(m.p. 139–141°) and by concentrating the mother liquor a second crop of 1.4 g. (m.p. 135–137°) was obtained. The combined yield was 95.6%. Recrystallization of a sample from isopropyl alcohol gave a product melting at 142.5–143.5°.

Anal. Calcd. for $C_{13}H_{17}O_4N$: C, 62.14; H, 6.82; N, 5.58. Found: C, 62.13; H, 6.83; N, 5.59.

***erythro*-β-Phenylserine Ethyl Ester Hydrochloride (V).**—*erythro*-N-Acetyl-β-phenylserine ethyl ester (56.5 g., 0.225 mole) was dissolved in 225 ml. of an approximately half saturated solution of hydrogen chloride in absolute alcohol and the clear solution was allowed to stand at room temperature. After 15 hours, heavy crystal deposition had occurred and 225 ml. of absolute ether was added to the mixture. It was cooled at 0° for 3 hours and filtered. The ethanol washed product weighed 35.0 g. (63.4%) and melted at 180–181° with decomposition.

Anal. Calcd. for $C_{11}H_{16}O_3NCl$: C, 53.77; H, 6.56; N, 5.70; Cl, 14.43. Found: C, 53.91; H, 6.56; N, 5.71; Cl, 14.42.

***erythro*-β-Phenylserine (VI). A. By Hydrolysis of *erythro*-β-Phenylserine Ethyl Ester Hydrochloride.**—A solution of 34.5 g. of *erythro*-β-phenylserine ethyl ester hydrochloride in 175 ml. of 10% hydrochloric acid was heated under reflux for two hours. After vacuum concentration to dryness the total weight of solid product was 30.4 g. (theory for β-phenylserine hydrochloride is 30.6 g.). The crystalline product was dissolved in 25 ml. of water, treated with Darco, and neutralized with concentrated ammonia. After standing at 0° overnight, a first crop of 15.0 g. was obtained. Concentration of the filtrate yielded an additional 8.2 g., resulting in a total yield of 92.0%.

The combined products were recrystallized from a mixture of water and dimethylformamide and 16.1 g. of pure material was obtained. A sample dried at 100° *in vacuo* melted with decomposition at 199–200°.

Anal. Calcd. for $C_9H_{11}O_3N$: N, 7.72. Found: N, 7.75.

B. By Acid Hydrolysis of *erythro*-N-Acetyl-β-phenylserine Ethyl Ester.—The procedure used was the same as for the hydrolysis of the ester hydrochloride (above). An over-all yield of 92% was obtained.

***threo*-β-Phenylserine Hydrochloride (VIII).**—Two grams of *erythro*-N-acetyl-β-phenylserine ethyl ester was added to 6 ml. of thionyl chloride at 0°. After five minutes, 40 ml. of absolute ether was added and the product separated in the form of fine needles. Filtration and washing with absolute ether gave 1.70 g. (79.5%) of the oxazoline hydrochloride (VII) as sticky white crystals.

This product was added to 30 ml. of 10% hydrochloric acid and heated under reflux for two hours. The solution was concentrated until crystallization occurred and the residue was placed in a vacuum desiccator over sodium hydroxide pellets. Dry, powdery *threo*-β-phenylserine hydrochloride (1.30 g., 94.9% based on the oxazoline hydrochloride) was obtained which melted with decomposition at 164–166°.

Esterification with absolute alcohol and hydrogen chloride yielded *threo*-β-phenylserine ethyl ester hydrochloride (92.8%) melting at 134–137°. Recrystallization from alcohol yielded material having the characteristic melting point of 137.5–139°.

Anal. Calcd. for $C_{11}H_{16}O_3NCl$: C, 53.77; H, 6.56; N, 5.70; Cl, 14.43. Found: C, 53.86; H, 6.69; N, 5.69; Cl, 14.42.

***threo*-N-Acetyl-β-phenylserine Ethyl Ester (X).**—To a mixture of 40 ml. of acetic acid, 15 g. (0.18 mole) of anhydrous sodium acetate and 40 g. (0.16 mole) of *threo*-β-phenylserine ethyl ester hydrochloride (prepared by method of Carrara and Weitnauer[14]), acetic anhydride (16.5 ml., 0.17 mole) was added slowly with agitation. The temperature rose to about 80° and the solution became thick with crystals. After standing at room temperature for two hours, the mixture was poured into 500 ml. of water. The white product, washed until chloride free, weighed 39.0 g. (95.0%) and melted at 173–175°. A sample recrystallized from dioxane melted at 175–176.5°.

Anal. Calcd. for $C_{13}H_{17}O_4N$: C, 62.14; H, 6.82; N, 5.58. Found: C, 62.36; H, 6.77; N, 5.56.

Treatment of *threo*-N-Acetyl-β-phenylserine Ethyl Ester with Thionyl Chloride.—This experiment, and the identification of products by formation of the ester hydrochlorides was carried out in the same manner as described for the preparation of *threo*-β-phenylserine hydrochloride. The *erythro*-ethyl ester hydrochloride is much less soluble in alcohol than its *threo* isomer.

(14) G. Carrara and G. Weitnauer, *Gazz. chim. ital.*, **79**, 856 (1949).

WEST POINT, PA.

[CONTRIBUTION FROM THE SQUIBB INSTITUTE FOR MEDICAL RESEARCH]

Streptomycin. X.[1] The Structure of Mannosidostreptomycin[2]

BY JOSEF FRIED AND HOMER E. STAVELY

RECEIVED APRIL 24, 1952

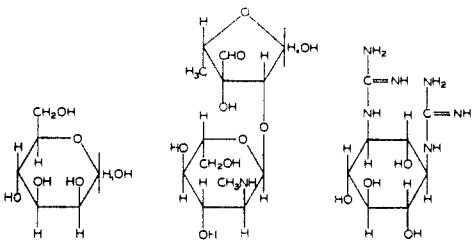
Evidence is presented to show that the glycosidic linkage between the D-mannose and N-methyl-L-glucosamine moieties in mannosidostreptomycin extends to position 4 of the latter and that the former most probably exists in the pyranoid form. Mannosidostreptomycin is therefore represented by formula IV. Proof for the site of attachment of the D-mannose to the N-methyl-L-glucosamine moiety rests on the degradation of fully methylated N-acetyldihydromannosidostreptomycin by acid hydrolysis followed by acetylation and chromatography to 2,4-diacetyl-(V) and 1,2,4-triacetyl-3,6-dimethyl-N-methyl-α-L-glucosamine (VI). Additional evidence is adduced by periodate oxidation of mannosido- and N-pentaacetyl-mannosidostreptomycin. The pyranose structure of the N-methyl-L-glucosamine moiety in streptomycin and mannosidostreptomycin is confirmed by degradation of fully methylated N-acetyldihydrostreptomycin to a trimethyl-N-methyl-L-glucosamine, which is shown to possess the pyranoid structure XI.

Mannosidostreptomycin has been shown by stepwise degradation[1] to be composed of D-mannose (I), streptobiosamine (II) and streptidine (III),[3] joined glycosidically in the order: D-mannose → N-methyl-L-glucosamine → L-streptose → streptidine. Moreover, it has been shown that the glycosidic linkage between the D-mannose and the N-methyl-L-glucosamine moieties, is of the α-type and involves one of the three hydroxyl groups of the

(1) Paper IX of this series: H. E. Stavely and J. Fried, THIS JOURNAL, **71**, 135 (1949).

