pound must be formed which is neither well crystallized nor a good catalyst. It should be observed that over the entire region bounded by the points 55-900, 45-700, 70-650, 95-700, 80-900 there is a high concentration of $Ba_8Fe_8O_{21}$ and that all of the catalysts within these limits are reasonably active. In this range there is little or no ferric oxide and widely varying amounts of barium oxide and barium carbonate. Some of the fluctuations in catalytic efficiency may be attributable to the presence of these substances or to variations in their promoter action on the perowskite-like substance.

A rather simple hypothesis may be advanced to explain the unusual catalytic efficiency of this substance. The compound corresponds approximately to the formula $Ba_8Fe_8O_{21}$ although the structure calls for the formula $BaFeO_3$ so that only one-fourth of the iron ions are in the oxidation state 4. Such a crystal could readily accommodate more oxygen ions without any fundamental change occurring in the lattice. Some of the tervalent iron merely has to be oxidized to the quadrivalent state.

Thus the reaction

$$Ba_8Fe_8O_{21} + O_2 \longrightarrow Ba_8Fe_8O_{23}$$

would cause three-fourths of the iron ions to be in the quadrivalent state. The upper limit of this reaction would be $BaFeO_3$ but all degrees of oxidation up to 4 are possible. The oxidation of the carbon monoxide at the surface of the oxidized crystal could readily occur.

$$Ba_8Fe_8O_{23} + 2CO \longrightarrow Ba_8Fe_8O_{21} + 2CO_2$$

The oxygen ions removed in this way would not necessarily be derived from the oxygen molecule. Such a mechanism would not require the presence of active centers since any part of the crystal surface would be available for the exchange.

Summary

The relative catalytic activity of the products of the solid phase reaction between barium carbonate and ferric oxide have been determined using the oxidation of carbon monoxide as a means of comparison. Good correlation between the catalytic properties and the composition of the catalysts as revealed by X-ray analysis has been obtained.

For the compounds $BaO \cdot 6Fe_2O_3$ and $BaO \cdot 2Fe_2O_3$ the maximum activity is obtained with mixtures in which it can be assumed that incipient formation of the compounds is occurring and that with increasing crystallinity or increasing concentration of these compounds, the catalytic properties of the mixtures decrease. This is in accord with the results of Hüttig. With mixtures containing the compound $Ba_8Fe_8O_{21}$, however, the catalytic activity increases as the concentration or crystallinity of the compound increases. An explanation of this phenomenon based upon the deficient lattice of the substance and the variable valence of the iron is advanced. A contour diagram correlating catalytic efficiency with temperature of preparation and composition of the original mixtures is shown to be useful in problems of this kind.

BROOKLYN, N. Y. RECEIVED MARCH 18, 1946

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

Streptomyces Antibiotics. X. The Degradation of Streptomycin and Dihydrostreptomycin with Ethyl Mercaptan

BY FREDERICK A. KUEHL, JR., EDWIN H. FLYNN, NORMAN G. BRINK AND KARL FOLKERS

The cleavage of streptomycin hydrochloride, I, by the action of methanolic hydrogen chloride to give streptidine[1] and the amorphous methyl streptobiosaminide dimethyl acetal hydrochloride, II, which upon acetylation was obtained as the crystalline tetraacetyl derivative, III, has been described.[2] The methyl streptobiosaminide dimethyl acetal hydrochloride was degraded further to a new hexosamine, N-methyl-*l*-glucosamine,[3] which was isolated as the pentaacetyl derivative, IV. These reactions and products may now be formulated according to the following graphic scheme. The pyranose ring structure for the N-methyl-*l*-glucosamine derivatives is written at present by analogy with the work of Neuberger relating to the ring structure of N-acetyl-α-methylglucosaminide.[4]

