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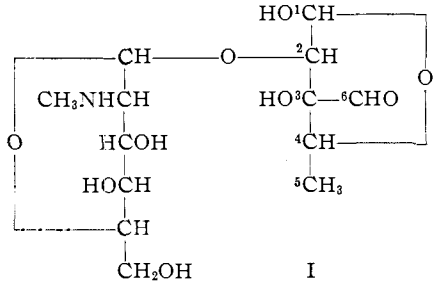
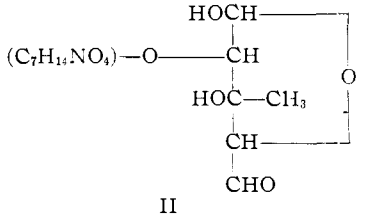
Streptomyces Antibiotics. XIII. The Structure of Streptobiosamine

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

The elucidation of the structure of tetraacetylbisdesoxystreptobiosamine has been described.[1] The determination of the structure of this degradation product, together with other evidence,[2,3,4] permitted the formulation of structures I, II, III and IV for streptobiosamine, the disaccharide which is combined with streptidine to constitute streptomycin.



I



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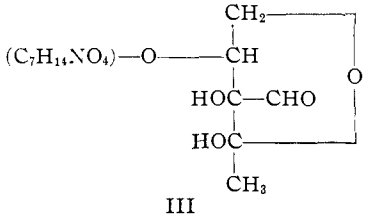

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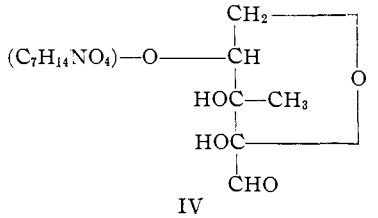


IV

New evidence has led to the establishment of one of these structures for streptobiosamine. The structures of certain related derivatives which are obtained from streptomycin by degradation are given. This evidence, together with previous information on the composition of streptomycin oxime,[4] the inactivation of streptomycin with various carbonyl reagents,[4,5] and the recently described methyl tetraacetylstreptobiosaminidic acid methyl ester,[6] has allowed a formulation of the position of attachment of streptidine to streptobiosamine.

The removal of the ethylmercapto groups of ethyl thiostreptobiosaminide diethyl mercaptal hydrochloride by hydrolysis with aqueous mercuric chloride has been described.[3] The reaction appeared to be accompanied by considerable decomposition of the streptobiosamine, presumably due to the action of the hydrochloric acid which was liberated in the formation of ethylmercaptomercuric chloride. Ethyl tetraacetylthiostreptobiosaminide diethyl mercaptal[3] (V), however, reacted satisfactorily with mercuric chloride in the presence of cadmium or strontium carbonate buffer. The product of hydrolysis was readily isolated by extraction with chloroform. Tetraacetylstreptobiosamine (VI), prepared in this manner, was obtained as a colorless, amorphous powder soluble in chloroform, acetone and alcohol, but insoluble in less polar solvents. It could not be obtained in crystalline form,[6a] and no further purification was attempted. The material reduced Fehling solution. Unlike streptomycin[7] and methyl streptobiosaminide dimethyl acetal,[4] tetraacetylstreptobiosamine did not give maltol when treated with alkali. The compound appeared to undergo decomposition during acid hydrolysis, since the solution invariably darkened, and only N-methyl-L-glucosamine[2] could be isolated.

Bromine oxidation of tetraacetylstreptobiosamine (VI) under the conditions employed in the oxidation of aldoses to aldonic acids yielded an acid which after reacetylation was obtained as the crystalline pentaacetyl derivative. The analytical data and the results of potentiometric titrations for this oxidation product, designated pentaacetylstreptobiosamic acid monolactone, were in best agreement with the formula $C_{13}H_{21}NO_{10}(CH_3CO)_5$. Pentaacetylstreptobiosamic acid monolactone was converted to the crystalline monomethyl ester by treatment of the silver salt of the acid with methyl iodide.

A solution of pentaacetylstreptobiosamic acid monolactone in 2.5 *N* hydrochloric acid was refluxed for four hours and evaporated to dryness. The residue gave on extraction with acetone a crystalline compound derived from the streptose moiety of streptobiosamine. This compound was

(1) Brink, Kuehl, Flynn and Folkers, THIS JOURNAL, **68**, 2405 (1946).

(2) Kuehl, Flynn, Holly, Mozingo and Folkers, *ibid.*, **68**, 536 (1946).

(3) Kuehl, Flynn, Brink and Folkers, *ibid.*, **68**, 2096 (1946).

