Journal of Medicinal and Pharmaceutical Chemistry

VOL. 2, No. 2 (1960)

Antituberculosis Agents—V.[1] Streptidine-β-Hexosides and Related Compounds

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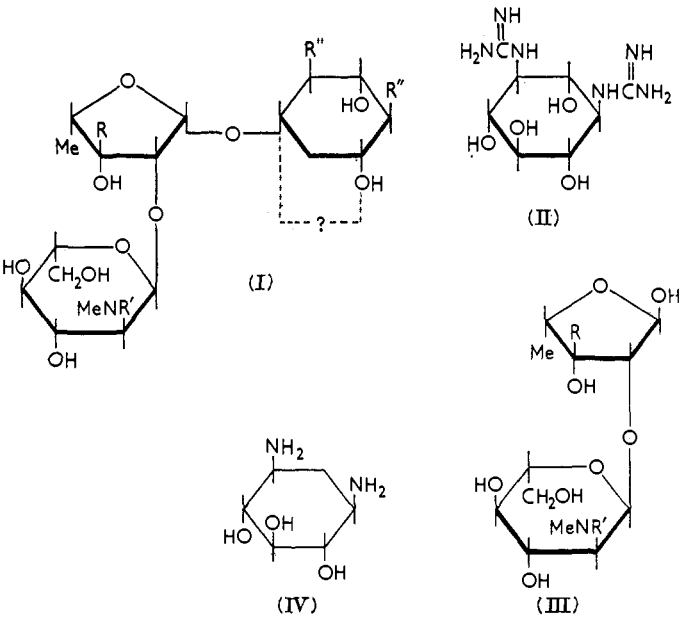
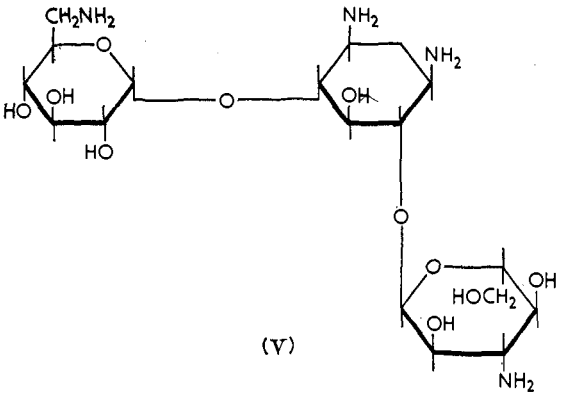
In Part IV[1] we reviewed some aspects of the chemotherapy of tuberculosis with streptomycin derivatives and described the preparation of *N*-acetyltrityldihydrostreptomycin (I; R = CH_2OCPh_3, R′ = Ac, R″ = $NHC(:NH)NH_2$). The latter showed only a low level of tuberculostatic activity compared with streptomycin (I; R = CHO, R′ = H, R″ = $NHC(:NH)NH_2$), dihydrostreptomycin (I; R = CH_2OH, R′ = H, R″ = $NHC(:NH)NH_2$) or *N*-acetyl derivatives. This supports the view that dihydrostreptomycin depends for its activity upon microbiological



oxidation to streptomycin,[2] and that the streptose–aldehydic group of the latter is essential. The role of the strongly basic guanidino groups has also been investigated with a range of Gram-positive and Gram-negative organisms, but not with mycobacteria. Thus, Abraham and Duthie[3] examined the influence of pH (of the test medium) upon the antibacterial activity of streptomycin, and observed that its activity decreased with increasing acidity of the media. From this they concluded that the antibiotic functions in its cationic form, competing with hydrogen ions for the same sites on the bacterial cells. On the other hand, both basic degradation products of streptomycin, streptidine (II) and streptobiosamine (III; R = CHO), are reported to be biologically inactive either individually[4, 5] or in admixture.[6] But since streptobiosamine (III; R = CHO), the *N*-methyl-L-glucosaminide of streptose, incorporates the all-important aldehydic group of streptomycin, its inactivity can only be attributed to the lack of the strongly basic streptidine unit.

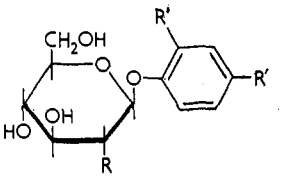
The view has been previously expressed by one of us[7] that the streptobiosamine fragment of streptomycin is merely responsible for concentrating, and possibly orientating, streptidine at the required site of action—a hypothesis which is supported by the evidence that aminohexoses, possibly D-glucosamine, are constituent units of both the lipoid-bound and somatic polysaccharides of *Mycobacterium tuberculosis*.[8] These ideas also appear to be underlined by the structures of the antituberculous antibiotics neomycin and kanamycin, which are derivatives of deoxystreptamine (IV), kanamycin being the bis-glucosaminide (V). Wolfrom and Polglase,[9] however, described the preparation of a crude sample of streptamine β-L-dihydrostreptobiosaminide (I; R = CH_2OH, R′ = H, R″ = NH_2), contaminated with barium chloride, which was said to be inactive. Since the commencement of this work a further communication on the same subject by Bodanszky[10] has become available to us[11] in which both strepturea β-L-dihydrostreptobiosaminide (I; R = CH_2OH, R′ = H, R″ = $NHCONH_2$) and streptamine β-L-dihydrostreptobiosaminide are shown to be inactive. We have also prepared these compounds independently of Bodanszky by the controlled hydrolysis of dihydrostreptomycin with barium hydroxide in a series of experiments to establish conditions for the degradation of the streptidine

glycosides described below, and to confirm the inactivity of the compounds against *M. tuberculosis*.