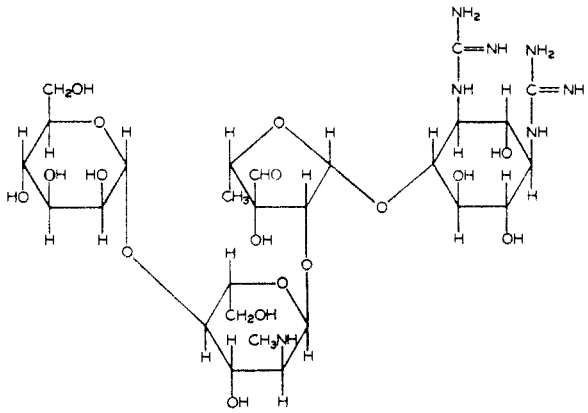
(2) Presented in part before the Division of Biological Chemistry of the American Chemical Society, Chicago, Ill., April, 1948.

(3) For a discussion of the structure of streptobiosamine and streptidine see the chapter "The Chemistry of Streptomycin" by R. V. Lemieux and M. L. Wolfrom in "Advances in Carbohydrate Chemistry," Vol. III, 1948, Academic Press Inc., New York, N. Y. Pertinent findings since the appearance of that review include the demonstration that streptose possesses the L-*lyxo*-configuration (F. A. Kuehl, Jr., M. N. Bishop, E. H. Flynn and K. Folkers, THIS JOURNAL, **70**, 2613 (1948); M. L. Wolfrom and C. W. DeWalt, *ibid.*, **70**, 3148 (1948)), that streptidine probably has the all-*trans*-configuration (M. L. Wolfrom, S. M. Olin and W. J. Polglase, *ibid.*, **72**, 1724 (1950); O. Wintersteiner and A. Klingsberg, *ibid.*, **70**, 885 (1948)) and that the anomeric structure of the L-streptose–streptidine linkage is of the β-type (M. L. Wolfrom, M. J. Cron and R. M. Husband, Abstr. of Papers 118th Meeting, Am. Chem. Soc., 7R, (1950)).



I II III

latter. More recently Peck, Hoffhine, Gale and Folkers[4] have demonstrated that the linkage between streptobiosamine and streptidine extends from carbon atom 1 of the L-streptose moiety to the hydroxyl group in position 4 of streptidine, as had previously been shown[5] to be the case with streptomycin. Mannosidostreptomycin is therefore, as is implied in the name, a D-mannoside of streptomycin, a conclusion which has received support by the finding of Perlman and Langlykke,[6] that this antibiotic is cleaved to streptomycin and D-mannose by the enzyme mannosidostreptomycinase. There remained to be determined the exact site of the α-glycosidic linkage joining D-mannose to the streptomycin molecule. The experimental evidence presented in this paper has led us to conclude that this linkage extends to carbon atom 4 of the N-methylglucosamine moiety, and that mannosidostreptomycin must therefore possess the structure represented by formula IV.



IV

In the course of adducing the evidence for that structure extensive use has been made of the N-acetyl derivatives of streptomycin and mannosidostreptomycin and of their dihydro products. A brief discussion of these derivatives is therefore in order. Acetylation of the nitrogenous groups occurred readily when the respective trihydrochlorides were treated with 3 equivalents of silver acetate and 6 equivalents of acetic anhydride in methanol at room temperature. The N-acetyl content of the resulting amorphous products consistently fell short of that calculated for the expected N-pentaacetates by an amount corresponding to 75–100% of one acetyl group. Undoubtedly these products do not constitute pure entities but represent mixtures containing mainly the pentaacetyl derivatives together with some of the partially or non-acetylated antibiotics. This was concluded from the behavior of N-acetylated streptomycin and mannosidostreptomycin in the Craig counter-current distribution system devised by Plaut and McCormack,[7] which employs aqueous stearate and pentasol as the immiscible phases. In contrast to streptomycin and mannosidostreptomycin, which in this system show peaks at tubes 21 and 9, respectively, the acetylated materials exhibited major maxima at tubes 8 and 1, respectively (Fig. 1), accounting for approximately 85% of the material and smaller peaks at the positions characteristic of the unacetylated antibiotics, accounting for the remaining 15%. It appears likely that the products giving rise to maxima at tubes 1 and 8 represent the N-pentaacetates of mannosidostreptomycin and streptomycin, respectively. The nature of the materials present in tubes 9 and 21 has not been further investigated. Treatment of the N-acetates of streptomycin and mannosidostreptomycin with alkali produced maltol in yields equivalent to those obtained with the free antibiotics, indicating that the labile streptose moiety had remained unchanged during acetylation. Since the conclusions arrived at in this paper would be valid even if only the methylamino group were acetylated, and this group has been shown to react readily with acetic anhydride in methanol[8] under the conditions used in this work, we felt justified in using the N-acetylation products without further purification.

Preliminary evidence regarding the site of attachment of D-mannose to the N-methylglucosamine moiety was obtained by periodate oxidation of mannosidostreptomycin and N-acetylmannosidostreptomycin. Whereas in the case of the latter the reaction came to a standstill after the consumption of 3.1 moles of the reagent the former consumed 5 atoms of oxygen within the first 2.5 hours and an additional atom during the ensuing 3.5 hours. In neither case was formaldehyde found among the oxidation products, indicating that the mannose moiety occurs in pyranoid form. As a reasonable interpretation of the above results it can be assumed that the action of periodate on N-acetylmannosidostreptomycin results in cleavage between carbon atoms 2, 3 and 4 of the mannose and between carbon atoms 3 and 6 of the streptose moieties, with a consumption of 3 moles of the reagent. The fact that the actual consumption amounted to approximately that value indicates that mannose must be attached to the N-acetylated N-methylglucosamine moiety in such a manner as to prevent attack by the reagent on the latter. This failure of the glucosamine moiety to be cleaved by periodate was confirmed by the isolation of its pentaacetyl derivative from the oxidation mixture after hydrolysis and acetylation. When a similar series of reactions was performed with mannosidostreptomycin itself no pentaacetyl-N-methyl-L-glucosamine could be isolated.

Providing that the N-methyl-L-glucosamine moi-

(4) R. L. Peck, C. E. Hoffhine, Jr., P. Gale and K. Folkers, THIS JOURNAL, **70**, 3968 (1948).

(5) R. L. Peck, F. A. Kuehl, C. E. Hoffhine, Jr., E. W. Peel and K. Folkers, *ibid.*, **70**, 2324 (1948).

(6) D. Perlman and A. F. Langlykke, *ibid.*, **70**, 3969 (1948).

(7) G. W. E. Plaut and R. B. McCormack, *ibid.*, **71**, 2264 (1949).

(8) F. A. Kuehl, Jr., E. H. Flynn, F. W. Holly, R. Mozingo and K. Folkers, *ibid.*, **69**, 3052 (1947).

ety in mannosidostreptomycin possesses pyranoid ring structure, an assumption which will be shown to be correct later in this paper, the above facts are compatible only with structure IV, in which D-mannose is joined to N-methylglucosamine *via* the 4-hydroxyl group of the latter. This formula explains both the failure to isolate N-methyl-L-glucosamine from periodate-oxidized mannosidostreptomycin, due to cleavage of the former between carbon atoms 2 and 3, and the ready isolation of that moiety after protection of the methylamino group by acetylation. If the attachment between D-mannose and N-methyl-L-glucosamine were effected through the 6-hydroxyl group of the latter, N-acetylmannosidostreptomycin would have suffered cleavage between carbon atoms 3 and 4 of the aminosugar moiety, which would have resulted in the consumption of 4 moles of periodate instead of the three actually observed, and no N-methyl-L-glucosamine could have been isolated from the oxidation mixture after acid hydrolysis. The only remaining alternative, attachment *via* the hydroxyl group in position 3, is likewise ruled out, since it would have required the isolation of N-methyl-L-glucosamine from unacetylated mannosidostreptomycin after oxidation and hydrolysis.