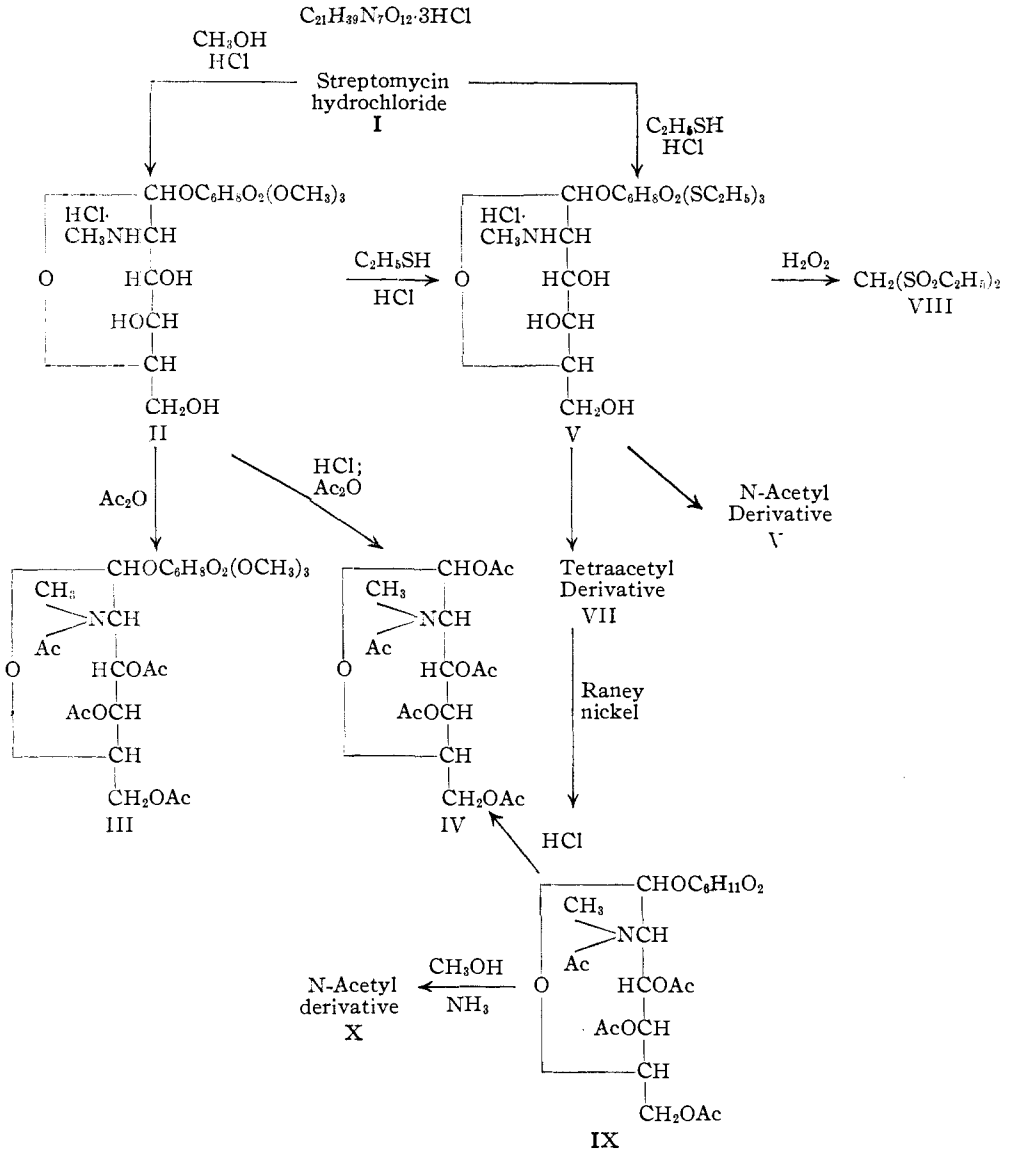
Streptomycin has now been cleaved with ethyl mercaptan and hydrogen chloride to streptidine and a new derivative of the disaccharide, ethyl thiostreptobiosaminide diethyl mercaptal hydrochloride, V. This degradation of streptomycin is accomplished by shaking overnight at 25° a suspension of streptomycin hydrochloride in ethyl mercaptan saturated with hydrogen chloride. After removing the excess ethyl mercaptan, the residue is dissolved in a small amount of water from which the mercaptal hydrochloride crystallizes, leaving the streptidine in solution.

(1) (a) Peck, Hoffhine, Peel, Graber, Holly, Mozingo and Folkers, THIS JOURNAL, **68**, 776 (1946); (b) Fried, Boyack and Wintersteiner, *J. Biol. Chem.*, **162**, 393 (1946); (c) Carter, Clark, Dickman, Loo, Skell and Strong, *Science*, **103**, 540 (1946).

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

(3) Kuehl, Flynn, Holly, Mozingo and Folkers, THIS JOURNAL, **68**, 536 (1946).

(4) Neuberger, *J. Chem. Soc.*, 47 (1941).



The degradation of streptomycin to ethyl thiostreptobiosaminide diethyl mercaptal hydrochloride offers certain advantages over the degradation to methyl streptobiosaminide dimethyl acetal hydrochloride. The method for the preparation of the mercaptal is simpler. The yield is about 80%, and the product is crystalline. The mercaptal derivative can also be prepared from streptomycin concentrate of about 400 U./mg. activity.

Treatment of the methyl streptobiosaminide dimethyl acetal hydrochloride, II, with ethyl mercaptan and hydrogen chloride readily yielded the mercaptal hydrochloride, V. It thus appears that the relationship between II and V is a simple one, involving merely the replacement of methoxyl groups by ethylmercapto groups on the same carbon skeleton.

When ethyl thiostreptobiosaminide diethyl mercaptal was acetylated with acetic anhydride in the presence of methanol, the crystalline ethyl N-acetylthiostreptobiosaminide diethyl mercaptal, VI, was obtained. Acetylation of ethyl thiostreptobiosaminide diethyl mercaptal hydrochloride with acetic anhydride–pyridine gave a crystalline tetraacetyl derivative, VII. This compound exhibited, in high concentration (about 50%) in tetrachloroethane solution, an infrared absorption band at 2.85 μ, which indicates the presence of a hydroxyl group.

