was boiled for a few minutes. After cooling, the residue was removed by filtration, washed with water and discarded. The filtrate was brought to *p*H 4.0–4.5 with acetic acid, and after standing several hours at 5° was filtered again. The resulting filtrate was then precipitated either with 20–30 volumes of acetone, or with barium hydroxide until precipitation was complete and a slight excess of the alkali remained. The solid from the acetone precipitation was usually of a very dark color and contained a large percentage of protein. It was hygroscopic but would not redissolve completely in water. Since the volume of acetone required was so great, barium precipitation was usually employed.

The barium precipitate was separated by centrifugation and washed several times with water (*p*H 9.5–10.0) and finally with a small amount of distilled water. It was then decomposed with excess 0.5 *M* sodium sulfate. The filtrate after removal of barium sulfate was usually golden yellow in color, with *p*H approximately 10. This preparation, after precipitation with acetone, filtration and drying of the precipitate *in vacuo*, contained one to five γ of pantothenic acid activity per mg. of organic solids.

By combining the above treatments with tungstic acid precipitation, adsorption with Lloyd reagent, and dialysis, concentrates containing up to 5% pantothenic acid activity have been obtained, based upon the total solid matter present. Further studies are in progress to accomplish the isolation of the pure conjugate.

Acknowledgment.—The authors wish to acknowledge with thanks, gifts of coenzyme A and alkaline phosphodiesterase from Drs. G. D. Novelli and F. Lippman, to Ruth S. Langdon and Eleanor C. Parsons for technical assistance, and to Dr. Rosalind Wulzen for helpful suggestions and the use of laboratory facilities for the testing of acetylcholine activity.

Summary

A conjugate of pantothenic acid (abbr. PAC) has been obtained in partially purified form from pork heart. It appears to be roughly twice as active for the growth of *A. suboxydans* as is the free vitamin. Growth of this organism is also more rapid with the conjugate than with pantothenic acid. The present conjugate is different from coenzyme A, the alkali-stable form of pantothenic acid or the protein conjugate found in blood.

From the behavior toward tungstic acid and dialysis, PAC appears to be an acidic molecule of molecular size considerably greater than pantothenic acid. Studies are in progress to accomplish the isolation of the pure material.

CORVALLIS, OREGON RECEIVED JULY 6, 1948

[CONTRIBUTION FROM THE DIVISION OF ORGANIC CHEMISTRY, THE SQUIBB INSTITUTE FOR MEDICAL RESEARCH]

Streptomycin. IX.[1] The Stepwise Degradation of Mannosidostreptomycin

BY HOMER E. STAVELY[2] AND JOSEF FRIED

Streptomycin concentrates have been shown to contain in addition to streptomycin a second antibiotic substance which has been termed streptomycin B or mannosidostreptomycin.[1] The degradation of this substance to derivatives of streptidine, streptobiosamine and D-mannose has been the subject of a preliminary communication.[3] The present paper gives a detailed account of those findings and, moreover, presents experimental data showing that in mannosidostreptomycin D-mannose is attached glycosidically to the streptobiosamine moiety through one of the three free hydroxyl groups of N-methyl-L-glucosamine.

Mannosidostreptomycin was isolated in the pure state as the crystalline reineckate which was converted to an amorphous hydrochloride.[1] Analyses of these two salts indicated that this antibiotic is a triacidic base of the composition $C_{27}H_{49}O_{17}N_7$. The difference in composition, $C_6H_{10}O_5$, between this substance and streptomycin appeared to be due to one hexose unit attached to the latter with the loss of one molecule of water. This assumption was substantiated[4] by the following degradation reactions.

When a solution of mannosidostreptomycin in 1.3 *N* methanolic hydrogen chloride was allowed to stand at room temperature for two days and the residue from the methanol solution, after careful neutralization with silver carbonate, was acetylated, an amorphous material was obtained, which upon chromatography on alumina afforded the two anomers of methyl tetraacetyl-D-mannopyranoside, as well as methyl tetraacetyl streptobiosaminide dimethyl acetal[5] and octaacetyl streptidine,[6] the latter two substances identical with the products obtained by similar degradation of streptomycin.

Confirmatory evidence as to the presence of D-mannose, streptobiosamine and streptidine in

(1) Paper VIII of this series: J. Fried and E. Titus, THIS JOURNAL, **70**, 3615 (1948).