(V)

The amino- and guanidino-phenyl-β-D-glucosides (VI; R = OH, R′ = NH_2) and (VI; R = OH, R′ = $NHC(:NH)NH_2$)[12] and the amino- and guanidino-phenyl-β-D-glucosaminides (VI; R = NH_2, R′ = NH_2) and (VI; R = NH_2, R′ = $NHC(:NH)NH_2$),[13] have been synthesised, and found to be ineffective *in vitro* against *M. tuberculosis*, but the corresponding streptidine- and streptamine-β-glucosides and -β-glucosaminides have not been examined. We now report the preparation of a number of streptomycin analogues of the latter type.

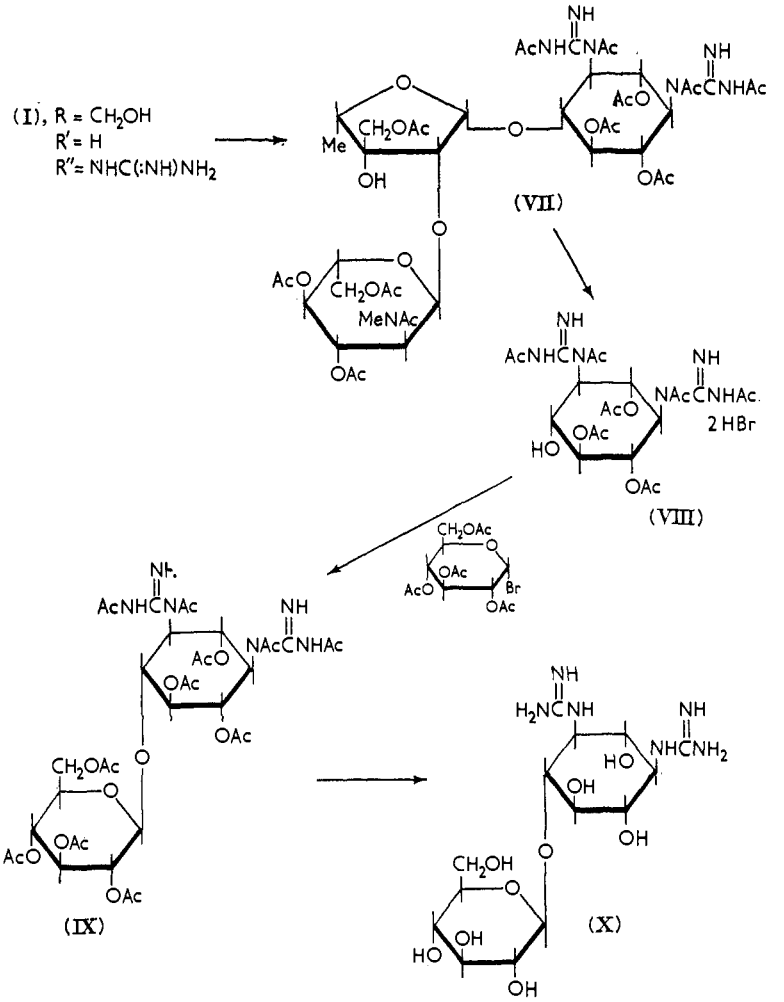


(VI)

Streptidine-β-glucopyranoside (X) was prepared from streptomycin as outlined in the scheme shown overleaf.

Dodeca-acetyldihydrostreptomycin, prepared as described in Part IV,[1] was cleaved with 31 per cent hydrobromic acid in glacial acetic acid–chloroform at 0° to yield hepta-acetylstreptidine dihydrobromide (VIII). The latter was characterised by conversion to hepta-acetylstreptidine with silver oxide, and by acetylation

with acetic anhydride in pyridine to the known octa-acetylstreptidine.[4] Hepta-acetylstreptidine dihydrobromide was also



obtained from undeca-acetylstreptomycin by the same method. Condensation of hepta-acetylstreptidine dihydrobromide (VIII) with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (aceto-

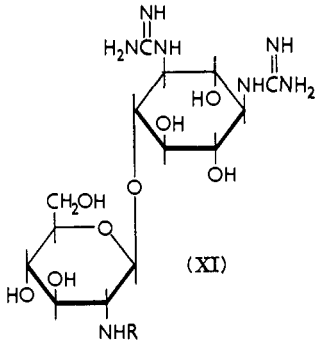
bromoglucose)[14] by the Koenigs-Knorr procedure,[15] in the presence of silver oxide and quinoline, yielded undeca-acetylstreptidine-β-D-glucopyranoside (IX). Preliminary experiments showed that the solvents most commonly used for this reaction, chloroform, benzene, carbon tetrachloride, ether, dioxan, and xylene, were unsuitable. However, reaction in *N*,*N*-dimethylformamide, under vacuum to remove water as it was formed, gave the required product in 22 per cent yield.

The mechanism of formation of glycosides by the Koenigs-Knorr reaction is based on the general theory of displacement reactions.[16] Ionisation of the halide to form a cation is considered to be the rate-controlling step,[17, 18] the cation combining with the hydroxyl group of the aglycone as rapidly as it is formed. The displacing group approaches the carbon atom on the opposite side to that from which the halogen departs, so that the reaction results in a Walden inversion. The product is, therefore, considered to be a β-D-glucopyranoside. Neighbouring group participation leads to *ortho*-ester formation with *O*-acetylglycosyl halides having the halogen at $C_{(1)}$ *trans* to the acetoxy group at $C_{(2)}$,[16, 19] so that the above method is not suitable for the preparation of the corresponding α-glucoside. Helferich and Brederick,[20] however, have obtained small yields of α-glycosides using quinoline alone (i.e. in the absence of silver oxide) as condensing agent. Condensation of hepta-acetylstreptidine dihydrobromide with acetobromoglucose under these conditions gave a partially deacetylated product, tetra-*O*-acetylstreptidine-α-D-glucopyranoside, likewise in very small yield. The α-configuration for this product was assigned on the basis of optical rotation.