The above evidence, although strongly suggestive of structure IV for mannosidostreptomycin cannot be entirely relied upon in view of the possibility that certain intermediates formed in the periodate oxidation might by appropriate cyclization spare from further attack some otherwise periodate-labile grouping. The formation of such a cyclized product has been postulated[5] in order to explain the failure of the streptidine nucleus in streptomycin to be cleaved between carbon atoms 5 and 6.[9]

Conclusive proof for the correctness of structure IV was adduced by means of the classical method of methylation (Chart I). N-Acetyldihydromannosidostreptomycin was methylated with dimethyl sulfate and sodium hydroxide according to the excellent procedure of West and Holden.[10] A single methylation furnished a product, in which, to judge from its methoxyl content, the three primary and seven secondary hydroxyl groups of dihydromannosidostreptomycin were replaced by methoxyl. Presumably, the tertiary hydroxyl group attached to carbon atom 3 of the streptose moiety was not methylated under these conditions. The liberation of trimethylamine during the reaction and the basic character of the methylated product suggested that the acetylated guanido groups had been at least partially degraded to amino- and subsequently methylated to dimethylamino groups in the strongly alkaline methylation medium. Analytical figures indicate that this product (and also the methylation product of N-acetyldihydrostreptomycin to be described below) probably represents a mixture of the dimethylamino- and ureido-derivatives. The methylated product was boiled with 2.5 *N* hydrochloric acid for 6 hours and the resulting hydrolysis mixture was acetylated with pyridine–acetic anhydride. Chromatography of the acetylated material

(9) H. E. Carter, Y. H. Loo and P. S. Skell, *J. Biol. Chem.*, **168**, 401 (1947).

(10) E. S. West and R. F. Holden, THIS JOURNAL, **56**, 930 (1934).

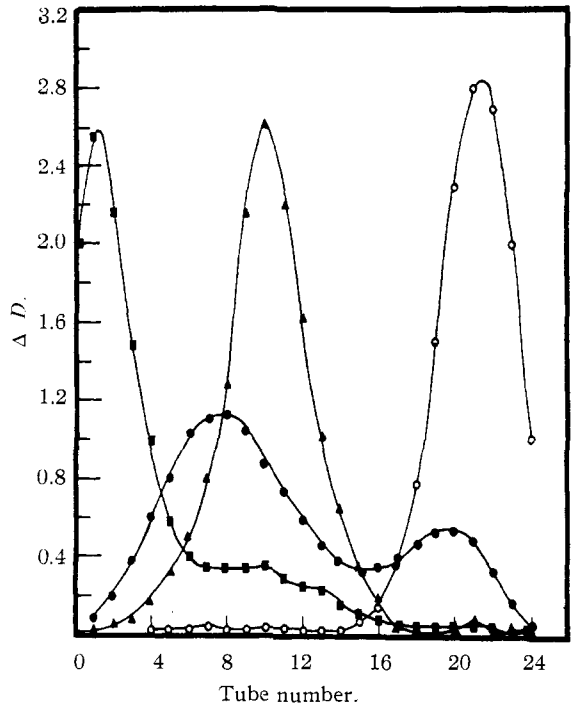


Fig. 1.—Countercurrent distribution curves: -O-O-O-, streptomycin; -●-●-●-, N-acetylated streptomycin; -▲-▲-▲-, mannosidostreptomycin; -■-■-■-, N-acetylated mannosidostreptomycin.

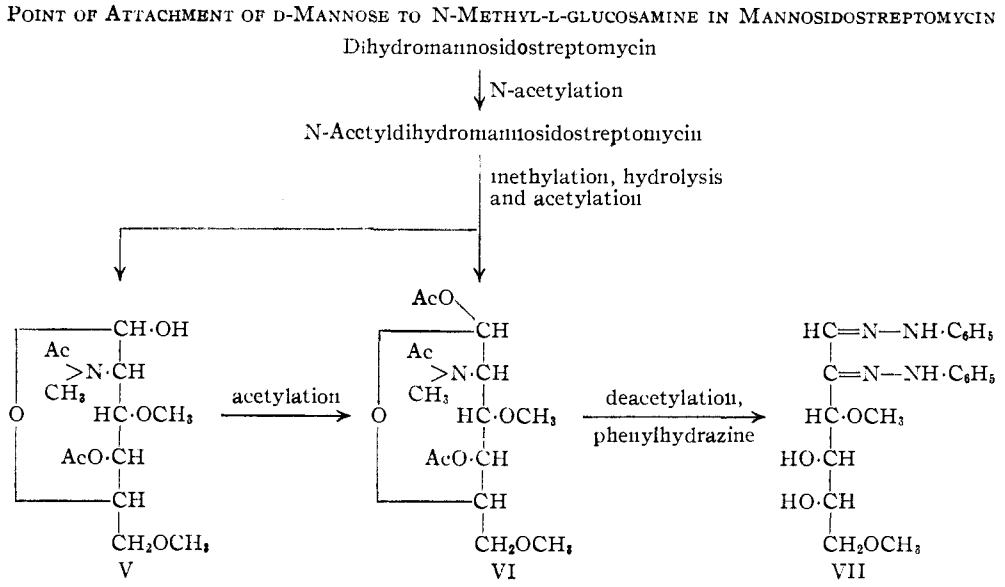
on acetic acid-washed alumina afforded in addition to a large amount of oily products two crystalline substances melting at 140 and 163°, respectively. The lower melting substance was also isolated when nonaacetyldihydromannosidostreptobiosamine (VIII),[1] prepared from dihydromannosidostreptomycin by partial hydrolysis and acetylation was methylated and the resulting methyl heptamethyl-N-acetyldihydromannosidostreptobiosaminide (IX) was subjected to hydrolysis followed by acetylation. Analytical data revealed the higher melting substance to be a diacetate (V) and the lower melting one to be a triacetate (VI) of a dimethyl-N-methylglucosamine. Acetylation of V, which exhibits strong mutarotation in water and therefore contains a free hemiacetalic hydroxyl group in position 1, yielded the non-mutarotating triacetate VI. The isolation of the incompletely acetylated derivative V is ascribed to partial saponification of VI during chromatography on acetic acid-washed alumina rather than to incomplete acetylation. A similar case involving facile deacetylation on acetic acid-washed alumina has recently been observed in this Laboratory in the conversion of the veratrine alkaloid germitrine to germerine.[11]

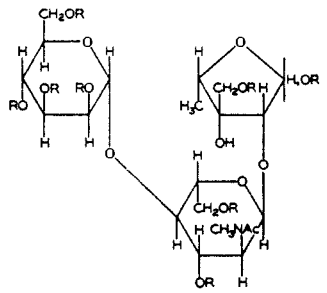
The assignment of structures V and VI to the two acetylated degradation products from methylated dihydromannosidostreptomycin is based on structure IV for mannosidostreptomycin, which up to this point rests on the preliminary evidence obtained by periodate oxidation of dihydromannosidostreptomycin and its N-acetyl derivative, and on the assumption that the N-methyl-L-glucosamine

(11) J. Fried, H. L. White and O. Wintersteiner, *ibid.*, **72**, 4621 (1950).