The ethyl thiostreptobiosaminide diethyl mercaptal hydrochloride, V, in aqueous solution, was treated with mercuric chloride for the preparation of streptobiosamine hydrochloride. An amorphous, levorotatory solid which reduced Fehling solution was obtained.

Ethyl thiostreptobiosaminide diethyl mercaptal was not hydrolyzed in 2 *N* sodium hydroxide when

the solution was refluxed for one and one-half hours. Treatment of the mercaptal hydrochloride at 90° with ethyl mercaptan and hydrogen chloride gave only starting material and tarry decomposition products. Ethyl thiostreptobiosaminide diethyl mercaptal hydrochloride did not reduce Fehling solution. The mercaptal hydrochloride was hydrolyzed by treatment with concentrated hydrochloric acid at the reflux temperature. Under these conditions, the streptose (nitrogen-free hexose) moiety of the molecule appeared to undergo extensive decomposition. However, the N-methyl-*l*-glucosamine[3] portion remained intact, since it was isolated from the hydrolytic reaction and characterized as the pentaacetyl derivative, IV.

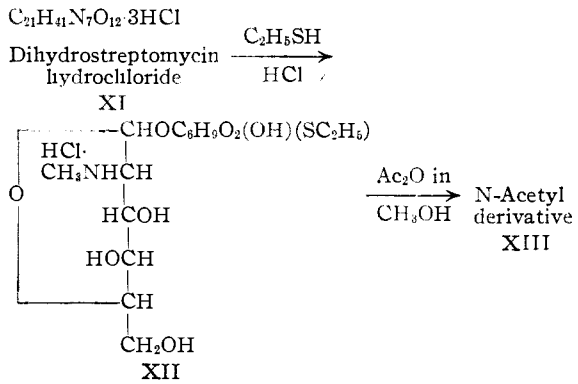
Oxidation of ethyl thiostreptobiosaminide diethyl mercaptal hydrochloride in glacial acetic acid solution with hydrogen peroxide yielded di-(ethylsulfonyl)-methane, VIII.

The hydrogenolysis of sulfides with Raney nickel catalyst[5] has been applied successfully to sugar mercaptals.[6] When ethyl tetraacetylthiostreptobiosaminide diethyl mercaptal in 70% ethanol was refluxed in the presence of freshly prepared Raney nickel, a crystalline, sulfur-free product having the composition $C_{13}H_{21}NO_7(CH_3CO)_4$, IX, was obtained. This formula is consistent with the replacement of the three ethylmercapto groups by hydrogen atoms. Because of this removal of two oxygen atoms from the original disaccharide by the hydrogenolysis of an intermediate mercaptal derivative, the product was designated tetraacetylbisdesoxystreptobiosamine.

Tetraacetylbisdesoxystreptobiosamine was partially deacetylated by treatment with a saturated solution of ammonia in methanol to the crystalline N-acetylbisdesoxystreptobiosamine, X. This water-soluble derivative showed no reducing action toward Fehling solution. Hence the linkage between the two hexose portions of the disaccharide is formulated as involving carbon atom one of N-methyl-*l*-glucosamine, as shown in structure IX.

Tetraacetylbisdesoxystreptobiosamine, IX, was hydrolyzed with concentrated hydrochloric acid by refluxing the solution for one and one-half hours. Although the streptose moiety of the molecule again was decomposed, N-methyl-*l*-glucosamine was obtained as the pentaacetyl derivative, IV, in good yields.

Dihydrostreptomycin hydrochloride,[7] XI, was treated with ethyl mercaptan and hydrogen chloride. The streptidine dihydrochloride which formed was removed from the reaction products by selective precipitation. The disaccharide fractions were chromatographed and an amorphous ethyl thiodihydrostreptobiosaminide hydrochloride, XII, was obtained. Partial acetylation gave crystalline ethyl N-acetylthiodihydrostreptobiosaminide, XIII.



Since dihydrostreptomycin reacts with only one equivalent of ethyl mercaptan, it is apparent that the carbonyl group in streptomycin which reacts with two moles of ethyl mercaptan (*i. e.*, the group from which the di-(ethylsulfonyl)-methane is derived) is the functional group which is reduced in the conversion of streptomycin to its dihydro derivative. Other evidence concerning the reactions of this carbonyl group has been described.[2,7]