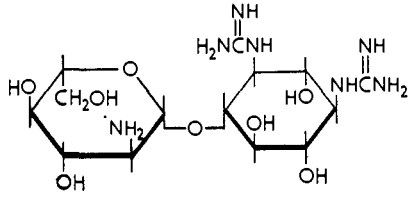
Undeca-acetylstreptidine-β-D-glucopyranoside was deacetylated at 0° in dry methanol saturated with dry ammonia to yield streptidine-β-D-glucopyranoside (X), which was characterised as its dipicrate. Streptidine-2-amino-2-deoxy-β-D-glucopyranoside (XI; R = H), streptidine-2-methylamino-2-deoxy-β-D-glucopyranoside (XI; R = Me) and streptidine-2-methylamino-2-deoxy-β-L-glucopyranoside (XII) have also been prepared by a similar series of reactions from hepta-acetylstreptidine dihydrobromide, and the appropriate tri-*O*-acetylbromoglucosamine hydrobromides. *O*-Acetylation of D-glucosamine with acetyl bromide yields tri-*O*-acetyl-2-amino-2-deoxy-α-D-glucopyranosyl bromide,[21, 22] an

intermediate which permits facile deacetylation, and avoids the difficulties of *N*-deacetylation described in Part IV.

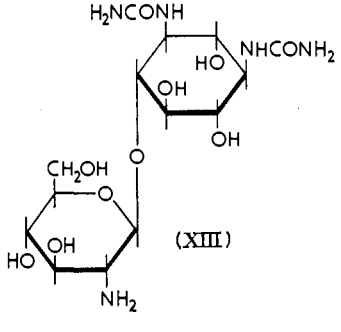
The sulphate of streptidine-2-amino-2-deoxy-β-D-glucopyranoside (XI; R = H) was degraded with 0·35 N barium hydroxide under reflux for one hour to yield strepturea-2-amino-2-deoxy-β-D-glucopyranoside (XIII). Further degradation with the same



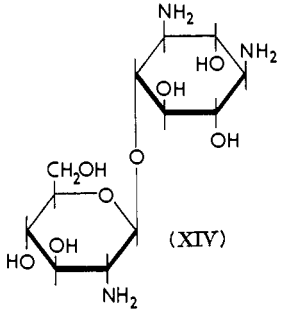
(XI)



(XII)



(XIII)



(XIV)

reagent under reflux for 24 h gave streptamine-2-amino-2-deoxy-β-D-glucopyranoside (XIV).

Anomerization of the glycosidic links of dihydrostreptomycin offers a further means of studying the influence of molecular shape on its biological specificity. β →α anomerisations are well-known and are usually effected by acidic catalysts. The β-streptidine–streptose link is acid-labile in aqueous media. Anomerisation of dodeca-acetyldihydrostreptomycin was, therefore, attempted in nonaqueous media with acidic catalysts, such as boron

trifluoride,[23] stannic chloride,[24] titanium tetrachloride,[24] and sulphuric acid–acetic anhydride,[25] the progress of the reaction being followed polarimetrically. The only products obtained were either unchanged or hydrolysed reactant. Similarly, attempted anomerisation with sodium hydroxide[26] in dry dioxan–ether resulted in partial deacetylation to a product, which was identified by reacetylation with acetic anhydride in pyridine to dodeca-acetyldihydrostreptomycin.

We are indebted to Glaxo Laboratories Ltd. for investigation of the tuberculostatic activity of the products listed in Table I against *M. tuberculosis* (human strain 666). The results for strepturea dihydrostreptobiosaminide and streptamine dihydrostreptobiosaminide confirm those of Bodanszky,[9] and establish the importance of the strongly basic guanidino groups for activity; the inactivity of the streptamine derivative suggests a mode of action completely different from that of kanamycin (V). The almost complete abolition of tuberculostatic activity which follows substitution of the streptobiosamine fragment by D-glucose, D-glucosamine, *N*-methyl-D-glucosamine and *N*-methyl-L-glucosamine further stresses the importance of the streptose unit (Part IV)[1]. As anticipated, none of the acetylated intermediates showed significant activity.

Experimental*†

Strepturea-β-L-dihydrostreptobiosaminide. Dihydrostreptomycin sulphate (0·756 g) in water (5 ml) was refluxed for one hour with 0·35N barium hydroxide solution (25 ml). (The ammonia was distilled off in a stream of nitrogen into excess 0·1 N hydrochloric acid, back titrated, and found to be equivalent to 2 moles). Excess barium hydroxide was precipitated by the addition of solid carbon dioxide and the solution centrifuged, and decolourised (charcoal). The solution was freeze-dried, to yield strepturea-β-L-dihydrostreptobiosaminide (0·51 g, 84·3 per cent) as a colourless microcrystalline solid (from aqueous-methanol and -ethanol), which, after drying *in vacuo* at 60° for 3 h, decomposed above 280°, $[\alpha]_D^{19}$ −90·1° (*c*, 1·32 in water).

* Analyses by Miss M. Buchanan, Mr. W. McCorkindale and Dr. A. C. Syme of this College, and by Drs. Weiler and Strauss, Oxford.

† Melting points are uncorrected.

Anal. Calcd. for $C_{21}H_{39}N_5O_{14}.2H_2O$: C, 40·6; H, 7·0; N, 11·3. Found: C, 40·8; H, 7·5; N, 11·6.