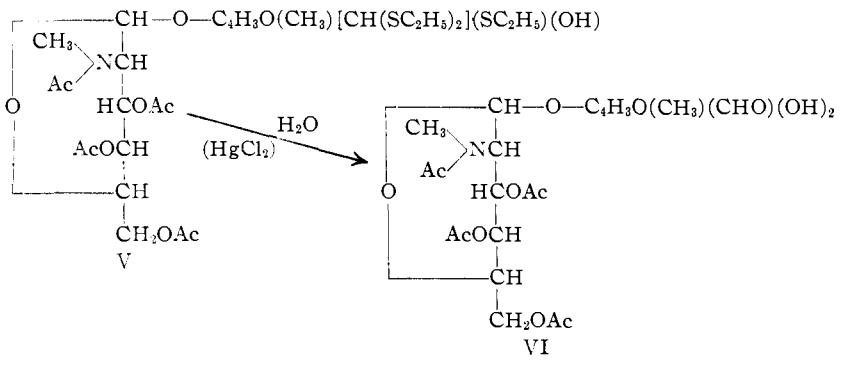
(4) Brink, Kuehl, Flynn and Folkers, *ibid.*, **68**, 2557 (1946).

(5) Donovick, Rake and Fried, *J. Biol. Chem.*, **164**, 173 (1946).

(6) Fried and Wintersteiner, Abstracts of the Chicago Meeting, A. C. S., Sept., 1946, p. 15B.

(6a) Footnote added in proof: Tetraacetylstreptobiosamine has now crystallized; m. p. 188–189°, $[\alpha]^{25}D$ −78° (*c*, 1.0 in chloroform).

(7) Schenck and Spielman, THIS JOURNAL, **67**, 2276 (1945).



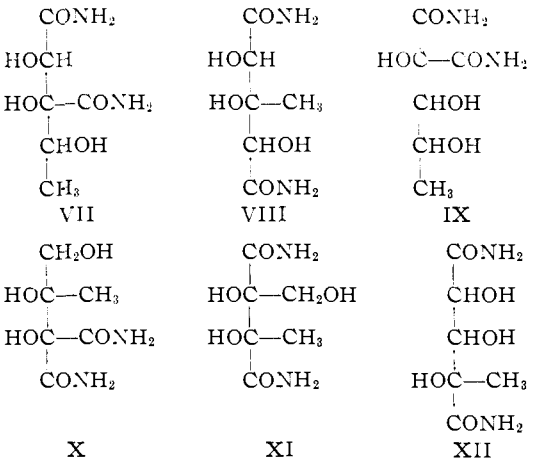
designated streptosonic acid monolactone. In early experiments, streptosonic acid monolactone was isolated as the diacetyl derivative. In this procedure, the hydrolysate was neutralized and acetylated, and an aqueous solution of the acetylation products was extracted with chloroform at neutrality to remove the pentaacetyl derivative of the N-methyl-L-glucosamine, after which the solution was acidified to *p*H 3 and extracted with ether to obtain diacetylstreptosonic acid monolactone.

Analyses of streptosonic acid monolactone showed a composition corresponding to the formula $C_6H_8O_6$. The formulation of this compound as a monolactone of a dibasic acid was based upon its behavior during potentiometric titration, when neutral equivalents of 175 and 87.5 were observed (theoretical values, 176 and 88); and upon infrared analysis, the compound showing absorption at 5.80 μ (carboxyl group) and at 5.62 μ (lactone). Further, a diamide having the formula $C_6H_{12}N_2O_5$ was prepared from this lactone, confirming the dibasic character of streptosonic acid. Chromic acid oxidation of streptosonic acid monolactone by the Kuhn–Roth method gave one mole of acetic acid, and the presence of the expected C-methyl group[4] was further evidenced by a positive iodoform reaction. When solutions of streptosonic acid monolactone and streptosonic acid diamide were treated with excess sodium metaperiodate, two moles of the oxidizing agent were consumed in each case, indicating the presence of three adjacent hydroxyl groups.

A degradation product with the properties of streptosonic acid eliminates structures III and IV for streptobiosamine, since neither of the structures would yield a six-carbon dibasic acid from the streptose moiety.

Any straight carbon skeleton for streptosonic acid is excluded by the presence of a methyl group and two carboxyl groups in the compound. This conclusion is in agreement with the results of the structure determination of bisdesoxystreptose, which showed that streptose has a branched carbon skeleton.[1] On the basis of the observations described above, and the known structure of bisdesoxystreptose,[1] only two structures (VII and VIII) were possible for streptosonic acid diamide. The results of the oxidative degradation of streptosonic acid diamide provided independent proof of the elimination of structures IX, X, XI and XII for the diamide, and permitted differentiation between the structures VII and VIII.