It was not possible to determine which of the two formulas for streptomycin, $C_{21}H_{37}N_7O_{12}$ or $C_{21}H_{39}N_7O_{12}$, was correct on the basis of the analytical data on the various crystalline salts[8] and the previously described crystalline derivative of streptobiosamine.[2] Ethyl thiostreptobiosaminide diethyl mercaptal hydrochloride, however, has an elementary composition such that a difference of two hydrogen atoms changes the analytical values for hydrogen more than is the case with the above-mentioned compounds. Analyses performed on a number of preparations of the mercaptal hydrochloride consistently favored the formula $C_{13}H_{22}NO_7(SC_2H_5)_3\cdot HCl$ for this derivative, rather than the alternative $C_{13}H_{20}NO_7(SC_2H_5)_3\cdot HCl$. On the basis of this formula, the cleavage of streptomycin hydrochloride may be represented by the equation

$$C_{21}H_{39}N_7O_{12}\cdot 3HCl + 3C_2H_5SH \longrightarrow C_8H_{18}N_6O_4\cdot 2HCl + C_{13}H_{22}NO_7(SC_2H_5)_3\cdot HCl + H_2O$$

This formula for streptomycin, $C_{21}H_{39}N_7O_{12}$, is substantiated by the observed analytical data on ethyl N-acetylthiostreptobiosaminide diethyl mercaptal. In like manner, analysis of ethyl N-acetylthiodihydrostreptobiosaminide showed a hydrogen content in best agreement with a formula $C_{21}H_{41}N_7O_{12}$ for dihydrostreptomycin.[7]

The hydrolysis of streptomycin to give streptidine ($C_8H_{18}N_6O_4$) and streptobiosamine ($C_{13}H_{23}NO_9$) may be represented by the equation

$$C_{21}H_{39}N_7O_{12} + H_2O \longrightarrow C_8H_{18}N_6O_4 + C_{13}H_{23}NO_9$$

The further hydrolysis of streptobiosamine to give a hexose-like molecule, $C_6H_{10}O_5$, and N-

(5) Mozingo, Wolf, Harris and Folkers, THIS JOURNAL, **65**, 1013 (1943).

(6) Wolfrom and Karabinos, *ibid.*, **66**, 909 (1944).

(7) Peck, Hoffhine, Mozingo and Folkers, *ibid.*, **68**, 1390 (1946).

(8) (a) Kuehl, Peck, Hoffhine, Graber and Folkers, *ibid.*, **68**, 1460 (1946); (b) Peck, Brink, Kuehl, Flynn, Walti and Folkers, *ibid.*, **67**, 1866 (1945).

methyl-*l*-glucosamine ($C_7H_{15}NO_5$) may be represented as

$$C_{13}H_{23}NO_9 + H_2O \longrightarrow C_6H_{10}O_5 + C_7H_{15}NO_5$$

The name streptose has been used to designate the molecule $C_6H_{10}O_5$. The degradation of tetraacetylbisdesoxystreptobiosamine, IX, to yield N-methyl-*l*-glucosamine establishes the presence of the two carbonyl groups of streptobiosamine which react with ethyl mercaptan (and with methanol) in the streptose moiety of the disaccharide. This degradation also shows that streptobiosamine is linked to streptidine through the streptose portion.

Experimental

Degradation of Streptomycin Hydrochloride to Ethyl Thiostreptobiosaminide Diethyl Mercaptal Hydrochloride (V).—A suspension of 10 g. of finely divided streptomycin hydrochloride[9] (activity, 800 U./mg.) in 40 ml. of ethyl mercaptan was saturated at 5° with hydrogen chloride and shaken overnight at room temperature. After removal of the excess ethyl mercaptan *in vacuo*, the residue was dissolved in 10 ml. of hot water. The mercaptal crystallized on cooling and was separated at 5° from the mother liquor by means of a filter stick. After two additional recrystallizations from water, 5.96 g. (75%) of the compound was obtained, m. p. 110–112°, $[\alpha]^{25}D$ − 262° (*c*, 1.50 in methanol).

Anal. Calcd. for $C_{19}H_{37}NO_7S_3 \cdot HCl$: C, 43.53; H, 7.31; N, 2.67; S, 18.3; Cl, 6.75; mol. wt., 524. Calcd. for $C_{19}H_{35}NO_7S_3 \cdot HCl$: C, 43.72; H, 6.95; N, 2.68; S, 18.3; Cl, 6.78; mol. wt., 522. Found: C, 43.53, 43.59; H, 7.28, 7.25; N, 2.89; S, 17.7; Cl, 7.10; eq. wt., 511 (potentiometric titration).

Analytical results for the hydrogen analysis on another preparation are as follows: H, 7.46, 7.35, 7.41. It may be seen that all analyses are in better agreement with the formula $C_{19}H_{37}NO_7S_3 \cdot HCl$.

Preparation of Ethyl Thiostreptobiosaminide Diethyl Mercaptal Hydrochloride (V) from Streptomycin Concentrate.—A suspension of 10 g. of finely divided streptomycin hydrochloride concentrate (activity 400 U./mg.) in 37 ml. of ethyl mercaptan saturated with hydrogen chloride was shaken overnight at room temperature. The product was obtained as described above except that in the third recrystallization, ethyl alcohol was used instead of water; yield, 3.0 g. (81%).