Deca-acetylstrepturea-β-L-dihydrostreptobiosaminide. Acetic anhydride (5 ml) was added slowly with stirring to a suspension of strepturea-β-L-dihydrostreptobiosaminide (0·26 g) suspended in dry methanol (10 ml) and pyridine (5 ml). When solution was complete (*ca.* 30 min), the solvent was removed *in vacuo* below 50°, the residue washed with dry ether, and then treated again with pyridine (5 ml), and acetic anhydride (5 ml) added slowly. The solution was maintained at room temperature for 40 h with occasional shaking, and then heated at 45° for 4 h with stirring. The solvent was removed *in vacuo* below 45°, and the residue triturated and washed with light petroleum (b.p. 40–60°) to yield *deca-acetylstrepturea-β-L-dihydrostreptobiosaminide* (0·97 g, from methanol–ethanol), m.p. 256–258°, $[\alpha]_D^{19}$ –73·3° (*c*, 0·96 in MeOH).

Anal. Calcd. for $C_{41}H_{59}N_5O_{24}.2H_2O$: C, 47·3; H, 6·1; N, 6·7. Found: C, 47·4; H, 6·0; N, 7·3.

Streptamine-β-L-dihydrostreptobiosaminide trihydrochloride. Dihydrostreptomycin sulphate (5·125 g) was heated under reflux over a water bath with 0·35 N barium hydroxide (350 ml) for 24 h. Excess barium hydroxide was precipitated by the addition of solid carbon dioxide, the solution filtered, and evaporated *in vacuo* below 50° to yield a pale yellow solid (2·85 g). The base was purified by adsorption on charcoal (2 g), impurities being removed by washing with water (3 × 10 ml) and methanol (3 × 10 ml). The charcoal was suspended in methanol (15 ml) at about 10°, the suspension stirred vigorously with N methanolic hydrochloric acid (3 ml) for 10 min, filtered, and the charcoal washed with methanol (2 × 10 ml). The combined filtrates were concentrated *in vacuo* to 10 ml, and slowly diluted with dry ether to precipitate the *trihydrochloride* (0·25 g), m.p. 194–196° (d.), $[\alpha]_D^{17}$ –88·6° (*c*, 0·94 in water).

Anal. (After drying at 80° for 3 h). Calcd. for $C_{19}H_{37}N_3O_{12}.3HCl.H_2O$: C, 36·4; H, 6·75; N, 6·7. Found: C, 36·3; H, 6·7; N, 7·2.

Deca-acetylstreptamine-β-L-dihydrostreptobiosaminide. The base (0·3 g) obtained in the previous experiment was acetylated as described for the preparation of deca-acetylstrepturea-β-L-dihydrostreptobiosaminide, and gave deca-acetylstreptamine-β-L-

dihydrostreptobiosaminide (0·192 g), m.p. 260–262°, $[\alpha]_D^{20} - 83°$ (*c*, 1·0 in MeOH). Wolfrom and Polglase[8] give m.p. 261·5–262·5°, $[\alpha]_D^{23} - 84°$.

Hepta-acetylstreptidine dihydrobromide. (a) Dodeca-acetyldihydrostreptomycin (2·053 g, 0·0019 moles)[1] in absolute chloroform (30 ml) was cooled in ice, and a 31 per cent solution of hydrogen bromide in glacial acetic acid (1·3 ml, 0·0058 moles) was added with stirring. After 24 h in the refrigerator the solution deposited a sticky solid. The chloroform was decanted and the residue washed with absolute chloroform (2 × 10 ml). The chloroform solution, treated again with 31 per cent hydrogen bromide in glacial acetic acid (0·4 ml, 0·0019 mole), yielded a further quantity of a similar solid during the next 24 h. The residue in each case was washed with dry ether (15 ml) and traces of acid were removed by adding chloroform (2 × 10 ml) and then evaporating *in vacuo*. The dry yellowish residues (1·068 g) were combined, dissolved in methanol (10 ml), the solution decolourised with charcoal (0·2 g), and then poured with stirring into dry ether (100 ml). The colourless micro-crystalline precipitate was washed with dry ether, absolute chloroform (2 × 10 ml) and finally dry ether to yield *hepta-acetylstreptidine dihydrobromide* (0·728 g, 54 per cent), m.p. 180–183° (d.), $[\alpha]_D^{19} - 5·4°$ (*c*, 0·918 in MeOH).

Anal. Calcd. for $C_{22}H_{32}N_6O_{11}.2HBr.H_2O$: C, 35·9; H, 4·9; N, 11·4; Br, 22·2; CH_3CO, 40·9. Found: C, 35·6; H, 4·7; N, 11·4; Br, 22·0; CH_3CO, 41·0.

(b) The dihydrobromide {m.p. 180–189° (d.)} was also obtained from undeca-acetylstreptomycin by the same method.

Anal. Calcd. for $C_{22}H_{32}N_6O_{11}.2HBr.H_2O$: N, 11·4. Found: N, 11·25.

1,3-Bis (diacetylguanidino)-4-hydroxy-2,5,6-triacetoxycyclohexane, (hepta-acetylstreptidine). Hepta-acetylstreptidine dihydrobromide (0·2 g) in methanol (3 ml) was treated with silver oxide (0·19 g), the suspension filtered, and the filtrate poured into dry ether (50 ml). The colourless precipitate, when dried *in vacuo* gave *hepta-acetylstreptidine*, m.p. 195–197° (d., micro-block).

Anal. Calcd. for $C_{22}H_{32}N_6O_{11}$: N, 15·1. Found: N, 14·9.

Octa-acetylstreptidine. Hepta-acetylstreptidine dihydrobromide (0·41 g) was acetylated in pyridine (5 ml) with acetic anhydride at room temperature for 24 h, and then at 50° for 1 h. The solution

was evaporated *in vacuo* to a viscous residue, which was triturated and washed with dry ether to yield octa-acetylstreptidine (0·232 g) (from chloroform–ether), m.p. 260–262° (micro-block). Peck *et al.*[4] give m.p. 260–262° (micro-block).