(2) Present address: Commercial Solvents Corporation, Terre Haute, Indiana.

(3) J. Fried and H. E. Stavely, THIS JOURNAL, **69**, 1549 (1947).

(4) The experimental evidence adduced in this paper does not permit any conclusions as to the position of attachment of the streptobiosamine moiety to streptidine in mannosidostreptomycin. It is most likely, however, that this linkage involves the 4-hydroxyl group of streptidine as has been shown to be the case in streptomycin by Kuehl, Peck, Hoffhine, Peel and Folkers, *ibid.*, **69**, 1234 (1947).

(5) N. G. Brink, F. A. Kuehl, Jr., E. H. Flynn and K. Folkers, *ibid.*, **68**, 2557 (1946).

(6) R. L. Peck, R. P. Graber, A. Walti, E. W. Peel, C. E. Hoffhine, Jr., and K. Folkers, *ibid.*, **68**, 29 (1946).

mannosidostreptomycin was adduced by mercaptolysis. In using the anhydrous conditions described by Kuehl, Flynn, Brink and Folkers[7] for the mercaptolysis of streptomycin incomplete reaction was often observed. However, when aqueous hydrochloric acid[8] was substituted for the gaseous hydrogen chloride reaction was complete after fifteen hours of shaking at room temperature. Chromatography of the crude acetylated product yielded octaacetyl streptidine, ethyl tetraacetyl-β-thiostreptobiosaminide diethyl mercaptal[8] and the α- and β-forms of ethyl tetraacetyl-1-thio-D-mannopyranoside.[9]

Following the identification of the cleavage products obtained from mannosidostreptomycin our efforts were directed toward ascertaining the site of the linkage joining D-mannose to the remaining portion of the molecule. On the assumption that the linkage between streptobiosamine and streptidine is identical for the two streptomycins, D-mannose could be attached glycosidally through one of the three free hydroxyl groups of the N-methyl-L-glucosamine moiety, through the 2-,5- or 6-hydroxyl groups of streptidine or, least probably, through the tertiary hydroxyl group of streptose. Moreover, the possibility of a linkage involving one of the nitrogenous groups could not be disregarded. Evidence that the D-mannose moiety is attached to N-methyl-L-glucosamine was adduced by partial cleavage to a product in which the linkage between these two moieties had remained intact. Initial trials to effect such an incomplete degradation by methanolysis of dihydromannosidostreptomycin[1] using a low enough concentration of hydrogen chloride to leave approximately half the starting material unreacted led to complete cleavage of the remaining portion into α- and β-methyl D-mannopyranosides, methyl-α-dihydrostreptobiosaminide[10] and streptidine, all isolated as the crystalline acetyl derivatives. Suitable experimental conditions were finally arrived at on the basis of the following observations. When a solution of mannosidostreptomycin or its dihydro-derivative in 1 *N* sulfuric acid was allowed to stand at 45° for twenty hours streptidine sulfate monohydrate separated in almost quantitative yield, but no mannose was detectable among the reaction products upon addition of phenylhydrazine to the neutralized hydrolysate. On the other hand, a quantitative yield of mannose phenylhydrazone was obtained when the hydrolysis was conducted at 100° for one hour. These findings indicated that D-mannose is attached to the streptobiosamine moiety and not to streptidine. Removal of the sulfate and excess chloride ions from the solution obtained by hydrolysis of dihydromannosidostreptomycin at 45°, followed by lyophilization, afforded a crude amorphous material which was acetylated immediately and purified by chromatography. The resulting product, although amorphous, gave analytical figures in accord with those calculated for the nonaacetate of a trisaccharide, which has been designated dihydromannosidostreptobiosamine on the basis of its cleavage by methanolysis into the anomeric methyl D-mannopyranosides and methyl dihydrostreptobiosaminide, and on the basis of further evidence discussed below. A differential acetyl determination on this nonaacetate indicated that eight of the acetyl groups were attached to oxygen and the remaining one to nitrogen, ruling out the possibility that D-mannose is linked to streptobiosamine via the N-methylamino group.