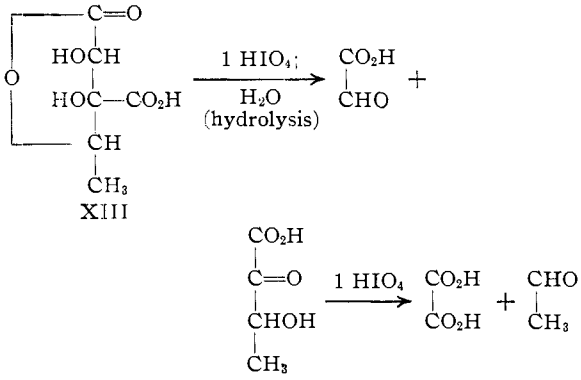
Investigation of the products obtained by the oxidation of the diamide with two moles of sodium metaperiodate showed that no volatile acid was produced. This result eliminated structures VIII and X, which would yield acetic acid on such treatment; and also structures IX and XII, from which formic acid would have been produced. From the products of the oxidation, a



volatile aldehyde was obtained, and was identified as acetaldehyde by conversion to the 2,4-dinitrophenylhydrazone. Of the six formulas for the diamide given above, only VII and IX would be expected to yield acetaldehyde upon periodate oxidation; but structure IX was already excluded because no volatile acid could be isolated. Formula VII, therefore, remained as the only structure in agreement with these results.

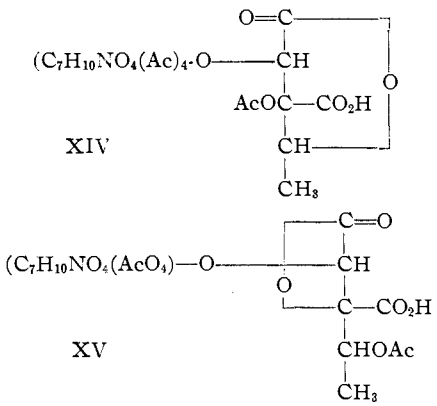
Confirmatory evidence for structure VII for streptosonic acid diamide was obtained by a study of the oxidation products of streptosonic acid monolactone. When this compound was oxidized with two moles of periodic acid, the remaining two cleavage products were identified. These were glyoxylic acid, isolated as the *p*-nitrophenylhydrazone of the methyl ester; and oxalic acid, isolated as the calcium salt. The suggested course of the oxidation of streptosonic acid monolactone (XIII) is shown below There is no evidence for the position of the lactone ring, but the γ-position is preferred on the basis of the structures of the known sugar lactones.

Unlike the other streptobiosamine derivatives, streptobiosamic acid monolactone forms a penta-



XIII

acetyl derivative, rather than a tetraacetyl compound. One must assume either that the ordinarily resistant tertiary hydroxyl group has been acetylated in this compound (structure XIV), or that the lactone ring has shifted from the γ- to the β-position during the acetylation, with the introduction of an acetyl group at carbon atom four (structure XV).

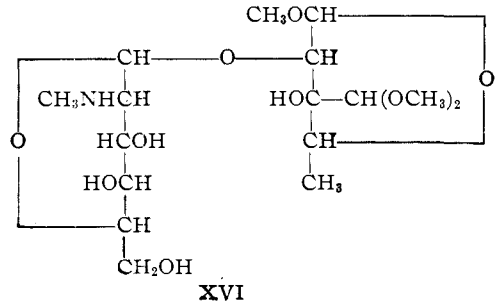


XIV

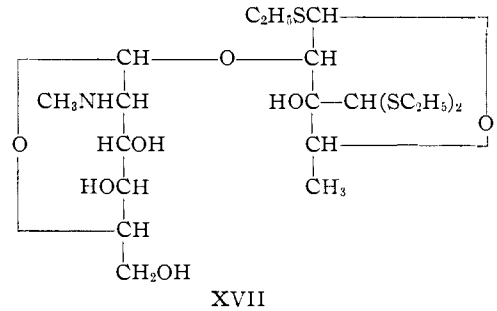
XV

Of the two remaining structures (I and II) formulated for streptobiosamine on the basis of the demonstrated structure of bisdesoxystreptose, only structure I is consistent with the formation of degradation products having the properties of the described streptosonic acid derivatives.

Complete structures for methyl streptobiosaminide dimethyl acetal and ethyl thiostreptobiosaminide diethyl mercaptal are shown in formulas XVI and XVII. It is also evident that the glycosidic hydroxyl group of tetraacetyldesoxystreptobiosamine is located at carbon atom one of

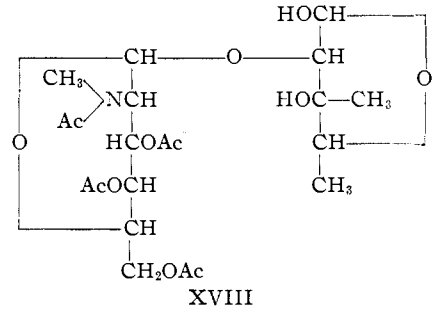


XVI



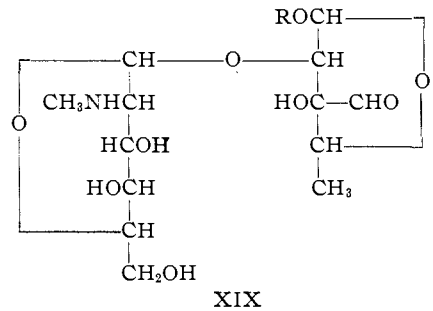
XVII

the streptose portion, and the structure of this compound may be represented by formula XVIII.