Conversion of Methyl Streptobiosaminide Dimethyl Acetal Hydrochloride (II) to Ethyl Thiostreptobiosaminide Diethyl Mercaptal Hydrochloride (V).—A solution of 368 mg. of methyl streptobiosaminide dimethyl acetal hydrochloride in 5 ml. of ethyl mercaptan was saturated with hydrogen chloride. After three hours at room temperature and forty-eight hours at 0°, the excess ethyl mercaptan was removed *in vacuo* and the reaction product was dissolved in 4 ml. of water. After standing at 5°, 312 mg. of ethyl thiostreptobiosaminide diethyl mercaptal hydrochloride was obtained.

Ethyl N-Acetylthiostreptobiosaminide Diethyl Mercaptal (VI).—A solution of 4.4 g. of the thiostreptobiosaminide and 10 g. of sodium carbonate in 120 ml. of water was extracted three times with chloroform. The chloroform extract was washed with water, dried, and concentrated *in vacuo*. The free base, 3.64 g., was dissolved in 200 ml. of methanol and 3.6 ml. of acetic anhydride was added. After standing for three hours at room temperature, the solvent was removed *in vacuo* to give a product which was dissolved in boiling ethyl acetate. On cooling, the N-acetyl derivative crystallized as colorless needles; yield, 3.46 g. (78%), m. p. 173–175°, $[\alpha]^{25}D$ − 250° (*c*, 1.55 in methanol). Recrystallization did not change the melting point.

(9) The powder was obtained by precipitating the streptomycin hydrochloride from methanol solution by the addition of acetone.

Anal. Calcd. for $C_{21}H_{39}NO_6S_3$: C, 47.62; H, 7.42; acetyl, 8.2. Found: C, 47.75; H, 7.41; acetyl, 10.5.

Ethyl Tetraacetylthiostreptobiosaminide Diethyl Mercaptal (VII).—A solution of 19.5 g. of ethyl thiostreptobiosaminide diethyl mercaptal hydrochloride in 100 ml. of pyridine was cooled to 5° and treated with 40 ml. of acetic anhydride. After standing overnight at room temperature, the acetylation mixture was concentrated *in vacuo* to a sirup. This crude product was poured into 200 ml. of ice water and the resulting mixture was extracted four times with chloroform. The chloroform extract was washed with dilute hydrochloric acid and with water, and then dried over anhydrous magnesium sulfate. The chloroform extract was concentrated *in vacuo* to a heavy sirup which was dissolved in 300 ml. of ether. After being cooled to the temperature of a Dry Ice-bath, the acetyl derivative crystallized and the liquid phase was removed at −60° by means of a filter stick. The product weighed 19.1 g., 71% yield. The tetraacetyl derivative was recrystallized from ether; m. p. 81–82°, $[\alpha]^{25}D$ −178° (*c*, 0.50 in chloroform).

Anal. Calcd. for $C_{27}H_{45}NO_{11}S_3$: C, 49.44; H, 6.92; N, 2.13; S, 14.6; acetyl, 26.2. Found: C, 49.58; H, 6.98; N, 2.18; S, 13.6; acetyl, 24.9.

Reaction of Ethyl Thiostreptobiosaminide Diethyl Mercaptal Hydrochloride (V) with Mercuric Chloride for the Preparation of Streptobiosamine Hydrochloride.—To a solution of 1.431 g. (2.73 millimoles) of ethyl thiostreptobiosaminide diethyl mercaptal hydrochloride in 10 ml. of water was added a solution of 2.296 g. (8.45 millimoles, 3.1 equivalents) of mercuric chloride in 25 ml. of hot water. The mixture was heated for ten minutes at 95° and then stirred for one hour at room temperature. The ethylmercaptomercuric chloride (2.178 g., 91%) was removed by filtration and the filtrate was saturated with hydrogen sulfide. The solution was filtered through charcoal and the clear yellow filtrate dried from the frozen state. The product, a pink, amorphous solid, weighed 0.903 g., and had a rotation of $[\alpha]^{25}D$ − 106° (*c*, 1.96 in water). It reduced Fehling solution, and gave a precipitate with a solution of 2,4-dinitrophenylhydrazine. Without further purification, the amorphous product was analyzed after drying for two hours *in vacuo* at 56°.

Anal. Calcd. for $C_{13}H_{23}NO_9 \cdot HCl$: C, 41.8; H, 6.5. Calcd. for $C_{13}H_{23}NO_9 \cdot HCl \cdot H_2O$: C, 39.9; H, 6.7. Found: C, 40.2; H, 6.5.

This product showed an activity of 1.2 U./mg. in the streptomycin assay using *B. subtilis*.