Anal. Calcd. for $C_{24}H_{34}N_6O_{12}$: N, 14·04. Found: N, 13·8.

2,3,4,6-Tetra-O-*acetyl*-α-D-*glucopyranosyl bromide*, (*acetobromoglucose*) was prepared by the procedure of Jeremias, Lucas and MacKenzie,[14] yield 85 per cent, m.p. 87–88° $[\alpha]_D^{19} + 198°$ (*c*, 1·03 in $CHCl_3$). Jeremias *et al.*[14] give m.p. 88–89°. The product decomposed on storage at room temperature, but was stable when kept under light petroleum (b.p.40–60°). It was recrystallized from carbon tetrachloride–light petroleum (b.p. 40–60°) before use.

*Tetra-acetyl-{4-*O-α-*(1,3-diguanidino-2,5,6-trihydroxycyclohexyl)*-α-D-*glucopyranoside}*, (*tetra-acetylstreptidine*-α-D-*glucopyranoside*). Hepta-acetylstreptidine dihydrobromide (0·453 g, 0·00063 mole) and acetobromoglucose (0·264 g, 0·00064 mole) were dissolved in dry quinoline (1·841 g). After keeping at 5° for 18 h, the excess quinoline was removed by trituration with dry ether. The ether-insoluble residue was shaken with silver oxide in chloroform (10 ml) and filtered. Evaporation of the solvent gave a residue, which was washed with ether and extracted with absolute chloroform (5 × 10ml). The chloroform extract on evaporation *in vacuo* gave an amorphous product (0·056 g), which was purified by precipitation from chloroform–ether, and then from acetone–light petroleum (b.p. 40–60°) to yield *tetra-acetylstreptidine*-α-D-*glucopyranoside* (0·037 g, 10 per cent), m.p. 140–142° (d., micro-block), $[\alpha]_D^{19} + 15·6°$ (*c*, 0·74 in $CHCl_3$).

Anal. Calcd. for $C_{22}H_{36}N_6O_{13}.2H_2O$: C, 42·0; H, 6·4; N, 13·4. Found: C, 41·9; H, 5·55; N, 13·1.

4-O-β-*{1,3-Bis(diacetylguanidino)-2,5,6-triacetoxycyclohexyl}tetra*-O-*acetyl*-D-*glucopyranoside*, (*undeca-acetylstreptidine*-β-D-*glucopyranoside*). Hepta-acetylstreptidine dihydrobromide (0·67 g, 0·00093 mole) was dissolved in *N*,*N*-dimethylformamide (10 ml) in a flask wrapped in black paper. The solution was kept *in vacuo* (0·2 mm) for 15 min with constant stirring. Acetobromoglucose (0·373 g, 0·00092 mole) was added, and the solution again maintained *in vacuo* for 10 min. Silver oxide (0·604 g) and quinoline (4 drops) were added simultaneously. Water liberated during the reaction was removed continuously by maintaining the

reactants under vacuum, and adding *N,N*-dimethylformamide when necessary to maintain volume during the reaction period. After 8 h the solvent was removed by distillation *in vacuo* at room temperature. The viscous residue was triturated and washed with dry ether (5 × 10 ml) and extracted with absolute chloroform (10 × 10 ml). The chloroform solution was kept in the refrigerator overnight, and hydrogen sulphide passed into the cold solution. The filtrate was evaporated and the tan-coloured residue precipitated from chloroform–ether. The precipitate was redissolved in acetone, the solution decolourised with charcoal (0·15 g), and poured slowly into light petroleum (b.p. 40–60°) to precipitate *undeca-acetylstreptidine-β-D-glucopyranoside*, as a colourless microcrystalline solid (0·184 g, 22 per cent), m.p. 164–167° (d.), $[\alpha]_D^{18}$ −25·5° (*c*, 2·56 in $CHCl_3$).

Anal. Calcd. for $C_{36}H_{50}N_6O_{20}.2H_2O$: C, 46·9; H, 5·9; N, 9·1. Found: C, 46·2; H, 6·3; N, 9·5.

4-O-β-(1,3-Diguanidino-2,5,6-trihydroxycyclohexyl) D*-glucopyranoside*, (*streptidine-β-*D*-glucopyranoside*). Undeca-acetylstreptidine-β-D-glucopyranoside (0·528 g) was dissolved in dry methanol (5 ml). The solution was saturated with ammonia at 0°, and kept for 15 h at room temperature. The solvent was removed *in vacuo* at room temperature and the residue washed with chloroform (3 × 20 ml). The chloroform-insoluble residue was dissolved in methanol (5 ml), the solution filtered and the filtrate added slowly to dry ether (50 ml), to precipitate *streptidine-β-*D*-glucopyranoside* (0·178 g, 70 per cent) m.p. 170–172° (d., micro-block) $[\alpha]_D^{20}$ −11·4° (*c*, 0·615 in MeOH).

Anal. Calcd. for $C_{14}H_{28}N_6O_9.H_2O$: C, 38·0; H, 6·8; N, 19·0. Found: C, 37·8; H, 7·0; N, 18·6.

Dipicrate, m.p. 260–262° (d., micro-block) (from aqueous methanol).

Anal. Calcd. for $C_{26}H_{34}N_{12}O_{23}.2H_2O$: C, 34·0; H, 4·0; N, 18·3. Found: C, 33·4; H, 4·3; N, 18·4.