The acetolysis procedure of Hann and Hudson[11] when applied to methyl pentaacetyl dihydrostreptobiosaminide resulted in the quantitative cleavage of this disaccharide into its component sugars,[12] only one of which (pentaacetyl N-methyl-L-glucosamine) was extractable from the neutralized reaction mixture by chloroform. Whereas the residue remaining after evaporation of this chloroform solution invariably crystallized, the analogous fraction obtained by acetolysis of the nonaacetate of dihydromannosidostreptobiosamine remained amorphgus even after chromatooraphic purification on alumina. Indications from analytical figures that this product represents the octaacetate of a disaccharide composed of D-mannose and N-methyl-β-L-glucosamine were confirmed by methanolysis and reacetylation, which readily afforded the two anomeric methyl tetraacetyl-D-mannopyranosides as well as methyl tetraacetyl-N-methyl-β-L-glucosaminide. The latter was identified by comparison with the product obtained by methanolysis and reacetylation of pentaacetyl N-methyl-α-L-glucosamine. On the basis of this cleavage reaction and of the above described degradation of dihydromannosidostreptobiosamine to derivatives of D-mannose and dihydrostreptobiosamine the amorphous acetolysis product is formulated as the octaacetate of a D-mannoside of N-methyl-L-glucosamine.

Information concerning the anomeric character of the glycosidic linkage between the two sugars can be derived from available data by the application of Hudson's rules of isorotation.[13] On the assumption that the linkage between streptobiosamine and streptidine is of the α-type, as has been shown for streptomycin,[14] the molecular rotation of mannosidostreptomycin can be expressed as $[M]_B = [M]_A + A_{St.} + B_{Man.}$, where $[M]_B$ and $[M]_A$ are the molecular rotations of mannosidostreptomycin and streptomycin, respectively, $A_{St.}$

(7) F. A. Kuehl, Jr., E. H. Flynn, N. G. Brink and K. Folkers, *ibid.*, 68, 2096 (1946).

(8) I. R. Hooper, L. H. Klemm, W. J. Polglase and M. L. Wolfrom, THIS JOURNAL, 68, 2120 (1946).

(9) J. Fried and D. E. Walz, *ibid.*, 71, 140 (1949).

(10) J. Fried and O. Wintersteiner, *ibid.*, 69, 79 (1947).

(11) R. M. Hann and C. S. Hudson, *ibid.*, 66, 2465 (1934).

(12) H. E. Stavely, O. Wintersteiner, J. Fried, H. L. White and M. Moore, *ibid.*, 69, 2742 (1947).

(13) C. S. Hudson, THIS JOURNAL, 31, 66 (1909).

(14) R. U. Lemieux, C. W. DeWalt and M. L. Wolfrom, *ibid.*, 69, 1838 (1947).

the contribution of carbon atom one of D-mannose to the molecular rotation of mannosidostreptomycin, and $B_{Man.}$ the contribution of the basal chain of the mannose moiety as calculated from the molecular rotations of α- and β-D-mannose ($A + B = +5380$, $-A + B = -2940$). $A_{St.}$, the quantity characteristic of the glycosidic linkage in question then becomes $A_{St.} = [M]_B - [M]_A - B_{Man.}$ or, after substitution of the experimental values, $A_{St.} = (871)(-47) - (691)(-86) - 1220 = +17180$. The positive numerical value for $A_{St.}$ and the fact that the mannose moiety in mannosidostreptomycin has the D-configuration indicate that the glycosidic linkage is of the α-D type. The absolute value of 17180 is in reasonable agreement with the value for A_{OMe} (14230) calculated from the molecular rotations of the two anomeric methyl D-mannopyranosides.

Experimental data establishing the exact site of the glycosidic linkage between D-mannose and the remaining portion of the molecule will be presented in a subsequent publication.

Experimental[15]

Methanolysis of Mannosidostreptomycin.—Mannosidostreptomycin trihydrochloride[1] (1.1 g.) was dissolved in 30 ml. of 1.3 *N* methanolic hydrogen chloride. After two days the spectrophotometric maltol assay[1] showed a decline from the original ΔE value of 126 to less than 3. An excess of silver carbonate was added to the flask and the mixture was stirred for four hours. After standing overnight the mixture was filtered and excess silver ion removed from the solution by means of hydrogen sulfide. After filtration through charcoal the filtrate was evaporated *in vacuo* and the residue (710 mg.) was acetylated in pyridine (5 ml.)-acetic anhydride (5 ml.) for twenty-four hours. After removal of the solvents *in vacuo* the residue was dissolved in chloroform, the solution washed with dilute hydrochloric acid, dilute sodium bicarbonate solution and water, and the chloroform extract dried over sodium sulfate. The residue from the chloroform solution was dried and extracted several times with dry ether, most of the streptidine octaacetate remaining undissolved. It was crystallized from ethanol, m. p. 255–257° (dec.).