CHART I

POINT OF ATTACHMENT OF D-MANNOSE TO N-METHYL-L-GLUCOSAMINE IN MANNOSIDOSTREPTOMYCIN





VIII, R = CH_3CO; IX, R = CH_3

moiety in mannosidostreptomycin possesses pyranoid structure. Conversely, unambiguous evidence that formulas V and VI actually represent the structures of the two degradation products, and the demonstration that the N-methyl-L-glucosamine moiety in mannosidostreptomycin possesses the pyranoid structure would constitute proof for structure IV for mannosidostreptomycin. It was felt that such proof would be available, if it could be shown that periodate oxidation of the de-O-acetylation product of VI, which might be expected to exist in equilibrium with the open aldehydo-form, yielded methoxyacetaldehyde by cleavage between carbon atoms 4 and 5. Since only very little of this product was available for study the readily accessible 2,3,6-trimethylglucose was used as a model for this reaction. When the latter compound was treated with periodate in neutral or acidic medium no reaction was observed for a period of 24 hours. In alkaline medium 2 moles was taken up in a slow reaction without a sharp end-point but no methoxyacetaldehyde could be isolated from the reaction mixture. It may be assumed from this result that in neutral and acidic medium, 2,3,6-trimethylglucose exists practically all in the hemiacetalic form thus barring attack by periodate between carbon atoms 4 and 5.[12] By analogy one would expect the N-acetate of 3,6-dimethyl-N-methyl-L-glucosamine likewise to exist in the hemiacetalic form only and therefore to be resistant to periodate.

This approach having proved unpromising it was decided to synthesize the D-enantiomorphs of V and VI by an unambiguous route from D-glucose and to compare their melting points and optical rotation values with those of the two products obtained by hydrolysis and acetylation of methylated N-acetylmannosidostreptomycin. The details of this synthesis are described in paper XI of this series.[13] The results of the comparison are summarized in Table I, which also includes the melting points of the osazone VII prepared from VI after deacetylation, and of 3,6-dimethyl-D-glucosazone prepared from 3,6-dimethyl-D-glucose.[13] These data show beyond doubt that the two sets of derivatives represent three enantiomorphic pairs, and that therefore the products derived from mannosidostreptomycin actually possess formulas V, VI and VII, as was previously inferred from the results of the periodate oxidation. Particularly convincing are the mutarotation values for the 2,4-diacetyl-3,6-dimethyl-N-methyl-D- and L-glucosamines, which change from the same absolute initial values to numerically identical equilibrium values of opposite sign, and the melting point of the mixture of 3,6-Dimethyl-D- and L-glucosazones which is approximately 40° higher than that of the individual components, obviously due to the formation of a racemate.

The identification of the partially methylated aminosugar from mannosidostreptomycin as 3,6-dimethyl-N-methyl-L-glucosamine permits two interpretations with respect to the attachment of D-mannose to the N-methylglucosamine moiety, *viz.*, a pyranoid ring structure of the glucosamine moiety and attachment of D-mannose *via* the 4-hydroxyl group of the former or a furanoid structure with attachment through C5. In deciding between these two alternatives it must be remembered that identical streptobiosamine derivatives

(12) This finding was utilized in the synthesis of 2,5-dimethyl-D-arabinose from 3,6-dimethyl-D-glucose; *cf.* ref. 13.

(13) J. Fried and D. E. Walz, THIS JOURNAL, **74**, 5468 (1952).

TABLE I

COMPARISON OF PARTIALLY METHYLATED L-GLUCOSAMINE DERIVATIVES FROM MANNOSIDOSTREPTOMYCIN WITH THEIR SYNTHETIC D-ENANTIOMORPHS

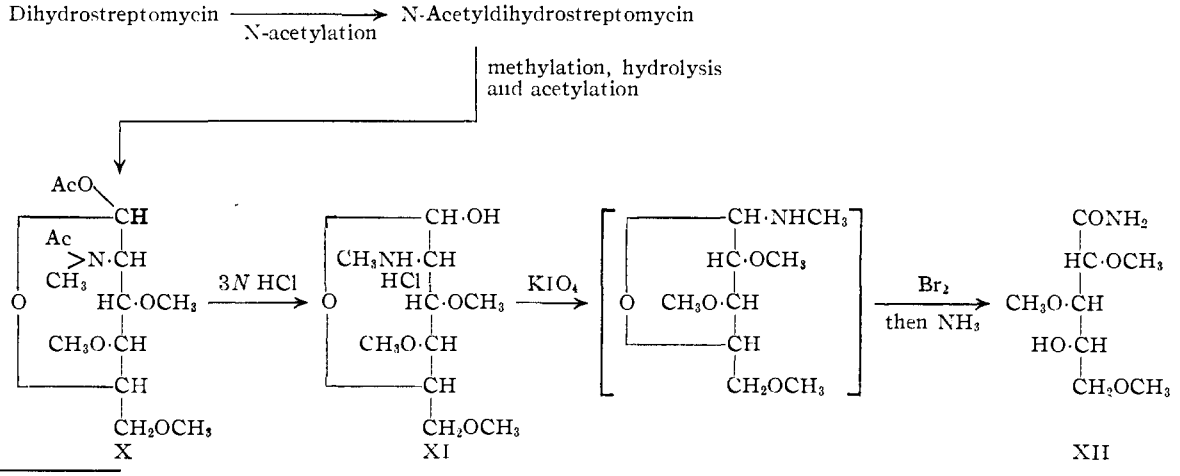
Substance	Source	M.p.	M.p. of mixture of enantiomorphs	$[\alpha]_D$
1,2,4-Triacetyl-3,6-dimethyl-N-methyl-D-glucosamine	D-Glucose	140–141	137.5–138	+110° ($CHCl_3$)
1,2,4-Triacetyl-3,6-dimethyl-N-methyl-L-glucosamine (VI)	Mannosidostreptomycin	139.5–140		−107 ($CHCl_3$)
2,4-Diacetyl-3,6-dimethyl-N-methyl-D-glucosamine	D-Glucose	162.5–163.5	143.5–144.5	+71 → +31 (H_2O)
2,4-Diacetyl-3,6-dimethyl-N-methyl-L-glucosamine (V)	Mannosidostreptomycin	162.5–163.5		−70 → −29 (H_2O)
3,6-Dimethyl-D-glucosazone	D-Glucose	115–116	155–158	−139 → −50[a]
3,6-Dimethyl-L-glucosazone (VII)	Mannosidostreptomycin	114.5–115.5		[b]

[a] Solvent: 2 parts pyridine + 3 parts absolute alcohol. [b] Insufficient amount of material for rotation.