Treatment of Ethyl Thiostreptobiosaminide Diethyl Mercaptal Hydrochloride (V) with Alkali.—A solution of 75 mg. of ethyl thiostreptobiosaminide diethyl mercaptal hydrochloride in 5 ml. of 2.5 *N* sodium hydroxide and 1 ml. of ethyl alcohol was refluxed for one and one-half hours. When the solution was cooled and acidified with hydrochloric acid, 73 mg. of crystalline starting material was obtained.

Treatment of Ethyl Thiostreptobiosaminide Diethyl Mercaptal Hydrochloride with Ethyl Mercaptan.—A solution of 96 mg. of ethyl thiostreptobiosaminide diethyl mercaptal hydrochloride in 5 ml. of ethyl mercaptan was saturated with hydrogen chloride at 0°. The solution was heated at 90° for fifteen hours in a sealed tube. After removing the excess ethyl mercaptan, the dark residue was dissolved in water and the aqueous solution extracted with ether to remove colored decomposition products. The aqueous layer was concentrated *in vacuo* to give 62 mg. of residue. After this residue was dissolved in a few drops of water and cooled, starting material was obtained, m. p. 108–109°.

Acid Hydrolysis of Ethyl Thiostreptobiosaminide Diethyl Mercaptal Hydrochloride (V) to Pentaacetyl-N-methyl-*l*-glucosamine (IV).—A solution of 300 mg. of ethyl thiostreptobiosaminide diethyl mercaptal hydrochloride in 3 ml. of concentrated hydrochloric acid was refluxed for one and one-half hours. The dark hydrolysate was extracted with chloroform to remove colored decomposition products. The aqueous solution was decolorized

with Darco and concentrated to dryness under reduced pressure to yield 154 mg. of a light brown product. This product was acetylated with pyridine–acetic anhydride. The mixture was concentrated *in vacuo* and the residue was dissolved in water. The solution was extracted with chloroform. Removal of the solvent and crystallization from ether gave 55 mg. of crystals, m. p. 155–157°. Two recrystallizations performed by first dissolving the crystals in a small amount of chloroform, then adding ether, gave a product melting at 160–161°. When this product was mixed with pentaacetyl-N-methyl-*l*-glucosamine, no depression in the melting point was observed.

Oxidation of Ethyl Thiostreptobiosaminide Diethyl Mercaptal Hydrochloride (V) to Di-(ethylsulfonyl)-methane (VIII).—A solution of 1.09 g. of ethyl thiostreptobiosaminide diethyl mercaptal hydrochloride in 5 ml. of glacial acetic acid was treated with 2 ml. of 30% hydrogen peroxide at 5°. After twenty-four hours at room temperature, the solvent was removed under reduced pressure, yielding a colorless residue weighing 1.34 g. The residue was triturated with ether and the ether-soluble portion was then dissolved in chloroform. The chloroform solution was washed successively with 5% aqueous sodium bicarbonate, 5% hydrochloric acid, and water. After drying with anhydrous magnesium sulfate, the chloroform was removed *in vacuo*. The resulting residue was dissolved in ether and 65 mg. of a crystalline product was obtained; m. p. 100–101°. No depression in the melting point occurred when this product was mixed with an authentic specimen of di-(ethylsulfonyl)-methane, m. p. 100–101°.

Anal. Calcd. for $C_5H_{12}O_4S_2$: C, 30.00; H, 6.00. Found: C, 29.75; H, 6.10.

Hydrogenolysis of Ethyl Tetraacetylthiostreptobiosaminide Diethyl Mercaptal (VII) with Raney Nickel.—About 2.5 g. of Raney nickel catalyst was added to a solution of 183 mg. of the tetraacetyl derivative in 20 ml. of 70% ethanol and the mixture was refluxed for four hours. The catalyst was removed by centrifugation and washed several times with hot ethanol. The supernatants were combined and concentrated *in vacuo* to a residue which was acetylated with pyridine–acetic anhydride. The crude acetylation product, 137 mg., when dissolved in a small amount of ether, yielded 80 mg. of crystals, m. p. 154–156°. After several recrystallizations from chloroform–ether, the tetraacetylbisdesoxystreptobiosamine (IX) melted at 159–160°, $[\alpha]^{25}D$ −85° (*c*, 1.00 in chloroform).

Anal. Calcd. for $C_{21}H_{33}NO_{11}$: C, 53.04; H, 7.00; N, 2.96; O-acetyl, 27.2. Found: C, 52.98; H, 6.75; N, 2.87; O-acetyl, 27.7.

N-Acetylbisdesoxystreptobiosamine (X).—A solution of 200 mg. of tetraacetylbisdesoxystreptobiosamine in 5 ml. of methanol was saturated with anhydrous ammonia at 0°. After standing two hours at room temperature, the solution was concentrated *in vacuo* to a colorless residue. This product was dissolved in 3 ml. of chloroform and 122 mg. of crystals was deposited; m. p. 195–196°. After three recrystallizations from the same solvent, the N-acetylbisdesoxystreptobiosamine melted at 196–197°.