Anal. Calcd. for $C_8H_{10}N_6O_4(COCH_3)_8$: N, 14.04. Found: N, 13.68.

The dried ether residue (874 mg.) was dissolved in benzene (25 ml.) and chromatographed on an acetic acid-washed alumina[16] column, 1.5 × 20 cm. The chromatogram was developed with 100 ml. of benzene. The first 25 ml. of eluate yielded 270 mg. of residue which crystallized from an ether solution on evaporation. It was recrystallized twice from ether-pentane, $[\alpha]^{24}D$ +49° (*c*, 1.1 in chloroform). The m. p. was 65–66°, not depressed on admixture of an authentic sample of methyl tetraacetyl-α-D-mannopyranoside.

Anal. Calcd. for $C_7H_{10}O_6(CH_3CO)_4$: C, 49.72; H, 6.12; CH_3O, 8.56; acetyl, 47.5. Found: C, 50.01; H, 6.08; CH_3O, 8.98; acetyl, 47.7.

The succeeding 25 ml. of benzene contained 30 mg. of a substance which likewise crystallized from ether but which melted unsharply from 130 to 150° and was probably a mixture of the α- and β-anomers of methyl tetraacetyl-D-mannopyranoside. Further elution with 50 ml. of benzene yielded only 22 mg., likewise crystalline from ether, melting from 100 to 110°.

The column was then eluted with a mixture of 70 ml. of benzene and 30 ml. of ether and the eluate collected in 20-ml. portions. The residues all crystallized from ether and melted unsharply between 100 and 125°. Elution with 50 ml. of ether then yielded 131 mg. of crystalline material which melted at 124–125° after two recrystallizations from ether-hexane; $[\alpha]^{25}D$ −122° (*c*, 0.56 in chloroform). There was no melting point depression on admixture of an authentic specimen of methyl tetraacetyl-α-streptobiosaminide dimethyl acetal.

Anal. Calcd. for $C_{13}H_{18}NO_7(OCH_3)_3(COCH_3)_4$: C, 50.97; H, 6.95; N, 2.48; CH_3O, 16.5; O-acetyl, 22.9. Found: C, 51.01; H, 6.74; N, 2.60; CH_3O, 16.9; O-acetyl,[17] 23.2.

The material which had been eluted with 30% ether in benzene was combined with the residue from the methyl tetraacetyl-α-streptobiosaminide dimethyl acetal mother liquor, dissolved in 15 ml. of benzene and rechromatographed on a column of alumina, 0.8 × 15 cm. Nothing could be eluted with benzene. Elution with 100 ml. of 30% ether in benzene afforded 62 mg. of a substance which attained a constant m. p. of 117–119° after several recrystallizations from ether-hexane. The specific rotation was −102°.

Anal. Found: C, 51.58; H, 6.98.

This product was probably a mixture of the α- and β-anomers of methyl tetraacetyl streptobiosaminide dimethyl acetal.

Elution of the column with ether yielded a further quantity (87 mg.) of methyl tetraacetyl-α-streptobiosaminide dimethyl acetal, m. p. 123–125°, $[\alpha]D$ −121°.

Mercaptolysis of Mannosidostreptomycin.—Mannosidostreptomycin (2.38 g.) was added to 10 ml. of ethyl mercaptan and 4.5 ml. of concentrated hydrochloric acid and the mixture was shaken vigorously for a period of eighteen hours at room temperature. The reagents were distilled off *in vacuo* at a bath temperature not exceeding 30° and the last traces of water were removed by twice distilling absolute alcohol off the residue. The latter was acetylated by shaking for sixteen hours with 25 ml. of pyridine and an equal volume of acetic anhydride. The dark red solution was concentrated to a sirup and the latter partitioned between ice-water and chloroform. The chloroform layer was washed with dilute hydrochloric acid, sodium bicarbonate and finally with water. After drying over sodium sulfate the chloroform extract was evaporated to dryness *in vacuo* and the residue taken up in 10 ml. of absolute alcohol. On standing in the refrigerator overnight the alcoholic solution deposited crystals, which were filtered off and washed with cold ethanol. The filtrate and washings were evaporated to dryness *in vacuo* and chromatographed as described below.