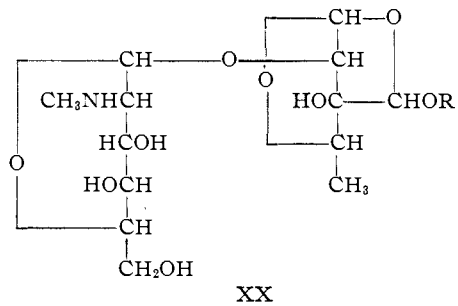


XVIII

Streptidine must be attached to streptobiosamine either at carbon atom one or six of the streptose moiety. It seems most probable that the attachment to streptobiosamine is through the carbonyl group responsible for the hemiacetal formation, carbon atom one, as indicated by formula XIX (R = $C_8H_{17}N_6O_4$, the streptidine moiety). Structure XX could, conceivably, also account for the formation of ethyl thiostreptobiosaminide diethyl mercaptal (XVII), the precursor of both bisdesoxystreptose and streptosonic acid monolactone. Both structures (XIX and XX)



XIX

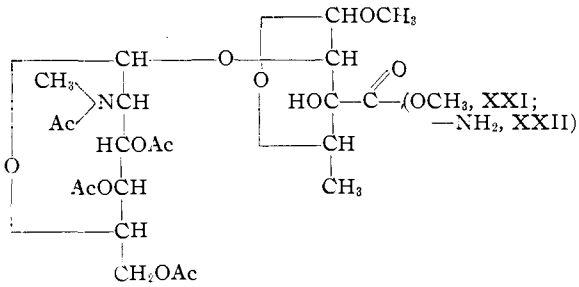


XX

have a furanose ring in the streptose moiety, which is consistent with the established 1,4-oxide ring in bisdesoxystreptose,[1] and there is no evidence at present to indicate that this particular ring structure in streptomycin has shifted in the degradation to bisdesoxystreptose.

Although both structures XIX and XX appear to account satisfactorily for many of the degradation products, a compound of structure XIX would give an oxime of the composition $C_{21}H_{40}N_8O_{12}\cdot 3HCl$, that observed for streptomycin oxime hydrochloride.[4] A compound of structure XX, however, would be expected to give an oxime containing an additional mole of water, but the analytical data were not in agreement with such a composition. Furthermore, streptomycin is reduced catalytically[6,8,9] and reacts with carbonyl reagents[4,5] in a manner which is more in agreement with structure XIX than with the complete acetal structure XX.

Fried and Wintersteiner[6] have described the oxidation of streptomycin with bromine water to streptomycinic acid, and the degradation of the new acid to methyl streptobiosaminidic acid methyl ester hydrochloride, $C_{12}H_{19\text{-}21}NO_7(OCH_3)(CO_2CH_3)\cdot HCl$. They converted the latter compound into a crystalline tetraacetyl methyl ester and an amide. Again, structure XIX for streptomycin accounts for the described properties and compositions of these new degradation products of streptomycinic acid better than does structure XX. On the basis of structure XIX for streptomycin, the methyl tetraacetylstreptobiosaminidic acid methyl ester would be represented by structure XXI and the corresponding amide by structure XXII.



All of this degradative evidence is in better agreement with partial structure XIX for streptomycin than with structure XX or other structures which can be written.

Treatment of streptomycin with alkali yields maltol.[7] There is no doubt that maltol arises from the streptose portion of streptomycin. If maltol is β-hydroxy-α-methyl-γ-pyrone, it possesses a linear carbon skeleton, while the streptose portions of the various streptobiosamine derivatives have branched carbon skeletons. It is evident that a carbon–carbon rearrangement must have occurred either during the degradation of streptomycin to maltol by alkali or during the degradation of streptomycin with methanol or ethyl mercaptan and hydrogen chloride. *A priori*, a rearrangement during the degradation to maltol seemed more likely, since sugars are known to undergo carbon–carbon rearrangements[10] when treated with alkali. The following experimental evidence is in agreement with this interpretation.