Anal. Calcd. for $C_{15}H_{27}NO_8$: C, 51.51; H, 7.78; N, 4.01; acetyl, 12.3. Found: C, 51.29; H, 7.95; N, 4.36; acetyl, 11.3.

Acid Hydrolysis of Tetraacetylbisdesoxystreptobiosamine (IX) to Pentaacetyl-N-methyl-*l*-glucosamine (IV).—A solution of 33 mg. of tetraacetylbisdesoxystreptobiosamine in 2 ml. of concentrated hydrochloric acid was refluxed for one and one-half hours. The oily decomposition products were removed by extraction with chloroform and the light yellow aqueous layer was concentrated *in vacuo* to an amorphous powder; yield, 23.3 mg. This product was acetylated with pyridine–acetic anhydride to give 25 mg. of an oil. This oil was dissolved in a few milliliters of ether and 9 mg. of crystals was deposited; m. p. 150–160°. Two recrystallizations from the same solvent gave pentaacetyl-N-methyl-*l*-glucosamine; m. p. 160–161°.

Degradation of Dihydrostreptomycin (XI) to Ethyl Thiodihydrostreptobiosaminide Hydrochloride (XII).—A suspension of 10 g. of dihydrostreptomycin trihydrochloride in 100 ml. of ethyl mercaptan was saturated with hydrogen chloride and then shaken overnight at room temperature. The excess ethyl mercaptan was removed *in vacuo* and the residue was dissolved in 10 ml. of methanol. Addition of 50 ml. of isopropyl alcohol precipitated most of the streptidine dihydrochloride, which was removed by filtration. The filtrate was concentrated *in vacuo* giving 2.30 g. of an amorphous powder, $[\alpha]^{25}D$ −166° (*c*, 0.70 in methanol). The isopropyl alcohol–insoluble material was again dissolved in 20 ml. of methanol and 100 ml. of acetone was added. After centrifuging, the supernatant solution was concentrated to dryness, giving 2.1 g. of material, $[\alpha]^{25}D$ −171° (*c*, 0.50 in methanol).

The two levorotatory fractions were combined and a portion chromatographed on acid-washed alumina. The material seemed to be homogeneous, as evidenced by the rotations of the various fractions. Without further purification, the amorphous product was analyzed.

Anal. Calcd. for $C_{15}H_{29}NO_8S \cdot HCl$: C, 42.90; H, 7.15. Found: C, 43.80; H, 7.44.

Ethyl N-Acetylthiodihydrostreptobiosaminide (XIII).—A solution of 1.06 g. of ethyl thiodihydrostreptobiosaminide hydrochloride in 75 ml. of methanol was shaken with excess silver carbonate and filtered through a pad of Darco. Acetic anhydride, 0.6 ml., was then added and the methanolic solution was allowed to stand at room temperature for four hours. The solvent was removed *in vacuo* and the residue was dissolved in a small amount of chloroform. On standing, the chloroform solution deposited 481 mg. of the N-acetyl derivative, m. p. 165–170°. After two recrystallizations from ethanol–chloroform, the compound melted at 170–171°.

Anal. Calcd. for $C_{17}H_{31}NO_9S$: C, 47.99; H, 7.36; N, 3.27; S, 7.52; acetyl, 10.1. Found: C, 47.96, 48.18; H, 7.67, 7.65; N, 3.54; S, 7.58; acetyl, 8.5.

Acknowledgment.—The authors wish to thank Dr. N. R. Trenner and his associates for the infrared measurements and potentiometric titrations, Mr. Richard N. Boos and his associates for microanalyses, and Mr. David Hendlin for assay data.

Summary

Streptomycin hydrochloride has been degraded by ethyl mercaptan and hydrogen chloride to ethyl thiostreptobiosaminide diethyl mercaptal hydrochloride. The same compound resulted from treatment of methyl streptobiosaminide dimethyl acetal hydrochloride with these reagents. In like manner, dihydrostreptomycin yielded ethyl thiodihydrostreptobiosaminide hydrochloride.

Acid hydrolysis of ethyl thiostreptobiosaminide diethyl mercaptal hydrochloride gave N-methyl-*l*-glucosamine, which was isolated as the pentaacetyl derivative. Oxidation of the mercaptal hydrochloride with hydrogen peroxide yielded di-(ethylsulfonyl)-methane. Hydrogenolysis of ethyl tetraacetylthiostreptobiosaminide diethyl mercaptal with Raney nickel afforded tetraacetylbisdesoxystreptobiosamine.

From the products of acid hydrolysis of tetraacetylbisdesoxystreptobiosamine, N-methyl-*l*-glucosamine was obtained. Treatment of tetraacetylbisdesoxystreptobiosamine with methanolic ammonia gave an N-acetyl derivative which did not reduce Fehling solution.

These degradations indicate that N-methyl-*l*-

glucosamine is linked to streptose through carbon atom one of the amino sugar, and that streptobiosamine is linked to streptidine through the streptose moiety of the disaccharide.