The crystals, which appeared to be a mixture, were extracted with warm ether. This treatment left undissolved a fraction which was difficultly soluble in most organic solvents. Recrystallization from a large volume of alcohol produced small needles which melted at 255–257°, and were identified as octaacetylstreptidine.

The combined ether extracts were evaporated to dryness and the crystalline residue was recrystallized three times from absolute alcohol. Prismatic needles, 110 mg., melting at 160–161°, were obtained. The melting point was raised to 161–162° by chromatography of a benzene solution of this material on alumina, followed by an additional recrystallization from alcohol, $[\alpha]^{25}D$ −67° (*c*, 0.51 in chloroform). The substance showed no depression in melting point when mixed with an authentic sample of ethyl tetraacetyl-1-thio-β-D-mannopyranoside.[8]

Anal. Calcd. for $C_8H_{12}O_5S(CH_3CO)_4$: C, 48.98; H, 6.17; S, 8.16; CH_3CO, 43.84. Found: C, 49.22; H, 6.91; S, 8.33; CH_3CO, 43.4.

(15) The melting points reported in this paper were taken in capillary tubes and have been corrected for stem exposure.

(16) The alumina used for all chromatograms reported in this paper was purchased from the Harshaw Chemical Company, Cleveland, Ohio. It was washed with dilute acetic acid and then washed repeatedly with distilled water until the *p*H of a water suspension of the alumina had risen to 6.0. It was then filtered and dried at 150° for thirty-six to forty-eight hours.

(17) J. F. Alicino, *Anal. Chem.*, **20**, 590 (1948).

The residue from the above alcoholic filtrate and washings was dissolved in 15 ml. of benzene and after the addition of an equal volume of hexane was chromatographed on a column containing 30 g. of alumina. A mixture of equal volumes of benzene and hexane eluted a crystalline fraction (100 mg.) which after several recrystallizations from hexane containing a small amount of ether melted at 107–108°, $[\alpha]^{24}D$ +94° (*c*, 1.06 in chloroform). These data are in accord[18] with the constants for ethyl tetraacetyl-1-thio-α-D-mannoside.[9] No melting point depression was observed on admixture of an authentic sample of the latter.

Anal. Calcd. for $C_8H_{12}O_5S(CH_3CO)_4$: C, 48.98; H, 6.17; S, 8.16; CH_3CO, 43.84; mol. wt., 392.4. Found: C, 48.91; H, 6.04; S, 8.17; CH_3CO, 44.2; mol. wt. (Rast), 378.

Subsequent elution of the alumina column with 850 ml. of benzene yielded a second crystalline substance (530 mg.), which after recrystallization from ether–hexane melted at 111–112°, $[\alpha]^{25}D$ −31° (*c*, 0.95 in chloroform). There was no depression in melting point when this substance was mixed with an authentic sample of ethyl tetraacetyl-β-thiostreptobiosaminide diethyl mercaptal. The latter has been reported[8] to have m. p. 111–111.5° and $[\alpha]^{25}D$ −29° (*c*, 3.0 in chloroform).

Anal. Calcd. for $C_{37}H_{45}NO_{11}S_3$: C, 49.44; H, 6.92; N, 2.14; S, 14.66. Found: C, 49.45; H, 7.01; N, 2.52; S, 14.81.

Methanolysis of Dihydromannosidostreptomycin.—A solution of 800 mg. of vacuum-dried dihydromannosidostreptomycin in 16 ml. of 0.8 *N* methanolic hydrogen chloride was allowed to stand at room temperature for forty hours. Upon addition of 32 ml. of anhydrous ether to this solution a copious precipitate formed, which was filtered, washed with ether and dried *in vacuo*. The dried powder weighed 610 mg. and had $[\alpha]^{25}D$ −46° (*c*, 1.51 in methanol). The ether–methanol filtrate was concentrated *in vacuo* to a thin sirup and precipitated with a large excess of ether. After two hours of standing in the refrigerator, the oily deposit was separated from the supernatant by decantation and dried *in vacuo*. A white powder (169 mg.) was obtained which had $[\alpha]^{26}D$ −77° (*c*, 1.05 in methanol). All but 11 mg. of this material was acetylated with 4 ml. of pyridine and 3 ml. of acetic anhydride and the resulting acetylation product chromatographed in benzene solution (10 ml.) on 7 g. of alumina. Benzene eluted an oil which crystallized readily when its ether solution was allowed to evaporate at room temperature. The material on recrystallization from absolute alcohol yielded prismatic needles which melted at 159.5–160.5° and had $[\alpha]^{24}D$ −50° (*c*, 0.68 in chloroform). The melting point of this substance was not depressed on admixture of an authentic sample of methyl tetraacetyl-β-D-mannopyranoside. The latter has been reported[19] to have m. p. 161° and $[\alpha]^{25}D$ −50.4° (*c*, 1.4 in chloroform).