The formation of maltol in 17% yield from methyl streptobiosaminide dimethyl acetal has been reported.[4] However, tetraacetylstreptobiosamine gave no maltol on treatment with alkali. Ethyl N-acetylthiostreptobiosaminide diethyl mercaptal[1] was subjected to hydrolysis of the ethylmercapto groups by treatment with aqueous mercuric chloride, and the solution containing N-acetylstreptobiosamine was made alkaline and warmed. Again, no maltol was formed. Since tetraacetyl- and N-acetylstreptobiosamine differ from streptomycin and methyl streptobiosaminide dimethyl acetal by having no group attached glycosidically at carbon atom one of the streptose moiety, it seemed possible that such a group was necessary for the maltol formation. Further, the failure[8] of dihydrostreptomycin to give maltol, and the relatively low yield of maltol from methyl streptobiosaminide dimethyl acetal, suggested that a free or potentially free streptose aldehydic group was necessary for the reaction to occur.

To test the necessity of a glycosidic group and an aldehydic group for maltol formation, methyl N-acetylstreptobiosaminide seemed to be a suitable compound. Ethyl tetraacetylthiostreptobiosaminide diethyl mercaptal was hydrolyzed with mercuric chloride and reacetylated. The product was treated with hydroxylamine and then with methanolic hydrogen chloride. The crude methyl N-acetylstreptobiosaminide oxime was treated with nitrous acid for liberation of the aldehydic group, and finally with alkali. Maltol was isolated and identified by melting point determinations.

Experimental

Pentaacetylstreptobiosamic Acid Monolactone.—Ten grams of ethyl tetraacetylthiostreptobiosaminide diethyl mercaptal was dissolved in 200 ml. of 50% acetone. To this solution was added 20 g. of cadmium carbonate, followed by a solution of 16.4 g. of mercuric chloride in 20 ml. of acetone. The mixture was then heated under reflux for forty minutes. Continuous stirring was necessary to prevent bumping. The reaction mixture was cooled and filtered to remove ethylmercaptomercuric chloride and excess cadmium carbonate. Acetone was removed from the filtrate by concentration under reduced pressure. The aqueous solution remaining, together with the precipitate which formed, was then extracted continuously for four hours with chloroform. The chloroform extract was filtered and concentrated *in vacuo* to dryness, yielding 7.2 g. of a colorless, amorphous product. A portion (4.5 g.) of this material was shaken with 100 ml. of water and 20 g. of strontium carbonate until it had dissolved. Four grams of bromine was added. The suspension was shaken occasionally until the bromine dissolved

(8) Peck, Hoffhine and Folkers, THIS JOURNAL, **68**, 1390 (1946).
(9) Bartz, Controulis, Crooks and Rebstock, *ibid.*, **68**, 2163 (1946).
(10) *Cf.* Nef, *Ann.*, **376**, 1 (1910); W. L. Evans, R. H. Edgar and G. P. Hoff, THIS JOURNAL, **48**, 2665 (1926).

and the solution was allowed to stand overnight in the dark. The excess strontium carbonate was removed by filtration and the filtrate was concentrated *in vacuo* until free of bromine. The colorless solution was shaken for one hour with excess silver carbonate to remove the strontium bromide, and then was treated with hydrogen sulfide to remove silver ion. After being filtered through a thin layer of Darco to remove silver sulfide, the colorless filtrate was concentrated under reduced pressure to give the strontium salt of the acid as a colorless amorphous solid. This material was acetylated by adding 20 ml. of pyridine and 10 ml. of acetic anhydride and allowing the mixture to stand overnight. The acetylation mixture was concentrated *in vacuo* to a sirup. The sirup was dissolved in water and the solution was acidified and extracted with chloroform. It was found desirable to use large volumes of water and chloroform during this process, since the strontium salt of the acetylated acid frequently crystallized at this point, complicating the manipulations.

Concentration of the chloroform extract to dryness gave a partially crystalline solid, which was recrystallized from 12 ml. of hot ethyl acetate, giving 3.2 g. (60%) of crystalline pentaacetylstreptobiosamic acid monolactone, m. p. 158–159°. Recrystallization raised the melting point to 162–163°; $[\alpha]^{25}D$ −79° (*c*, 0.70 in methanol).

Anal. Calcd. for $C_{23}H_{31}NO_{15}$: C, 49.19; H, 5.57; N, 2.49; acetyl, 37.41; mol. wt., 562. Found: C, 49.16, 49.40; H, 5.54, 5.51; N, 2.71; acetyl, 38.84; mol. wt., 556 (potentiometric titration).