*3,4,6-Tri-O-acetyl-2-amino-2-deoxy-α-*D*-glucopyranosyl bromide hydrobromide*, (*acetobromoglucosamine hydrobromide*) was prepared essentially by the methods of Irvine, McNicoll and Hynd,[21] and Fodor and Ötvös.[22] Acetyl bromide (15·4 g, 0·127 mole) was stirred with D-glucosamine hydrochloride (5·2 g, 0·024 mole) for three days at room temperature. The excess reagent was removed

in vacuo, and the dry residue washed with ether (25 ml) and then dissolved in warm chloroform (50 ml). The filtered solution was concentrated to *ca.* 35 ml, diluted to turbidity with ether and cooled in the refrigerator to yield acetobromoglucosamine hydrobromide (6·01 g, 55 per cent) as colourless needles (from chloroform–ether), m.p. 151–153° (d.), $[\alpha]_D^{22}+148°$ (*c*, 1·05 in acetone). Irvine, McNicoll and Hynd[21] give m.p. 149–150·, $[\alpha]_D^{22}+148·4°$ (*c*, 1·05 in acetone).

4-O-β-{1,3-Bis(diacetylguanidino)-2,5,6-triacetoxycyclohexyl}tri-O-acetyl-2-amino-2-deoxy-D-glucopyranoside, (*deca-acetylstreptidine-2-amino-2-deoxy-β-D-glucopyranoside*) was prepared from hepta-acetylstreptidine dihydrobromide (1·652 g, 0·0023 mole), tri-*O*-acetyl-2-amino-2-deoxy-α-D-glucopyranosyl bromide hydrobromide (1·06 g, 0·00236 mole) silver oxide (3·018 g) and dry quinoline (0·2 ml), as described for the preparation of undeca-acetylstreptidine-β-D-glucopyranoside. The product (0·4 g, 20·6 per cent) crystallised from methanol–ether, m.p. 132–134° (d.), $[\alpha]_D^{19}-12°$ (*c*, 0·88 in $CHCl_3$).

Anal. Calcd. for $C_{34}H_{49}N_7O_{18}.2H_2O$: C, 46·4; H, 6·1; N, 11·1. Found: C, 46·3; H, 6·4; N, 11·3.

4-O-β-(1,3-Diguanidino-2,5,6-trihydroxycyclohexyl) 2-amino-2-deoxy-D-glucopyranoside trihydrochloride, (*streptidine-2-amino-2-deoxy-β-D-glucopyranoside trihydrochloride*). Streptidine-2-amino-2-deoxy-β-D-glucopyranoside (0·50 g, 87·3 per cent) was prepared from deca-acetylstreptidine-2-amino-2-deoxy-β-D-glucopyranoside (1·145 g) as described for the preparation of streptidine-β-D-glucopyranoside. The crude product (120 mg) was dissolved in water and adsorbed on charcoal (1 g). The latter was washed twice with water, with dry methanol, and then suspended in dry methanol (5 ml) acidified with N methanolic hydrochloric acid. After shaking for a few minutes, the solution was separated by centrifugation, the charcoal washed twice with methanol (10 ml), and the combined methanolic solutions concentrated *in vacuo* to *ca.* 5 ml. Precipitation with dry ether (75 ml) gave the hygroscopic *trihydrochloride*, m.p. 160–162° (d.), $[\alpha]_D^{19}-17·6°$ (*c*, 1·02 in H_2O).

Anal. Calcd. for $C_{14}H_{29}N_7O_8.3HCl$: C, 31·6; H, 6·05; N, 18·4. Found, after drying at 80° *in vacuo* for 3 h: C, 32·0; H, 5·8; N, 17·8.

Sulphate (70 mg) prepared from the crude base (80 mg) in dry methanol (5 ml), cooled and acidified with N sulphuric acid to pH 3, had $[\alpha]_D^{20} - 17 \cdot 1°$ (*c*, 1·07 in H_2O).

Anal. Calcd. for $C_{14}H_{29}N_7O_8 . 1\frac{1}{2}H_2SO_4$: N, 17·2. Found after drying at 80° for 3 h, N, 17·2.

Trihelianthate. The crude base (120 mg, 0·00028 mole) was dissolved in water, the solution acidified with 0·1 N hydrochloric acid, and methyl orange (301 mg, 0·0003 mole) dissolved in warm 50 per cent aqueous methanol (27 ml) added slowly. The flocculent precipitate was redissolved at 50° by the addition of 50 per cent aqueous methanol (10 ml), and gave needle crystals of *streptidine*-β-D-*glucopyranoside trihelianthate* (96 mg), m.p. 236–239° (d.).

Anal. Calcd. for $C_{56}H_{77}N_{16}O_{17}S_3 . 2H_2O$: C, 48·75; H, 5·6; N, 16·3; S, 7·0. Found: C, 48·0; H, 5·9; N, 16·0; S, 6·7.

2-N-Methylamino-2-deoxy-α-D-glucopyranose hydrochloride was prepared by the method of Kuehl, Flynn, Holly, Mozingo and Folkers.[27]

3,4,6-Tri-O-acetyl-2-N-methylamino-2-deoxy-α-D-glucopyranosyl bromide hydrobromide. Acetyl bromide (6·15 g, 0·05 mole) was stirred with 2-*N*-methylamino-2-deoxy-α-D-glucopyranose hydrochloride (3·1 g, 0·00135 mole) for two days at room temperature. Excess of the reagent was removed *in vacuo*, and the residue washed with dry ether (25 ml). The residue was dissolved in chloroform (50 ml), the solution filtered, concentrated to 10 ml, and poured into dry ether. The precipitate dried over P_2O_5 *in vacuo* gave *3,4,6-tri-O-acetyl-2-N-methylamino-2-deoxy-α-D-glucopyranosyl bromide hydrobromide* (2·1 g, 33·6 per cent), m.p. 120–122° (d.), $[\alpha]_D^{23} + 149 \cdot 5°$ (*c*, 1·25 in acetone).

Anal. Calcd. for $C_{13}H_{20}BrNO_7 . HBr$: C, 32·4; H, 4·8; Br, 33·2; N, 2·9. Found: C, 32·7; H, 4·7; Br, 32·9; N, 3·1.

