hydrolyzed by refluxing for 48 hours with 6 N alkali yielding four moles of ammonia and a new base, for which we propose the name streptamine. This base is readily isolated as the slightly soluble sulfate by neutralizing the hydrolysis mixture with sulfuric acid and adding an equal volume of methanol. The sulfate thus obtained is purified by recrystallization from aqueous methanol.

Streptamine sulfate: Dec. ca. 340°. *Anal.* Calcd. for $C_6H_{14}N_2O_4 \cdot H_2SO_4$: C, 26.10; H, 5.83; N, 10.13; S, 11.60. Found: C, 26.67; H, 6.10; N, 9.91 (Micro Dumas), 10.03 (Van Slyke amino nitrogen); S, 11.55.

The hydrolysis of streptidine evidently proceeds as shown in the following equation:

$$\underset{\text{Streptidine}}{C_8H_{18}N_6O_4} + 4H_2O \rightarrow \underset{\text{Streptamine}}{C_6H_{14}N_2O_4} + 4NH_3 + 2CO_2$$

These results, coupled with titration data and the Sakaguchi test, establish with some certainty that the six nitrogen atoms of streptidine are present as two monosubstituted guanidine groups which are replaced by two primary amino groups in streptamine.

Streptamine is converted into a mixture of polybenzoyl derivatives by the Schotten-Baumann procedure. Further treatment with benzoyl chloride in pyridine yields a product melting at 350–351°, the analyses of which agree fairly well for hexabenzoylstreptamine.

Anal. Calcd. for $C_{48}H_{38}N_2O_{10}$: C, 71.79; H, 4.77; N, 3.49. Found: C, 71.02; H, 4.94; N, 3.55.

Hexabenzoylstreptamine is converted into N,N-dibenzoylstreptamine by refluxing with 0.5 N methanolic sodium hydroxide. The N,N-dibenzoyl derivative melts at 276–277° and gives a negative ester test.

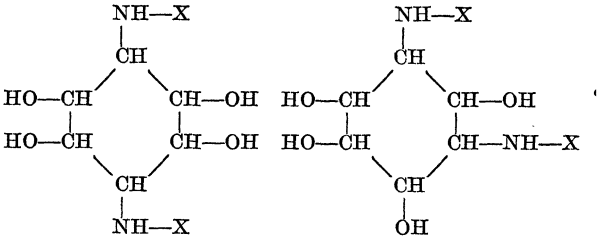
Anal. Calcd. for $C_{20}H_{22}N_2O_6$: C, 62.14; H, 5.75; N, 7.25. Found: C, 60.05, 60.64; H, 5.78, 6.05; N, 6.93, 7.20 (Micro Dumas), 0.0 (Van Slyke amino nitrogen).

Hexa-acetylstreptamine is produced by heating streptamine sulfate with sodium acetate and acetic anhydride, the crude product being purified by sublimation under reduced pressure. Hexa-acetylstreptamine is relatively insoluble in organic solvents and sublimes below 350° when heated on a hot stage.

Anal. Calcd. for $C_{18}H_{26}N_2O_{10}$: C, 50.23; H, 6.09; N, 6.51. Found: C, 50.18; H, 6.20; N, 6.66.

Periodate oxidation of streptidine, streptamine, and their derivatives: Since these substances are polyhydroxy compounds, a study of their behavior toward periodate seemed promising. As a model compound guanidoethanol sulfate was investigated. In contrast to ethanolamine, the guanidine derivative did not reduce periodate at pH 2–7. Streptidine reduced two moles of periodate; streptamine, six; dibenzoylstreptamine, two. No formaldehyde was formed from any of these compounds.

The fact that streptamine requires six moles of periodate indicates that the four hydroxyl and two amino groups are located on adjacent carbon atoms. The absence of formaldehyde production and the utilization of six moles of periodate both strongly point to a cyclic structure, since an open chain molecule should yield at least two moles of formaldehyde and require only five moles of periodate. On the basis of these results and the analytical data, streptamine is best formulated as a diaminotetrahydroxycyclohexane. Of the three possible arrangements of the amino groups (1,2; 1,3; 1,4) the 1,2-isomer is excluded by virtue of the fact that streptidine and N,N-dibenzoylstreptamine consume only two moles of periodate, whereas the 1,2-compound would use three moles. Streptidine and streptamine may be provisionally assigned one of the two following formulae:



Streptamine X = H

Streptidine X = $-C\begin{matrix}=NH\\-NH_2\end{matrix}$

The isolation of the products resulting from the periodate oxidations is in progress and should distinguish between these possibilities.

References

1. Brink, Norman G., Kuehl, Frederick A., Jr., and Folkers, Karl. *Science*, 1945, **102**, 506–507.
2. Carter, H. E., Clark, R. K., Jr., Dickman, S. R., Loo, Y. H., Skell, P. S., and Strong, W. A. *J. biol. Chem.*, 1945, **160**, 337.

Scanning Science—

Helmholtz, Hertz, and Kundt, the three greatest physicists of modern Germany, have died within two years, and the friends of German science feared that this loss would be followed by a standstill in physics, or at least by a lack of really important discoveries. But now we have Professor W. Röntgen's investigations in the physical laboratory of the University in Würzburg, the importance of which does not stand behind the famous electrical discoveries of Hertz in Bonn. Röntgen has found a new kind of rays—he calls them the X-rays—which though invisible to the eye, affect the photographic plate; which produce fluorescent phenomena; which pass through wood, metal and the human body; which are neither broken by prism and lenses nor reflected.

The chief facts about the X-rays are the following: It is well known that the discharges of a large Ruhmkorff induction coil produce in a vacuum tube, such as Crookes' or Hittorf's, colored rays which go in straight lines from the cathode to the glass of the tube. These cathode rays, which have been much studied, are visible to the eye and are well characterized by the fact that the magnet changes their direction; they do not pass thick cardboard, wood, etc. The place where these cathode rays reach the glass of the tube is the centre of Röntgen's X-rays. They are not visible and are not turned aside by a magnet; in short, they are not cathode rays, but are produced by them. Prisms and lenses do not diffract the rays, nor do prisms of hard rubber or aluminum. Lenses do not refract the rays and therefore ordinary photography is not possible; the pictures of the objects are only shadows. But these shadow-pictures can be taken in the closed window box of the camera in a light room, as the sunlight of course does not pass through the wood while the X-rays do. In this way Röntgen took photographs of a set of metal weights in a wooden box and of a thick wire wound as a spiral around a wooden stick; the wood was pervious, the metal of that thickness not, and so the shadows of the weights and of the wire are seen in the photograph, those of the wood scarcely at all. In the same manner he took the picture of a compass needle in the closed box. The door between two rooms did not hinder the chemical effect.

With regard to the nature of the X-rays it seems too early to say anything definite. Röntgen emphasizes the fact that they show no refraction and probably therefore move in all substances with equal velocity and are transmitted by a medium which exists everywhere and in which are the molecules of the substances.

—31 January 1896