Methyl Pentaacetylstreptobiosamate Monolactone.—A solution of 346 mg. of pentaacetylstreptobiosamic acid monolactone in 14 ml. of 70% methanol was stirred with excess silver oxide until it was no longer acid. The silver oxide was removed by filtration and the filtrate was concentrated to dryness under reduced pressure, giving 380 mg. of the silver salt of the acid. A 311-mg. portion of this product was treated with 3 ml. of methanol and 10 ml. of methyl iodide, and the mixture was heated under reflux for fifteen minutes. The solvent was removed, the residue dissolved in methanol, and the solution was then filtered through a layer of Darco. The resulting yellow filtrate was concentrated *in vacuo*, and the product dissolved in chloroform. This chloroform solution was shaken with silver oxide to remove iodine, filtered and concentrated to dryness. The product, 227 mg., was dissolved in 1 ml. of chloroform and 10 ml. of ether and adsorbed on 3 g. of acid-washed alumina. The column was eluted with 10 ml. of ether, followed by 10 ml. of chloroform. The ether eluate gave an oil which deposited 98 mg. of the crystalline methyl ester, while the chloroform eluate gave an oil which deposited 74 mg. of crystals when dissolved in ether. Both fractions melted at 161–162°. The fractions were combined and recrystallized from chloroform–ether to a melting point of 162–163°.

Anal. Calcd. for $C_{24}H_{33}NO_{15}$: C, 50.08; H, 5.79; N, 2.43; acetyl, 37.40; methoxyl, 5.23. Found: C, 50.47; H, 6.09; N, 2.61; acetyl, 36.9; methoxyl, 4.68.

Diacetylstreptosonic Acid Monolactone.—A solution of 3.12 g. of pentaacetylstreptobiosamic acid monolactone in 45 ml. of 2.5 *N* hydrochloric acid was heated under reflux for four hours. The hydrolysate was evaporated to dryness *in vacuo* and the residue redissolved in water. Excess strontium carbonate was added and, after the solution was neutralized, the unreacted strontium carbonate was removed by filtration. The filtrate was concentrated to dryness under reduced pressure and the solid obtained was acetylated with pyridine and acetic anhydride at 5°. After standing overnight, the pyridine and excess acetic anhydride were removed *in vacuo;* the resulting sirup was dissolved in 40 ml. of water. Pentaacetyl-N-methyl-L-glucosamine was removed by extracting with chloroform. The aqueous fraction was carefully acidified with dilute hydrochloric acid, and extracted with six portions of ether. The total ether extract was washed with water, dried with anhydrous magnesium sulfate, and concentrated *in vacuo*. Seven hundred and fifty-eight milligrams of partially crystalline product was obtained. This was triturated with carbon tetrachloride which left 722 mg. (50%) of crystals melting at 178–184°. Recrystallization from chloroform–carbon tetrachloride gave pure diacetylstreptosonic acid monolactone, m. p. 186–188°, $[\alpha]^{25}D$ +26° (*c*, 0.45 in methanol).

Anal. Calcd. for $C_{10}H_{12}O_8$: C, 46.15; H, 4.61; acetyl, 33.8; mol. wt., 260. Found: C, 45.95; H, 4.48; acetyl, 29.0; mol. wt., 254 (potentiometric titration).

Streptosonic Acid Monolactone.—The dried residue, obtained as described above by the hydrolysis of 4.74 g. of pentaacetylstreptobiosamic acid monolactone was dissolved in 6 ml. of 80% methanol, and the N-methyl-L-glucosamine hydrochloride was precipitated by the addition of 40 ml. of acetone. After centrifuging the mixture, the supernatant was concentrated to dryness and then treated with 20 ml. of acetone. The insoluble portion was triturated thoroughly and removed by filtration, and the filtrate was again concentrated to dryness. The residue was dissolved in 25 ml. of methyl *n*-propyl ketone by warming slightly, and then the solution was filtered through a layer of Darco. The clear solution obtained was concentrated once more and the sirup was dissolved in chloroform; on standing, 630 mg. of streptosonic acid monolactone was obtained, m. p. 142–144°. The product was purified by recrystallization from a mixture of methyl *n*-propyl ketone and chloroform. The pure material melted at 146–148°, and had $[\alpha]^{25}D$ −37° (*c*, 0.67 in water); yield 42%.

Hydrolysis of Diacetylstreptosonic Acid Monolactone.—A solution of 166 mg. of diacetylstreptosonic acid monolactone in 4 ml. of 5% hydrochloric acid was heated under reflux for one hour. The hydrolysate was concentrated *in vacuo* and traces of hydrogen chloride were removed by drying *in vacuo* over potassium hydroxide. The crystalline residue was dissolved in 0.5 ml. of methyl *n*-propyl ketone and after 2 ml. of chloroform was added, 91 mg. (81%) of streptosonic acid monolactone was deposited as colorless crystals, m. p. 144–146°. Recrystallization of this material from the same solvents gave a product which melted at 146–148°, $[\alpha]^{25}D$ −38° (*c*, 0.40 in water).