have been obtained by degradation of both streptomycin[14] and mannosidostreptomycin.[15] Since the glycosidic linkage between N-methyl-L-glucosamine and streptose is not affected in any of the degradation reactions leading to derivatives of streptobiosamine, the ring size of the N-methyl-L-glucosamine moiety in streptobiosamine must be the same in both streptomycin and mannosidostreptomycin. Evidence for the pyranose structure of the aminosugar moiety in streptobiosamine has been adduced by Brink, Kuehl, Flynn and Folkers.[16] These workers found that no formaldehyde was formed in the reaction of N-acetylbisdesoxystreptobiosamine with sodium periodate, a finding which is consistent only with a pyranose structure for the N-methylglucosamine moiety. In view of the important bearing which the ring size of this moiety has on the position of attachment of the D-mannose moiety it was felt that direct degradative proof concerning this point was desirable. For this purpose dihydrostreptomycin was subjected to a series of reactions, outlined in Chart 2, similar to those described above for dihydromannosidostreptomycin. N-Acetyldihydrostreptomycin upon methylation according to West and Holden, followed by hydrolysis with 2.5 *N* hydrochloric acid yielded after acetylation and chromatography the crystalline diacetate of a trimethyl-N-methyl-α-L-glucosamine (X). The latter substance was also obtained when the crude product resulting from the action of methanolic hydrogen chloride on pentaacetyl-N-methyl-α- and β-L-glucosamine (methyl tetraacetyl-α- and β-L-glucosaminides) was subjected to the same series of reactions. X upon hydrolysis with 2.5 *N* hydrochloric acid yielded the crystalline hydrochloride of the parent trimethyl-N-methyl-L-glucosamine (XI). The position of the methoxy groups in this substance was ascertained by oxidation with 1 mole of potassium periodate, followed by oxidation with bromine water, which led to the known 2,3,5-trimethyl-L-arabonolactone. The latter was converted into the crystalline amide XII, which proved to be identical with an authentic sample of 2,3,5-trimethyl-L-arabonamide. This series of reactions fully confirms the finding of Brink, Kuehl, Flynn and Folkers and leaves no doubt that in streptomycin, and by the above reasoning also in mannosidostreptomycin, the N-methylglucosamine moiety possesses pyranoid structure. Only one mode of attachment of the mannose moiety to the N-methyl-L-glucosamine moiety is in accordance with all the experimental evidence adduced above, that is attachment *via* position 4 of the latter as pictured in formula IV.

CHART II

RING STRUCTURE OF THE N-METHYLGLUCOSAMINE MOIETY IN STREPTOMYCIN AND MANNOSIDOSTREPTOMYCIN



(14) N. G. Brink, F. A. Kuehl, Jr., E. H. Flynn and K. Folkers, THIS JOURNAL, **68**, 2551 (1946).

(15) J. Fried and H. E. Stavely, *ibid.*, **69**, 1549 (1947).

(16) N. G. Brink, F. A. Kuehl, Jr., E. H. Flynn and K. Folkers, *ibid.*, **68**, 2405 (1946).

Experimental[17]

N-Acetylmannosidostreptomycin.—Mannosidostreptomycin trihydrochloride (9.26 g.) and silver acetate (5.87 g.) were suspended in dry methanol (80 ml.) containing acetic anhydride (9.26 ml.). The mixture was shaken on the machine for 4 hours, filtered and the filtrate evaporated to dryness *in vacuo*. The residue was taken up in water (100 ml.), the resulting solution cleared with charcoal and the filtrate lyophilized. The yield was 10.83 g. The material showed $\Delta E^{1\%}_{1\,cm.}$ 70 in the maltol test.[18]

The low ΔE value for this sample is due to the absence after boiling with alkali of the by-product showing a maximum at 330 mμ (curve C in ref. 18). The actual maltol yield thus very closely approximates that obtained from mannosidostreptomycin itself. The countercurrent distribution curve for this material is shown in Fig. 1.

Anal. Calcd. for $C_{27}H_{44}N_7O_{17}(CH_3CO)_5$: acetyl, 22.7. Found: total acetyl, 17.8.

N-Acetylstreptomycin.—Streptomycin trihydrochloride (3.0 g.) was N-acetylated for 4 hours in dry methanol (30 ml.) with acetic anhydride (2.40 ml.) in the presence of silver acetate (2.17 g.). The mixture was worked up as in the preceding experiment. The acetylated product (2.9 g.) showed ΔE 70 in the maltol test. Its countercurrent distribution curve is shown in Fig. 1. Two different preparations were analyzed after drying at 100° *in vacuo*.

Anal. Calcd. for $C_{21}H_{34}N_7O_{12}(CH_3CO)_5$: acetyl, 27.2. Found: total acetyl, 22.8, 23.4.

N-Acetyldihydromannosidostreptomycin. — Dihydromannosidostreptomycin trihydrochloride (9.26 g.) was N-acetylated for 4 hours in dry methanol (80 ml.) with acetic anhydride (9.26 ml.) in the presence of silver acetate (5.87 g.). The reaction mixture was worked up as described above. The yield was 10.83 g.

Anal. Calcd. for $C_{27}H_{46}N_7O_{17}(CH_3CO)_5$: acetyl, 22.5. Found: total acetyl, 18.3.

N-Acetyldihydrostreptomycin.—This substance was prepared exactly as described for N-acetylstreptomycin.

Anal. Calcd. for $C_{21}H_{36}N_7O_{12}(CH_3CO)_5$: acetyl, 27.1. Found: total acetyl, 23.7.

Titration of Mannosidostreptomycin and N-Acetylmannosidostreptomycin with Periodate.—The accurately weighed samples (20–25 mg.) were dissolved in 0.013 *M* potassium periodate (10 ml.) and 1-ml. aliquots titrated with 0.01 *N* sodium thiosulfate at the time intervals listed, according to the procedure of Rappaport, Reifer and Weinmann.[19] The results are expressed in terms of moles of periodate consumed per mole of substance:

	Time (minutes)						
	5	15	20	40	95	130	145
Mannosidostreptomycin	2.1	3.2		3.9	4.6		4.9
N-Acetylmannosidostreptomycin	1.4		2.2	2.8		3.1	

Isolation of Pentaacetyl-N-methyl-L-glucosamine from Periodate Oxidized N-Acetylmannosidostreptomycin.—A solution of N-acetylmannosidostreptomycin (747 mg.) and periodic acid (600 mg., 3.2 mole equivalents) in water (150 ml.) was allowed to stand at room temperature. At the end of 18 hours all the periodic acid had been consumed and the light yellow mixture was neutralized with barium carbonate and lyophilized. The residue was extracted with two 40-ml. portions of dry methanol by shaking for periods of five hours each. The methanol extracts were evaporated to dryness *in vacuo* and the residue was hydrolyzed with 1 *N* sulfuric acid (4 ml.) for 15 hours at 45°. The mixture was then placed in the refrigerator for several hours and the resulting precipitate of streptidine sulfate was filtered off and washed with a small amount of water. To the filtrate was added concentrated hydrochloric acid (4 ml.) and the solution was refluxed for 10 hours. The hydrolysate was diluted with water (10 ml.), decolorized with charcoal and the sulfate ion removed by precipitation with barium hydroxide. The sulfate-free solution was concentrated to dryness *in vacuo* and the residue was acetylated with pyridine (5 ml.)–acetic anhydride (5 ml.). The acetylated product was chromatographed on alumina (4 g.) from benzene (6 ml.)–hexane (2 ml.) and the column was eluted with the same solvent mixture (300 ml.). Crystals were obtained, which after three recrystallizations from ethyl acetate–ether melted at 157–158°. The melting point of this substance was not depressed on admixture of an authentic sample of pentaacetyl-N-methyl-α-L-glucosamine, which has been reported to melt at 160.5–161.5° (micro-block).[8]

Oxidation of Mannosidostreptomycin with Periodic Acid.—A solution of mannosidostreptomycin hydrochloride (760 mg.) and periodic acid (1.28 g., 6.1 mole equivalents) in water (250 ml.) was allowed to stand at room temperature for 15 hours. The oxidation mixture was worked up exactly as described in the previous experiment and the resulting acetylation product was chromatographed on alumina (4 g.) from benzene (6 ml.)–hexane (2 ml.). No material could be eluted from the column with benzene–hexane or benzene. Benzene–ether eluted oily products, which did not crystallize when seeded with pentaacetyl-N-methyl-α-L-glucosamine.