4-O-β-{1,3-Bis(diacetylguanidino)-2,5,6-triacetoxycyclohexyl}-tri-O-acetyl-2-N-methylamino-2-deoxy-D-glucopyranoside, (deca-acetylstreptidine-2-N-methylamino-2-deoxy-β-D-glucopyranoside) was prepared from hepta-acetylstreptidine dihydrobromide (1·35 g, 0·0018 mole), tri-*O*-acetyl-2-*N*-methylamino-2-deoxy-α-D-glucopyranosyl bromide hydrobromide (0·8 g, 0·00175 mole), silver oxide (1·57 g) and dry quinoline (0·15 ml), as described for the preparation of undeca-acetylstreptidine-β-D-glucopyranoside.

The product (0·3 g, 18·5 per cent) had m.p. 168–171° (d.), $[\alpha]_D^{20} - 11°$ (*c*, 0·9 in $CHCl_3$).

Anal. Calcd. for $C_{35}H_{51}N_7O_{18}.2H_2O$: C, 47·0; H, 6·2; N, 11·0. Found: C, 46·1; H, 5·4; N, 10·6.

4-O-β-(1,3,Diguanidino-2,5,6-trihydroxycyclohexyl) 2-N-methylamino-2-deoxy-D-glucopyranoside carbonate, (streptidine-2-N-methylamino-2-deoxy-β-D-glucopyranoside carbonate). Deca-acetylstreptidine-2-*N*-methylamino-2-deoxy-β-D-glucopyranoside was deacetylated as described for the preparation of streptidine-β-D-glucopyranoside. The chloroform-insoluble amorphous product (0·240 g, 77 per cent) gave $[\alpha]_D^{22} - 16°$ (*c*, 0·95 in H_2O) and decomposed slowly above 180°.

Anal. Calcd. for $C_{15}H_{31}N_7O_8.1\frac{1}{2}H_2CO_3.H_2O$: C, 36·1; H, 6·6; N, 17·9. Found: C, 36·15; H, 6·3; N, 18·2.

Sulphate. The carbonate (125 mg) was dissolved in water (2 ml), the solution acidified to pH 4·8 with 0·1 N sulphuric acid, and freeze-dried. The sulphate, dried over phosphorus pentoxide, had $[\alpha]_D^{20} - 16·5°$ (*c*, 0·8 in H_2O).

Anal. Calcd. for $C_{15}H_{31}N_7O_8.1\frac{1}{2}H_2SO_4$: N, 16·8. Found: N, 16·4.

Tripicrate. The carbonate (30 mg) in water (0·5 ml) treated with picric acid (39 mg) in water (3·6 ml) gave the *tripicrate*, m.p. 269–271° (d., micro-block).

Anal. Calcd. for $C_{33}H_{40}N_{16}O_{29}.2H_2O$: C, 34·2; H, 3·8; N, 19·3. Found: C, 33·4; H, 4·2; N, 18·8.

2-N-Methylamino-2-deoxy-α-L-glucopyranose hydrochloride was prepared by the procedure of Kuehl, Flynn, Holly, Mozingo and Folkers.[27]

3,4,6-Tri-O-acetyl-2-N-methylamino-2-deoxy-α-L-glucopyranosyl bromide hydrobromide, was prepared by the reaction of acetyl bromide (3·3 g, 0·027 mole) with 2-*N*-methylamino-2-deoxy-α-L-glucopyranose hydrochloride (1·165 g, 0·0051 mole) at room temperature for 24 h, and thereafter as described for the preparation of 3,4,6-tri-*O*-acetyl-2-*N*-methylamino-2-deoxy-D-glucopyranosyl bromide hydrobromide. The amorphous hygroscopic product (1·1 g, 47 per cent) had m.p. 114–116° (d.), $[\alpha]_D^{22} - 148°$ (*c*, 0·9 in acetone).

Anal. Calcd. for $C_{13}H_{20}BrNO_7.HBr.H_2O$: Br, 33·2; N, 2·9. Found: Br, 32·7; N, 3·0.

4-O-β-{*1,3-Bis(diacetylguanidino)-2,5,6-triacetoxycyclohexyl*} *tri*-O-*acetyl-2*-N-*methylamino-2-deoxy*-L-*glucopyranoside*, (*deca-acetylstreptidine-2*-N-*methylamino-2-deoxy*-β-L-*glucopyranoside*), was prepared from hepta-acetylstreptidine (1·40 g, 0·0019 mole), 3,4,6-tri-*O*-acetyl-2-*N*-methylamino-2-deoxy-α-L-glucopyranosyl bromide hydrobromide (0·9 g, 0·0019 mole), silver oxide (1·25 g) and dry quinoline (0·12 ml) as described for the preparation of undeca-acetylstreptidine-β-D-glucopyranoside. The amorphous product (0·51 g, 30 per cent) had m.p. 171–173° (d.), $[\alpha]_D^{21} - 20°$ (*c*, 8·4 in $CHCl_3$).

Anal. Calcd. for $C_{35}H_{51}N_7O_{18}.2H_2O$: C, 47·0; H, 6·2; N, 11·0. Found: C, 46·5; H, 5·5; N, 11·1.

4-O-β-(*1,3-Diguanidino-2,5,6-trihydroxycyclohexyl*)-*2*-N-*methylamino-2-deoxy*-L-*glucopyranoside trihydrochloride*, (*streptidine-2*-N-*methylamino-2-deoxy*-β-L-*glucopyranoside trihydrochloride*). Deca-acetylstreptidine-2-*N*-methylamino-2-deoxy-β-L-glucopyranoside (0·41 g) was deacetylated as described for the preparation of streptidine-β-D-glucopyranoside, yielding 0·175 g of amorphous base. The latter was dissolved in water (3 ml), the solution acidified with 0·1 N hydrochloric acid to pH 4·8 and freeze-dried, to yield the colourless hygroscopic *trihydrochloride*, m.p. 178–180° (d.), $[\alpha]_D^{22} - 29·5°$ (*c*. 0·8 in H_2O).