Anal. Calcd. for $C_6H_8O_6$: C, 40.88; H, 4.54; C-methyl, 8.5; mol. wt., 176. Found: C, 40.68; H, 4.21; C-methyl, 7.5; mol. wts., 87.5 and 175.

Streptosonic Acid Diamide.—A solution of 112 mg. of streptosonic acid monolactone in 10 ml. of methanol containing 2% of hydrogen chloride was heated under reflux for three hours. The solution was concentrated *in vacuo* to a residue which was dissolved in 2 ml. of chloroform. The chloroform solution was passed through 2 g. of acid-washed alumina and then the column was eluted with 10 ml. of chloroform, followed by 10 ml. of acetone. The two fractions were combined, giving 81 mg. of a neutral product. This product was dissolved in 10 ml. of methanol and the solution was saturated with ammonia. After three hours at room temperature, the solvent was removed *in vacuo* and the product dissolved in methanol–chloroform. Forty-three milligrams of crystalline diamide was obtained, m. p. 135–136°, $[\alpha]^{25}D$ +14° (*c*, 0.5 in water). After several recrystallizations from methanol the diamide melted at 143–144°.

Anal. Calcd. for $C_6H_{12}N_2O_5$: C, 37.53; H, 6.30; N, 14.59. Found: C, 37.65; H, 6.14; N, 14.58.

Determination of Periodate Consumption by Streptosonic Acid Monolactone and Streptosonic Acid Diamide.—A 5-ml. aqueous solution of 5.33 mg. of streptosonic acid monolactone and 23.76 mg. of sodium metaperiodate was allowed to stand for one hour at room temperature. The periodate consumption was determined by the standard arsenite method.[11] A 2-ml. aliquot portion required 3.835 ml. of sodium arsenite solution (1 ml. = 1.09 mg. of sodium metaperiodate) corresponding to the consumption of 2.05 moles of periodate. A similar titration after standing overnight showed that 2.16 moles of periodate was consumed.

A 5-ml. aqueous solution of 5.30 mg. of streptosonic acid

(11) Jackson, "Organic Reactions," Vol. II, p. 361.

diamide and 22.6 mg. of sodium metaperiodate was allowed to stand for one hour at room temperature. A 2-ml. aliquot portion required 3.70 ml. of sodium arsenite solution, corresponding to 2.2 moles of periodate consumed.

Acetaldehyde from Streptosonic Acid Diamide.—A solution of 182 mg. of streptosonic acid diamide and 406 mg. of sodium metaperiodate in 20 ml. of water was allowed to stand at room temperature for one hour. The aqueous solution was distilled at 40° under reduced pressure, and the distillate collected in a trap at Dry Ice temperature. A solution of 2,4-dinitrophenylhydrazine in 1 *N* hydrochloric acid was added to the distillate and the crystals of the dinitrophenylhydrazone were removed by filtration; yield 48 mg., m. p. 140–145°. Recrystallization of the derivative from ethanol gave pure acetaldehyde 2,4-dinitrophenylhydrazone, m. p. and mixed m. p. 149–151°.

Anal. Calcd. for $C_8H_8N_4O_4$: C, 42.86; H, 3.57; N, 25.00. Found: C, 42.77; H, 3.64; N, 25.38.

Isolation of the Periodate Oxidation Products of Streptosonic Acid Monolactone.—A solution of 385 mg. of streptosonic acid monolactone and 995 mg. of paraperiodic acid in 18.2 ml. of water was allowed to stand at room temperature for one and one-fourth hours. The solution was shaken with excess strontium carbonate and then filtered to remove strontium iodate and excess strontium carbonate. The filtrate was concentrated under reduced pressure to a volume of 2 ml. and ethanol was added until the solution became turbid. Some strontium iodate precipitated and was removed by filtration. The alcohol was removed by concentrating the solution under reduced pressure, after which the volume was adjusted to 5 ml. with water. A solution of 666 mg. of *p*-nitrophenylhydrazine in 23 ml. of water and 3 ml. of 10% hydrochloric acid was added. After standing for two hours at 0°, the precipitate was filtered, washed with water and dried. The dry material weighed 823 mg. The product failed to crystallize, even though chromatographic purification was attempted. All fractions from the chromatogram were combined, dissolved in 20 ml. of 5% methanolic hydrogen chloride, and heated under reflux for one hour. When the solvent was removed *in vacuo* and the product dissolved in 2 ml. of methanol, 166 mg. of orange crystals was deposited, m. p. 205–208°. Recrystallization of this product to constant melting point gave the pure *p*-nitrophenylhydrazone, m. p. 214–215°. No depression was observed when a mixed melting point determination was made with an authentic sample of the *p*-nitrophenylhydrazone of methyl glyoxylate, m. p. 213–215°.