Estimation of Formaldehyde Resulting from Periodate Oxidation of Mannosidostreptomycin and Dihydromannosidostreptomycin.—Mannosidostreptomycin trihydrochloride (74 mg.) was oxidized with periodate according to the procedure of Reeves.[20] The amounts of reagents were those employed by that author in the oxidation of 18 mg. of glucose. There was no precipitation upon addition of dimedon. Dihydromannosidostreptomycin trihydrochloride (70 mg.) under identical conditions yielded 10.8 mg. of the dimedon derivative of formaldehyde, m.p. 188–189°; no depression when mixed with an authentic sample. This amount represents 0.037 millimole of formaldehyde or 0.45 mole per mole of dihydromannosidostreptomycin.

Methylation of N-Acetyldihydromannosidostreptomycin.—To a vigorously stirred solution of N-acetyldihydromannosidostreptomycin (4.2 g.) in water (8 ml.) was added a mixture of dimethyl sulfate (15 ml.) and carbon tetrachloride (21 ml.). During this and the subsequent addition of alkali the temperature of the bath in which the reaction vessel was immersed was kept at 55°. Sixty per cent. alkali (66 ml.) was then added at a rate of one drop every two seconds for the first 5 min., one drop per second for the next 5 minutes and finally 3 drops per second until all the alkali had been added. The bath temperature was then raised slowly to 70–75° and dimethyl sulfate (27 ml.) was added at the rate of 3–4 drops per second. After this addition the water-bath was heated to boiling and kept at that temperature for 30 min. The temperature was then lowered to 50° and the mixture was stirred vigorously with chloroform (100 ml.) for 15 min. The mixture was filtered and the sodium sulfate precipitate was washed with chloroform (40 ml.). The washed sodium sulfate and the aqueous portion of the filtrate were combined and re-extracted twice more with chloroform as described above. The resulting chloroform extracts were combined, washed with small portions of water and dried with sodium sulfate. Evaporation of the solvent *in vacuo* left an amber gum (4.26 g.) soluble in both chloroform and water. A sample was dried *in vacuo* at 100°.

Anal. Calcd. for $C_{25}H_{32}NO_7(CH_3CO)(OCH_3)_{10}(N(CH_3)_2)_2$: OCH_3, 34.5; N, 4.67; N-acetyl, 4.78. Calcd. for $C_{25}H_{32}NO_7(CH_3CO)(OCH_3)_{10}(NHCONH_2)_2$: OCH_3, 33.3; N, 7.53; N-acetyl, 4.62. Found: OCH_3, 33.6; N, 6.72; total acetyl, 4.05.

Degradation of Methylated N-Acetyldihydromannosidostreptomycin to 3,6-Dimethyl-N-methyl-L-glucosamine.—Methylated N-acetyldihydromannosidostreptomycin (2.118 g.) was refluxed with 2.5 *N* hydrochloric acid (32 ml.) for 6 hours. The brown solution was decolorized with Darco G60 and the clear filtrate was evaporated to dryness *in vacuo*. The dry residue was acetylated with acetic anhydride (15 ml.)–pyridine (15 ml.) at room temperature for 16 hours. The acetylation mixture was concentrated to a sirup *in vacuo*, the sirup distributed between ice-water and chloroform and the chloroform solution washed with dilute hydrochloric acid, sodium bicarbonate and water. After drying with sodium sulfate the solvent was removed *in vacuo* and the residue was dissolved in benzene (16 ml.) and hexane (4 ml.). This solution was chromatographed on acetic

(17) The melting points reported in this paper were taken in capillary tubes and are corrected for stem exposure.

(18) J. Fried and E. Titus, THIS JOURNAL, **70**, 3615 (1948).

(19) F. Rappaport, J. Reifer and H. Weinmann, *Mikrochim. Acta*, 240 (1937).

(20) R. E. Reeves, THIS JOURNAL, **63**, 1476 (1941).

acid-washed alumina (30 g.). A mixture of benzene (8 parts) and hexane (2 parts) eluted at first a good deal of oily material, which was followed by a crystalline fraction. Three crystallizations of this fraction from ethyl acetate–hexane yielded the pure substance, which melted at 139.5–140° and had $[\alpha]^{25}D$ −107° (*c* 0.98 in chloroform).

Anal. Calcd. for $C_7H_{10}O_3N(COCH_3)_3(OCH_3)_2$: C, 51.86; H, 7.26; N, 4.03; OCH_3, 17.9. Found: C, 52.12; H, 7.67; N, 3.79; OCH_3, 16.4.

The above product was shown to be 1,2,4-triacetyl-3,6-dimethyl-N-methyl-α-L-glucosamine (VI) by comparison with its optical antipode,[13] which melted at 140–141° and had $[\alpha]^{25}D$ +110° (*c* 0.98 in chloroform). The product obtained by recrystallization of equal amounts of the D- and L-forms from ethyl acetate–hexane melted at 137.5–138° and had $[\alpha]^{25}D$ +2.6° (*c* 1.1 in chloroform).

Continued elution of the column with benzene yielded only non-crystalline products. A second band containing crystalline material was obtained when a mixture of equal volumes of benzene and U.S.P. ether was used as the eluting agent. To separate the crystals from oily by-products the eluates were evaporated to dryness, the residues taken up in a small amount of ethyl acetate and treated with hexane to incipient turbidity. The resulting crystals were sparingly soluble in ethyl acetate and were obtained by recrystallization from that solvent as fine long needles, which melted with decomposition at 162.5–163.5° and had $[\alpha]^{25}D$ −79° (*c* 0.76 in chloroform). The substance mutarotates rapidly in water from an initial value of $[\alpha]^{25}D$ −70° (*c* 1.04) to a constant value of $[\alpha]^{25}D$ −29° at the end of one hour.

Anal. Calcd. for $C_7H_{11}O_3N(COCH_3)_2(OCH_3)_2$: C, 51.10; H, 7.54; N, 4.59; OCH_3, 20.3; O-acetyl, 14.1; total acetyl, 28.2. Found: C, 51.50; H, 7.86; N, 4.52; OCH_3, 20.5; O-acetyl, 19.0[21]; total acetyl, 31.5.

The substance was identified as 2,4-diacetyl-3,6-dimethyl-N-methyl-α-L-glucosamine (V) by comparison with synthetic 2,4-diacetyl-3,6-dimethyl-N-methyl-α-D-glucosamine which melts at 162.5–163.5° and mutarotates within an hour from an initial value of $[\alpha]^{25}D$ +71° to a constant value of +31°.[13] The mixture of the two optical antipodes melted at 143.5–144.5°.