Anal. Calcd. for $C_7H_{10}O_6(CH_3CO)_4$: C, 49.72; H, 6.12; OCH_3, 8.56; CH_3CO, 47.5. Found: C, 49.61; H, 6.08; OCH_3, 8.35; CH_3CO, 48.3.

Subsequent elution of the column with a mixture of equal volumes of benzene and ether gave fractions which crystallized spontaneously upon evaporation of the solvents *in vacuo*. Recrystallization from absolute alcohol yielded a product (m. p. 192–193°; $[\alpha]^{24}D$ −119° (*c*, 0.48 in chloroform)) which proved to be identical with an authentic sample of methyl pentaacetyl-α-dihydrostreptobiosaminide prepared from dihydrostreptomycin.

Anal. Calcd. for $C_{14}H_{22}NO_9(CH_3CO)_5$: C, 51.14; H, 6.62; O-acetyl, 30.6. Found: C, 51.10; H, 6.64; O-acetyl, 30.4.

(18) The specific rotation of ethyl tetraacetyl-1-thio-α-D-mannoside prepared directly from D-mannose is +104°. The difference between this value and the one obtained for the substance prepared from mannosidostreptomycin is probably due to the presence of a small amount of the β-anomer in the latter.

(19) T. L. Harris, E. L. Hirst and C. E. Wood, *J. Chem. Soc.*, 2108 (1932).

The vacuum-dried ether precipitate (610 mg.) described above was acetylated with 5 ml. of acetic anhydride and 10 ml. of pyridine. The resulting acetylation product was dissolved in 5 ml. of absolute alcohol and allowed to stand in the refrigerator overnight. A crystalline precipitate formed, which consisted for the most part of octaacetylstreptidine. It was separated off by centrifugation and extracted several times with small portions of dry ether. The combined alcohol–ether filtrates were evaporated to dryness and the residue chromatographed in benzene solution on alumina. The two crystalline fractions obtained from the column proved to be identical with those described above.

Aqueous Acid Hydrolysis of Mannosidostreptomycin.—A solution of 1.08 g. of crude mannosidostreptomycin hydrochloride in 20 ml. of 1 *N* sulfuric acid was heated to 45° for twenty-four hours. After standing in the refrigerator for one day streptidine sulfate (0.45 g., 92% of the theoretical amount) was removed by filtration and the sulfate ion was removed from the filtrate by the addition of excess barium carbonate. To the supernate 0.4 g. of phenylhydrazine hydrochloride and 0.6 g. of sodium acetate were added. After standing for four hours the slight precipitate which had formed was centrifuged, washed and dried. It weighed only 26 mg. and could not be crystallized. Increasing the time of hydrolysis to sixty-five hours did not change the result. A quantitative yield of D-mannose phenylhydrazone was obtained, when the reaction was conducted at 100° for one hour.[20]

Nonaacetyl-dihydromannosidostreptobiosamine.—A solution of 8.1 g. of dihydromannosidostreptomycin trihydrochloride in 160 ml. of 1 *N* sulfuric acid was kept at 45° for twenty hours. After standing in the refrigerator for two days 3.28 g. (96% of the theoretical amount) of streptidine sulfate was removed by filtration. A sample was analyzed after drying at 100° for two hours.

Anal. Calcd. for $C_8H_{18}O_4N_6 \cdot H_2SO_4 \cdot H_2O$: C, 25.40; H, 5.86; N, 22.20; S, 8.46. Found: C, 25.42; H, 5.73; N, 22.14; S, 8.33.