Anal. Calcd. for $C_9H_9NO_4$: C, 48.43; H, 3.99; N, 18.84; OCH_3, 13.9. Found: C, 48.50; H, 4.21; N, 19.14; OCH_3, 12.9.

A solution containing 103 mg. of streptosonic acid monolactone and 267 mg. of paraperiodic acid was allowed to stand for one hour at room temperature. The water was removed *in vacuo*. The residue was sublimed *in vacuo* at 40°, and the sublimate dissolved in 3 ml. of water. When a solution of 100 mg. of calcium chloride in 5 ml. of water was added, 11 mg. of calcium oxalate crystallized from the solution. The calcium salt was washed with water, and dried at 200° for analysis.

Anal. Calcd. for C_2O_4Ca: C, 18.75; CaO, 43.8. Found: C, 18.99; H, 0.5; CaO, 43.8.

Conversion of Tetraacetylstreptobiosamine to Maltol.—Tetraacetylstreptobiosamine, obtained by the hydrolysis of 9.0 g. of ethyl tetraacetylthiostreptobiosaminide diethyl mercaptal, was acetylated with acetic anhydride and pyridine. The acetylation mixture, when treated in the usual manner, gave 5.29 g. of a colorless, glassy product. This product was converted to a white powder, $[\alpha]^{25}D$ −92° (*c*, 1.3 in chloroform), by dissolving the material in chloroform and precipitating it by the addition of petroleum ether. A solution of 1.89 g. of this material and 246 mg. of hydroxylamine hydrochloride in 10 ml. of pyridine was allowed to stand overnight at room temperature. The solvent was removed *in vacuo* and the residue was taken up in chloroform. The chloroform solution was washed with dilute hydrochloric acid and with water, dried, and concentrated *in vacuo* to a colorless glass, 1.86 g., $[\alpha]^{25}D$ −75° (*c*, 0.8 in chloroform). A solution of 1.80 g. of this product in 25 ml. of 1% methanolic hydrogen chloride was allowed to stand overnight at room temperature. After removal of the solvent, 1.33 g. of crude product was obtained, and this, in methanol–chloroform solution, was further purified by chromatography on acid-washed alumina. Methyl N-acetylstreptobiosaminide oxime thus prepared was obtained as a colorless powder, 400 mg., $[\alpha]^{25}D$ −140° (*c*, 0.6 in methanol), which was used for the next step without further purification.

Anal. Calcd. for $C_{16}H_{27}N_2O_{10}$: N, 6.9; OCH_3, 7.6. Found: N, 5.6; OCH_3, 6.1.

A solution of 444 mg. of methyl N-acetylstreptobiosaminide oxime and 150 mg. of sodium nitrite in 10 ml. of 0.1 *N* hydrochloric acid was allowed to stand for five minutes at room temperature. The solution was then made alkaline by the addition of 1.5 ml. of 2.5 *N* sodium hydroxide and heated on the steam-bath for one-half hour. After acidification with 2 ml. of 2.5 *N* hydrochloric acid, the cooled aqueous solution was extracted continuously with chloroform. Removal of the chloroform left 29 mg. (21%) of a brown crystalline residue. After several recrystallizations from toluene, the product melted at 159–160°, and no depression of melting point was observed upon admixture with an authentic specimen of maltol, m. p. 160–161°.

Treatment of N-Acetylstreptobiosamine with Alkali.—A solution of 250 mg. of ethyl N-acetylstreptobiosaminide diethyl mercaptal in 10 ml. of 50% aqueous acetone was refluxed with 1 g. of strontium carbonate and 0.5 g. of mercuric chloride for one-half hour. The solution was cooled, filtered, treated with hydrogen sulfide, filtered again and concentrated *in vacuo* to a colorless residue. The material, when treated with alkali under conditions employed for the formation of maltol for streptomycin, gave no evidence of maltol formation.

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Summary

Treatment of ethyl tetraacetylthiostreptobiosaminide diethyl mercaptal with aqueous mercuric chloride solution gave tetraacetylstreptobiosamine, which upon oxidation and acetylation yielded crystalline pentaacetylstreptobiosamic acid monolactone. Hydrolysis of this disaccharide acid led to the isolation of streptosonic acid monolactone, $C_6H_8O_6$. This new acid was converted into streptosonic acid diamide and diacetylstreptosonic acid monolactone.

The structure of streptosonic acid has been elucidated through studies of the periodate oxidation of the monolactone and of the diamide of this acid. This information, in conjunction with the results of previously described investigations, has led to the establishment of the structures of streptobiosamine, methyl streptobiosaminide dimethyl acetal, ethyl thiostreptobiosaminide diethyl mercaptal and tetraacetyldesoxystreptobiosamine.

A possible partial structure of streptomycin is presented.

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