The empirical formulas of the degradation products were consistently in best agreement with the formula $C_{21}H_{39}N_7O_{12}$ for streptomycin.

RAHWAY, NEW JERSEY RECEIVED JUNE 28, 1946

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, KANSAS STATE COLLEGE]

Dehydration of Cholic Acid

BY ARTHUR W. DEVOR[1] AND H. W. MARLOW

When cholic acid is dehydrated with anhydrous zinc chloride in acetic acid solution it yields an unsaturated compound by removal of the hydroxyl group from carbon number 7 and the hydrogen from number 8 carbon atom. The double bond rearranges to some other position, not yet fully agreed upon, yielding apocholic acid as the main product. Fieser[2] and Strain[3] have recently reviewed this work.

The object of this investigation was to study the effect of solvent and temperature upon the dehydration of cholic acid with zinc chloride. Of the number of solvents tried, acetone gave the best results.

Experimental

Dehydration in Acetone.—Fifty grams of anhydrous zinc chloride were dissolved in 225 g. of C. P. acetone; 50 g. of C. P. cholic acid[4] was added and the solution distilled over a steam-bath until it changed to a thin light orange-colored sirup. The flask was immediately weighed and, by difference in weight, found to contain 60 g. of acetone. Nine hundred and fifty ml. of cold water, acidified with three ml. of acetic acid, was added slowly while shaking with a whirling motion. The mixture was allowed to stand until the precipitate was crystalline; the water solution was decanted through a suction filter and the residue washed nearly free of chlorides. The precipitate was transferred back to the flask and the product dissolved in 100 ml. of boiling alcohol, then precipitated as before. After a third precipitation the product was dried in a non-oxidizing atmosphere.

The dry product was taken up in 300 ml. of hot absolute alcohol and filtered into a round bottom flask. The alcohol was evaporated over an oil-bath until the mixture had changed to a thin sirup. After a second alcohol treatment, 100 ml. of *m*-xylene was added rapidly while the solution was shaken with a whirling motion. After standing overnight, the crystalline mass was thoroughly broken up, filtered at the pump, and washed with xylene. The adhering xylene was removed by carefully heating to 134–140° in a non-oxidizing atmosphere. Thirty-five and five-tenths grams (75% yield) of the white product was obtained which was apparently nearly free of xylene. The yield of crystalline dehydrated product was decreased if heating was continued too long but when the heating period was too brief the product was found to contain unchanged cholic acid.

Properties of Product.—M. p.[5] 166–168°; mixed with product from acetic acid method (see below) the m. p. was unchanged; reacted rapidly with bromine and dilute alkali potassium permanganate showing unsaturation; neut. equiv. 396 (theoretical for apocholic acid, 390.5); X-ray diffraction photograph was the same as that from the acetic acid method.

Eight grams (17%) more of the crystalline product, melting at 154–159°, was obtained from the xylene filtrate by evaporating most of the xylene, adding absolute alcohol and crystallizing as above.

Dehydration in Acetic Acid.[6]—Five grams of anhydrous zinc chloride was dissolved in 150 ml. of glacial acetic acid, 50 g. of C. P. cholic acid added and the mixture refluxed forty minutes. It was then cooled and the product precipitated by adding, while shaking, 800 ml. of 2.5 *M* sodium hydroxide solution. After standing a few hours, the water solution was filtered off and the precipitate taken up in 600 ml. of 4% sodium hydroxide solution. The product was precipitated with hydrochloric acid, filtered off and washed free of chlorides. The remainder of the preparation was the same as the above procedure with acetone. The yield was 23.5 g. (48.6%). The properties of the slightly colored product were the same as those for the product from the acetone method.

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Summary

1. A simple method for dehydration of cholic acid with zinc chloride in acetone has been worked out which gives an excellent yield of dehydrated product.

2. From the melting point, unsaturation reactions, reaction-rate with iodine and bromide X-ray diffractions patterns the product seems to be the same as that obtained when cholic acid was dehydrated with zinc chloride in acetic acid solution. The acetone preparations, however, were whiter than those obtained by using the usual acetic acid method.

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(1) Present address: University of Southern California, Los Angeles, Calif.

(2) L. F. Fieser, "Chemistry of Natural Products Related to Phenanthrene," 2d ed., Monograph no. 70, Reinhold Publishing Company, New York, N. Y., 1937, pp. 123, 132 and 368.

(3) "Organic Chemistry, An Advanced Treatise," Henry Gilman, Editor-in-chief, Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1943, pp. 1416.

(4) The cholic acid was purchased from a reliable company and since the neutralization equivalent was 410, it was apparently nearly free of solvent (theoretical neut. equiv. 408.5).

(5) Melting point in this paper are uncorrected.

(6) A modified procedure of Boedecker, *Ber.*, **53**, 1852 (1920); *ibid.*, **54**, 2489 (1921).