Acetylation of the above 2,4-diacetyl-3,6-dimethyl-N-methyl-α-L-glucosamine (18 mg.) with pyridine (0.3 ml.)–acetic anhydride (0.3 ml.) yielded the triacetyl derivative melting at 139.5–140° and $[\alpha]^{25}D$ −102° (*c* 0.615 in chloroform).

Preparation of 3,6-Dimethyl-L-glucosazone (VII) from 1,2,4-Triacetyl-3,6-dimethyl-N-methyl-α-L-glucosamine.—1,2,4-Triacetyl-3,6-dimethyl-N-methyl-α-L-glucosamine (100 mg.) was refluxed for 2.5 hours with a mixture of concentrated hydrochloric acid (1 ml.) and water (2.8 ml.). The hydrolysate was decolorized with Darco G60 and the filtrate was evaporated to dryness. The amorphous residue (80 mg.) consisting of the hydrochloride of 3,6-dimethyl-N-methyl-L-glucosamine was dissolved in water (2 ml.) and the solution neutralized with solid sodium acetate. Glacial acetic acid (0.04 ml.) and freshly distilled phenylhydrazine (0.26 ml.) were then added and the mixture was refluxed for 6 hours with a stream of carbon dioxide passing through the solution. The reaction mixture was then extracted with three 10-ml. portions of benzene and the dried benzene extracts were passed through an alumina column containing 4 g. of acetic acid-washed alumina. Dark colored impurities were removed from the column by elution with benzene (60 ml.) and with a mixture of 3 parts of benzene and 1 part of hexane (90 ml.). Subsequent elutions with benzene containing 3% of 95% ethanol (80 ml.) and with benzene containing 6% of alcohol (120 ml.) yielded a yellow oil which crystallized upon seeding with a trace of synthetic 3,6-dimethyl-D-glucosazone.[13] Three recrystallizations from benzene yielded material (2 mg.) melting at 114.5–115.5°. 3,6-Diimethyl-D-glucosazone prepared from 3,6-dimethyl-D-glucose melted at 115–116°.[13] A mixture of equal amounts of the D- and L-forms melted at 155–158°.

Methylation of Nonaacetyldihydromannosidostreptobiosamine (VIII).—Nonaacetyldihydromannosidostreptobiosamine[1] (2.5 g.) was dissolved in water (10 ml.) and placed in a water-bath maintained at 55°. Vigorous stirring was applied throughout the reaction. A mixture of dimethyl sulfate (9 ml.) and carbon tetrachloride (15 ml.) was added quickly, and then 60% sodium hydroxide (40 ml.) was added dropwise over a 20-minute period. The bath temperature was raised rapidly to 75° and after the last of the carbon tetrachloride had distilled out of the flask a further quantity of dimethyl sulfate (16 ml.) was added at a rate of about three drops per second. After the addition was complete the water-bath was heated to boiling for 30 minutes. Extraction with warm chloroform yielded 1.61 g. of crude product. It was dissolved in benzene (25 ml.) and chromatographed on a column of acetic acid-washed alumina 2.5 × 23 cm. Elution with benzene (150 ml.) yielded only traces of material. Elution with 100-ml. portions of ether, ethyl acetate and 5% methanol in ethyl acetate eluted 275, 160 and 830 mg. of material, respectively, all having specific rotations of −43 ± 2°. They were combined and lyophilized from benzene solution to an amorphous powder, $[\alpha]^{25}D$ −44° (*c* 0.88 in chloroform).

Anal. Calcd. for $C_{19}H_{26}O_8N(COCH_3)(OCH_3)_8$: N, 2.14; OCH_3, 37.9. Found: N, 2.48; OCH_3, 36.7.

Hydrolytic Cleavage of Methyl Heptamethyl-N-acetyldihydromannosidostreptobiosaminide (IX) to 3,6-Dimethyl-N-methyl-L-glucosamine.—Methyl heptamethyl-N-acetyldihydromannosidostreptobiosaminide (345 mg.) was suspended in 3 *N* hydrochloric acid (20 ml.) and heated on the steam-bath for 6 hours. The reaction mixture was evaporated to dryness *in vacuo* and the residue was acetylated with acetic anhydride (5 ml.)–pyridine (5 ml.) for 17 hours. The crude acetylation product was dissolved in warm benzene and chromatographed on an acetic acid-washed alumina column 1 × 15 cm. Benzene eluted an oil (107 mg.) which would not crystallize and probably contained 1-acetyl-2,3,4,6-tetramethyl-D-mannose. Elution with a mixture of 3 parts of ether and 7 parts of benzene yielded crude crystals (69 mg.) which after two recrystallizations from ether melted at 140–141°, $[\alpha]^{25}D$ −106° (*c* 0.89 in chloroform).

Anal. Calcd. for $C_7H_{10}O_3N(COCH_3)_3(OCH_3)_2$: C, 51.86; H, 7.26; N, 4.03; O-acetyl, 24.8; OCH_3, 17.9. Found: C, 52.17; H, 7.44; N, 3.95; O-acetyl, 32.0[21]; OCH_3, 19.2.

The identity of the above product with that obtained by degradation of methylated N-acetyldihydromannosidostreptomycin is apparent from a comparison of their melting points and specific rotations. A mixture of the two products showed no depression in melting point.

Methylation of N-Acetyldihydrostreptomycin.—N-Acetyldihydrostreptomycin (2.09 g.) was methylated as outlined above for N-acetyldihydromannosidostreptomycin. 2.114 g. of amorphous material was obtained from the chloroform extract. It was analyzed after drying *in vacuo* at 100°.

Anal. Calcd. for $C_{19}H_{25}NO_5(OCH_3)_7(CH_3CO)(NHCONH_2)_2$: N, 9.64; OCH_3, 29.9. Calcd. for $C_{19}H_{25}NO_5(OCH_3)_7(CH_3CO)(N(CH_3)_2)_2$: N, 5.93; OCH_3, 30.6. Found: N, 7.90; OCH_3, 30.6.

1,2-Diacetyl-3,4,6-trimethyl-N-methyl-α-L-glucosamine (X) from Methylated N-Acetyldihydrostreptomycin.—The above methylated N-acetyldihydrostreptomycin (2 g.) was refluxed with 3 *N* hydrochloric acid (30 ml.) for six hours. The solution was decolorized with Darco G60, filtered and evaporated to dryness *in vacuo*. The residue was acetylated with pyridine (14 ml.)–acetic anhydride (14 ml.) and the acetylated product was chromatographed from benzene solution (20 ml.) on acetic acid-washed alumina (40 g.). Benzene eluted crystalline material, which after recrystallization from ethyl acetate–hexane (40 mg.) melted at 118–119° and had $[\alpha]^{25}D$ −142° (*c* 0.42 in chloroform).

Anal. Calcd. for $C_7H_{10}O_2N(COCH_3)_2(OCH_3)_3$: C, 52.65; H, 7.89; N, 4.39; O-acetyl, 13.5; OCH_3, 29.2. Found: C, 52.60; H, 8.10; N, 4.45; O-acetyl, 14.7; OCH_3, 28.3.

3,4,6-Trimethyl-N-methyl-L-glucosamine Hydrochloride (XI).—1,2-Diacetyl-3,4,6-trimethyl-N-methyl-α-L-glucosamine (X) (100 mg.) was refluxed with 3 *N* hydrochloric acid (3.7 ml.) for 2.5 hours. The solution was decolorized with Darco G60 and evaporated to dryness *in vacuo*. Two recrystallizations of the semi-crystalline residue from alcohol–ethyl acetate yielded the pure material (60 mg.) as large prisms which began charring at 160° and were decomposed completely at 210°, $[\alpha]^{24}D$ −108° (*c* 0.52 in water) (no mutarotation).