The filtrate was neutralized with barium hydroxide, the barium sulfate removed by filtration, and silver carbonate was added with shaking until the *p*H attained a value of 6 to 6.5. The supernatant liquid was immediately lyophylized and the resulting amorphous solid acetylated with pyridine (50 ml.)–acetic anhydride (30 ml.) for two days at room temperature. The solvents were then removed under reduced pressure and the residue taken up in chloroform, which was washed with dilute hydrochloric acid, dilute sodium bicarbonate solution and water, and dried over sodium sulfate. The dried residue from the chloroform solution was dissolved in benzene and chromatographed on an acetic acid-washed alumina column, 3 × 20 cm. Material eluted with benzene (200 ml.) weighed 0.83 g. and had a specific rotation of −93°. It was discarded. Subsequent elutions with benzene–ether mixtures, ether and ethyl acetate yielded fractions which all had specific rotations of −45° ± 7°, and these were combined. Since this material could not be induced to crystallize it was lyophylized from benzene solution to an amorphous powder, $[\alpha]^{25}D$ −48° (*c*, 1.05 in chloroform), which was dried at 60° *in vacuo*.

Anal. Calcd. for $C_{19}H_{26}O_{14}N(COCH_3)_9$: C, 50.51; H, 6.04; N, 1.59; O-acetyl, 39.2; total acetyl, 44.0. Found: C, 50.58; H, 6.15; N, 1.91; O-acetyl, 37.9; total acetyl, 44.7.

Methanolysis of Nonaacetyl-dihydromannosidostreptobiosamine.—The acetylated trisaccharide (320 mg.) was dissolved in 10 ml. of 1.5 *N* methanolic hydrogen chloride. After standing for two days the acid was removed by shaking with excess silver carbonate followed by centrifugation. The supernatant liquid was evaporated to dryness *in*

(20) This experiment was carried out by Mr. J. Kowald, and provides the basis for an analytical procedure for the determination of mannosidostreptomycin, a detailed account of which will be published shortly by Dr. E. T. Stiller and Mr. J. Kowald of the Division of Chemical Development.

vacuo and the residue acetylated overnight in pyridine (5.0 ml.)-acetic anhydride (3.0 ml.). The dried acetylation product (307 mg.) worked up as in the acetylations described above was extracted with benzene, leaving undissolved 46 mg. of crystals, which after recrystallization from acetone-pentane had a m. p. of 191–193°, not depressed on admixture of authentic methyl pentaacetyl-α-dihydrostreptobiosaminide.

The benzene solution was chromatographed on an acetic acid-washed alumina column, 1 × 18 cm. Elution with 75 ml. of benzene yielded 57 mg. of methyl tetraacetyl-α-D-mannopyranoside, m. p. 63–65°. Further elution with 50 ml. of 30% ether in benzene afforded first 26 mg. of a crystalline material which melted over a wide range, 60 to 140°, and then 26 mg. of methyl tetraacetyl-β-D-mannopyranoside which melted at 153–155° after recrystallization from ether-pentane. The m. p. of a mixture with an authentic sample of m. p. 160° was 153–157°. A further quantity of methyl pentaacetyl dihydrostreptobiosaminide, 85 mg., m. p. 188–191°, was obtained by elution with 50 ml. of ether.

Acetolysis of Nonaacetyl-dihydromannosidostreptobiosamine.—In a preliminary experiment 90 mg. of the acetylated trisaccharide was dissolved in a mixture of 7.0 ml. of acetic anhydride, 3.0 ml. of acetic acid and 0.1 ml. of concentrated sulfuric acid. The initial rotation, $[\alpha]^{25}D$ −48°, dropped to −19° in five hours and remained constant at −10° after sixteen hours. The solution was then poured into a beaker containing crushed ice and solid sodium bicarbonate and shaken and stirred vigorously until the acids were neutralized. Extraction with chloroform yielded 75 mg. of a residue which was taken up in benzene and chromatographed on an alumina column, 1 × 15 cm. Elution with 40% ether in benzene afforded pentaacetyl-N-methyl-L-glucosamine (11 mg., m. p. 156°), but the bulk of the material (51 mg.) could be eluted only with ether. Since it could not be induced to crystallize, it was lyophilized from benzene solution and dried at 60° *in vacuo*, $[\alpha]^{25}D$ −31° (*c*, 0.6 in methanol).

Anal. Calcd. for $C_{15}H_{17}O_{10}N(COCH_3)_5$: N, 2.03; O-acetyl, 43.6; total acetyl, 49.8. Found: N, 1.92; O-acetyl, 40.8; total acetyl, 51.6.

Subsequent acetolyses on a larger scale always gave as the principal product an amorphous material having $[\alpha]D$ −32 ± 2°. The small amounts of pentaacetyl-N-methyl-L-glucosamine were probably derived from the estimated 5–10% streptomycin present in the mannosidostreptomycin used for degradation.