Anal. Calcd. for $C_{15}H_{31}N_7O_8.3HCl.H_2O$: C, 31·9; H, 6·4; N, 17·4. Found: C, 31·3; H, 6·2; N, 17·5.

Tripicrate, m.p. 268–270° (d., micro-block) (from aqueous methanol.

Anal. Calcd. for $C_{33}H_{40}N_{16}O_{29}.2H_2O$: C, 34·2; H, 3·8; N, 19·3. Found: C, 33·6; H, 3·5; N, 18·9.

4-O-(*1,3-Diureido-2,5,6-trihydroxycyclohexyl*)-*2-amino-2-deoxy*-β-D-*glucopyranoside sulphate*, (*streptur*ea-*2-amino-2-deoxy*-β-D-*glucopyranoside sulphate*). Streptidine-2-amino-2-deoxy-β-D-glucopyranoside sulphate (0·42 g) in water (3 ml) was heated under reflux over a water-bath with 0·35 N barium hydroxide (15 ml) for 1 h. Excess barium hydroxide was precipitated by the addition of solid carbon dioxide and the solution centrifuged and decolourized (charcoal). The solution was freeze-dried, the product redissolved in water (2 ml), acidified with N sulphuric acid to pH 4·9, and the precipitate of barium sulphate removed by centrifugation. The solution was diluted with methanol (25 ml) and

Table I. Inhibition of *M. tuberculosis* (human strain 666) in Dubos Liquid Medium after incubation at 37° for 14 days

	Concentration μg/ml										
	100	50	25	12·5	6·25	3·12	1·56	0·78	0·4	0·2	0·1
Streptomycin (I; R=CHO, R′=H, R″=NHC(:NH)NH_2)	−	−	−	−	−	−	−	−	−	++	++
Strepturea dihydrostreptobiosaminide (I; R=CH_2OH, R′=H, R″=$NHCONH_2$)	−	−	−	±	+	++	++	++	++	++	++
Streptamine dihydrostreptabiosaminide (I; R=CH_2OH, R′=H, R″=NH_2)	++	++	++	++	++	++	++	++	++	++	++
Streptidine-β-D-glucopyranoside (XIII)	−	−	++	++	++	++	++	++	++	++	++
Streptidine-2-amino-2-deoxy-β-D-glucopyranoside sulphate (XIV; R=H)	++	++	++	++	++	++	++	++	++	++	++
Streptidine-2-*N*-methylamino-2-deoxy-β-D-glucopyranoside sulphate (XIV; R=Me)	++	++	++	++	++	++	++	++	++	++	++
Streptidine-2-*N*-methylamino-2-deoxy-β-L-glucopyranoside trihydrochloride (XV)	++	++	++	++	++	++	++	++	++	++	++
Strepturea-2-amino-2-deoxy-β-glucopyranoside sulphate (XVI)	++	++	++	++	++	++	++	++	++	++	++
Streptamine-2-amino-2-deoxy-β-D-glucopyranoside (XVII)	++	++	++	++	++	++	++	++	++	++	++

ether (100 ml) to yield the *sulphate* (0·25 g, 71 per cent), which decomposed slowly above 220°.

Anal. Calcd. for $C_{14}H_{27}N_5O_{10}\cdot\frac{1}{2}H_2SO_4\cdot 2H_2O$: C, 33·0; H, 6·3; N, 13·7. Found: C, 32·6; H, 5·9; N, 13·8.

4-O-(1,3-Diamino-2,5,6-trihydroxycyclohexyl)-2-amino-2-deoxy-β-D-glucopyranoside, (streptamine-2-amino-2-deoxy-β-D-glucopyranoside). Streptidine-2-amino-2-deoxy-β-D-glucopyranoside (0·452 g) was heated under reflux over a water bath with 0·35 N barium hydroxide (50 ml) for 24 h. Barium hydroxide was removed as described above, the solution decolourized (charcoal) and passed through a column of De-Acidite FF (5 g). The column was washed with water (10 ml) and the combined eluates freeze-dried to give the amorphous base (0·181 g, 67 per cent), m.p. 160–162° (d.).

Anal. Calcd. for $C_{12}H_{25}N_3O_8$: C, 42·5; H, 7·4; N, 12·4. Found, after drying at 80° *in vacuo* for 3 h, C, 42·2; H, 7·1; N, 12·5.

Tuberculostatic Activity

The compounds were dissolved in water (1,000 μg/ml), the solutions diluted in Dubos liquid medium, and the dilution inoculated with *M. tuberculosis* (human strain 666). The results recorded after 14 days incubation at 37°C are shown in Table I.

Summary. Streptidine-β-D-glucopyranoside, streptidine-2-amino-deoxy-β-D-glucopyranoside, streptidine-2-methylamino-2-deoxy-β-D- and L-glucopyranosides, strepturea-β-D-glucopyranoside and streptamine-β-D-glucopyranoside have been prepared and shown to have negligible *in vitro* tuberculostatic activity, thus confirming the importance of the streptose fragment for activity in this series. The inactivity of strepturea dihydrostreptobiosaminide and streptamine dihydrostreptobiosamide has been confirmed and further establishes the importance of the strongly basic guanidino group for activity.

Acknowledgements. The bacteriological testing was carried out by Glaxo Laboratories Ltd., whom we wish to thank for a generous gift of streptomycin and dihydrostreptomycin, and also for a maintainance grant to one of us (H.C.M.).

(*Received 4 December, 1959*)

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