(21) The high value found with this substance in the O-acetyl determination of Kunz and Hudson has been observed with other derivatives of N-methylglucosamine in which the hemiacetalic hydroxyl group is either free or substituted by an alkali-labile group.

Anal. Calcd. for $C_7H_{12}O_2N(OCH_3)_3 \cdot HCl$: C, 44.19; H, 8.15; Cl, 13.05; OCH_3, 34.2. Found: C, 44.21; H, 7.93; Cl, 13.06; OCH_3, 34.5.

Preparation of 3,4,6-Trimethyl-N-methyl-L-glucosamine Hydrochloride (XI) from a Mixture of Pentaacetyl-N-methyl-α- and β-L-glucosamine.—A mixture of α- and β-pentaacetyl-N-methyl-L-glucosamine was prepared by acetolysis of crude methyl pentaacetyldihydrostreptobiosaminide according to the procedure of Stavely, *et al.*,[22] with the exception that the amount of acetolysis mixture was reduced to 25 ml. per gram of material. 2.32 g. of methyl dihydrostreptobiosaminide yielded 2.67 g. of crystalline mixed pentaacetates. This product (2.67 g.) was converted into a mixture of the anomeric methyl N-acetyl-N-methylglucosaminides by refluxing with 2% methyl alcoholic hydrogen chloride (65 ml.) for two hours. After removal of the hydrogen chloride by means of silver carbonate and of excess silver ion by hydrogen sulfide the methanol solution was evaporated to dryness. The crude residue (1.6 g.) was methylated in 3.2 ml. of water as described above, with a total of 14.4 ml. of carbon tetrachloride, 21.2 ml. of dimethyl sulfate and 26.7 ml. of 60% sodium hydroxide. The methylated product (1.33 g.) was removed by extraction with chloroform and was hydrolyzed with 3 *N* hydrochloric acid (19 ml.) for 2.5 hours. Evaporation of the hydrochloric acid after treatment with charcoal yielded a semi-crystalline residue, which upon recrystallization from alcohol–ethyl acetate yielded 3,4,6-trimethyl-N-methyl-L-glucosamine hydrochloride (XI) (519 mg.) in analytically pure form. This product as well as the α-diacetate of m.p. 118–119° prepared from it were identical with the products obtained from methylated N-acetyldihydrostreptomycin.

Degradation of 3,4,6-Trimethyl-N-methyl-L-glucosamine Hydrochloride (XI) to 2,3,5-Trimethyl-L-arabonamide (XII).—In a preliminary experiment 3,4,6-trimethyl-N-methyl-L-glucosamine hydrochloride (6.014 mg., 0.0221 millimole) was dissolved in potassium periodate solution and the utilization of periodate was followed iodometrically.[17] At the end of 18 hours 0.0216 millimole of periodate had been consumed, that is 0.98 mole of periodate per mole of substrate. In a subsequent preparative experiment 3,4,6-trimethyl-N-methyl-L-glucosamine hydrochloride (400 mg.) and finely powdered potassium periodate (339 mg.) were shaken with water (20 ml.) for 18 hours at room temperature. The undissolved potassium periodate (76 mg.) was filtered off and the aqueous filtrate was evaporated to dryness *in vacuo*. The residue was extracted with ethyl acetate and the filtered extract was evaporated to dryness *in vacuo*. A light yellow, low melting crystalline solid (150 mg.) remained, which was oxidized without further purification with bromine (0.3 ml.) in water (3 ml.) for 16 hours at room temperature. The excess bromine was removed by aeration under reduced pressure and the bromide ion was precipitated with silver carbonate. The resulting aqueous solution was carefully evaporated to dryness *in vacuo* so as not to lose the volatile 2,3,5-trimethylarabonolactone. The sirupy residue was transferred to a microdistillation apparatus and the product distilled at a bath temperature of 100–110° and a vacuum of 20 mm. The distillate was transformed into the amide by treating it for 15 hours at room temperature with methanol saturated with ammonia at −15°. Removal of the solvent *in vacuo* left behind a crystalline residue, which was recrystallized twice from acetone. The product (25.9 mg.) melted at 137° and had $[\alpha]^{25}D$ +17.4 (*c* 1.3 in water).

Anal. Calcd. for $C_8H_{17}O_5N$: C, 46.37; H, 8.35; N, 6.76; OCH_3, 44.9. Found: C, 46.56; H, 8.22; N, 6.91; OCH_3, 44.8.

2,3,5-Trimethyl-L-arabonamide has been reported[23] to melt at 132° and to have $[\alpha]D$ +15.8° (*c* 0.75 in water). An authentic sample of 2,3,5-trimethyl-L-arabonamide prepared from methyl L-arabofuranoside[24] by the procedure of Humphreys, Pryde and Waters[23] melted at 137.5–138° and had $[\alpha]^{23}D$ +16.9 (*c* 2.0 in water). A mixture of this material with that obtained by degradation of 3,4,6-trimethyl-N-methyl-L-glucosamine showed no depression in melting point. A mixture of the latter material with 2,3,5-trimethyl-D-arabonamide[13] began to sinter at 133–134° then resolidified and melted at 148.5–149.5°, the melting point characteristic for the D,L-form.[13]

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[CONTRIBUTION FROM THE SQUIBB INSTITUTE FOR MEDICAL RESEARCH]

Streptomycin. XI.[1] Synthesis of 3,6-Dimethyl-N-methyl-D-glucosamine[2]

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The synthesis of 1,2,4-triacetyl-(VIII) and 2,4-diacetyl-3,6-dimethyl-N-methyl-D-glucosamine (IX) is described. 3,6-Dimethyl-D-glucose (II) was degraded by means of lead tetraacetate to produce in excellent yield 2,5-dimethyl-D-arabinose (III). The latter was converted by a cyanhydrin synthesis in the presence of methylamine into a mixture of 3,6-dimethyl-N-methyl-D-glucosaminic acid (V) and what appears to be the corresponding mannosaminic acid. The former after reduction with sodium amalgam followed by acetylation yielded the triacetate VIII. Chromatography of VIII on acetic acid-washed alumina produced the diacetate IX. 3,4,6-Trimethyl-N-methyl-D-glucosaminic acid (VI) prepared from 2,3,5-trimethyl-D-arabinose could not be reduced with sodium amalgam.

In the preceding paper we have described the degradation of methylated N-acetyldihydromannosidostreptomycin to the di- and triacetates of a O-dimethyl-N-methyl-L-glucosamine and the identification of the latter two substances as 2,4-diacetyl- and 1,2,4-triacetyl-3,6-dimethyl-N-methyl-L-glucosamine by comparison with the corresponding D-enantiomorphs prepared by synthesis. The present paper records this synthesis starting from 3,6-dimethyl-D-glucose.

The preparation of 3,6-dimethylglucose has been described by Bell.[3] Starting with 1,2-monoacetone-3-methyl-D-glucose[4] this author effected introduction of the methyl group in position 6 by treat-

(1) Paper X of this series: J. Fried and H. E. Stavely, THIS JOURNAL, **74**, 5461 (1952).

(2) Presented in part before the Division of Biological Chemistry of the American Chemical Society in Chicago, Ill., April, 1948.

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