Methanolysis of Octaacetyl-D-mannosido-N-methyl-L-glucosamine.—A solution of 360 mg. of the acetylated disaccharide in 20 ml. of 1.1 *N* methanolic hydrogen chloride was allowed to stand at room temperature for forty-eight hours, and the mixture of products was isolated and acetylated as described above for the methanolysis of mannosidostreptomycin.

The acetylation product was dissolved in 20 ml. of benzene and chromatographed on a column of alumina, 1 × 18 cm. Elution with benzene (100 ml.) gave 52 mg. of crude methyl tetraacetyl-α-D-mannopyranoside, which melted at 64–66° after recrystallization from ether-pentane. Further elution with 40% ether in benzene yielded successively 30 mg. of methyl tetraacetyl-β-D-mannopyranoside, m. p. 157–159°, and 93 mg. of a crystalline substance which melted at 106–108° after several recrystallizations from ether-pentane, $[\alpha]^{25}D$ −4.0° (*c*, 0.5 in ethanol).

Anal. Calcd. for $C_7H_{10}O_4N(COCH_3)_4(OCH_3)$: C, 51.19; H, 6.71; N, 3.73; CH_3O, 8.27; O-acetyl, 34.4. Found: C, 51.28; H, 6.71; N, 3.58; CH_3O, 8.06; O-acetyl, 35.7.

There was no depression in m. p. on admixture of methyl tetraacetyl-N-methyl-β-L-glucosaminide, m. p. 105–108°, which had been prepared by methanolysis and reacetylation of pentaacetyl-N-methyl-α-L-glucosamine.

Methyl Tetraacetyl-N-methyl-β-L-glucosaminide.—Pentaacetyl-N-methyl-α-L-glucosamine (140 mg.) was dissolved in 10 ml. of 1.1 *N* methanolic hydrogen chloride. After two days standing the solution was stirred with excess silver carbonate for thirty minutes, then filtered, and the filtrate evaporated to dryness *in vacuo*. The residue was acetylated overnight in acetic anhydride (2 ml.)-pyridine (2 ml.). The crude acetylation product was crystallized several times from ether-pentane, m. p. 103–105°, $[\alpha]^{25}D$ −4.2° (*c*, 0.5 in ethanol).

Acknowledgment.—The authors wish to express their appreciation to Dr. O. Wintersteiner for his interest and helpful advice in the course of this work. They are also indebted to Miss Mary Berestecki and Miss Doris E. Walz for their able technical assistance and to Mr. Joseph F. Alicino, Miss Anne C. Crickenberger and Miss Ruth Karitzky for the microanalytical determinations.

Summary

Mannosidostreptomycin has been cleaved by methanolic hydrogen chloride to streptidine, methyl α-streptobiosaminide dimethyl acetal, and the α- and β-forms of methyl D-mannopyranoside, and by ethyl mercaptan–hydrochloric acid to the corresponding ethyl thio-derivatives. The cleavage products were isolated as the crystalline acetates.

Dihydromannosidostreptomycin has been degraded by methanolic hydrogen chloride to the α- and β-anomers of methyl D-mannopyranoside and methyl dihydrostreptobiosaminide, all isolated as the crystalline acetates.

Hydrolysis of dihydromannosidostreptomycin with 1 *N* sulfuric acid at 45° yielded streptidine sulfate and a trisaccharide, dihydromannosidostreptobiosamine, isolated as the amorphous nonaacetate. This product was cleaved by methanolic hydrogen chloride to methyl β-D-mannopyranoside and methyl α-dihydrostreptobiosaminide, both isolated as the crystalline acetates.

Acetolysis of nonaacetyl dihydromannosidostreptobiosamine gave a disaccharide acetate, octaacetyl mannosido-N-methyl-L-glucosamine, which was cleaved by methanolic hydrogen chloride to methyl β-D-mannopyranoside and methyl N-methyl-β-L-glucosaminide, both isolated as the crystalline tetraacetates.

The conclusion is drawn from these data that mannosidostreptomycin is a D-mannosido-N-methyl-α-L-glucosaminido-L-streptoside of streptidine. The linkage between the D-mannose and the N-methyl-L-glucosamine moieties appears to be α.

NEW BRUNSWICK, NEW JERSEY

RECEIVED AUGUST 3, 1948