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SHORT COMMUNICATION

Dihydrostreptomycin derivatives: glycosides of dihydrostreptobiosamine

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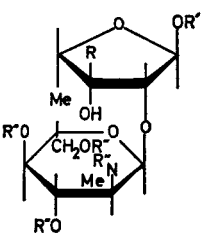
THE *in vitro* activity against *Mycobacterium tuberculosis* of streptomycin derivatives, in particular of certain glycosides of streptidine, has been investigated by Comrie, Mital & Stenlake (1960). In this communication we describe some glycosides of dihydrostreptobiosamine (I). This has allowed us to evaluate transglycosidation as a method of synthesising streptomycin-like compounds with the same stereochemistry as the natural antibiotic.

The anomeric mixture of methyl dihydrostreptobiosaminide hydrochlorides (II) was prepared by methanolysis (Fried & Wintersteiner, 1947), and separated into α- and β-methyl penta-acetyldihydrostreptobiosaminide (III), $[\alpha]_D^{20}$–117° and −34° respectively, by differential solubility of the acetates in ether (Brink, Kuehl, Flynn & Folkers, 1946). This original anomeric assignment has been confirmed by nmr and optical rotation evidence from streptomycin and various of its derivatives, in particular from the α- and β-methyl *N*-acetyldihydrostreptobiosaminides (McGilveray & Rinehart, 1965).



I	R = CH_2OH, R′ = R″ = H.
II	R = CH_2OH, R′ = CH_3, R″ = H.
III	R = CH_2OAc, R′ = CH_3, R″ = Ac.
IV	R = CH_2OH, R′ = CH_2Ph, R″ = H.
V	R = CH_2OAc, R′ = CH_2Ph, R″ = Ac.
VI	R = CH_2OH, R′ = CH_2CH_2Br, R″ = H.
VII	R = CH_2OH, R′ = Ph, R″ = H.

Benzyl (IV) and 2-bromoethyl (VI) dihydrostreptobiosaminide hydrochlorides have been prepared by treatment of the anomeric mixture of methyl dihydrostreptobiosaminide hydrochlorides with the appropriate alcohol in the presence of hydrogen chloride for 48 hr at 50°. The product in each case was a hygroscopic solid.

The phenyl glycoside (VII) was prepared by fusion of methyl dihydrostreptobiosaminide with phenol in the presence of hydrogen chloride.

The benzyl derivative was also obtained as outlined in Scheme I. Carefully dried dihydrostreptomycin sulphate was treated (96 hr) with benzyl alcohol containing 2N hydrogen chloride. Streptidine was filtered off and the solution concentrated *in vacuo*: the addition of

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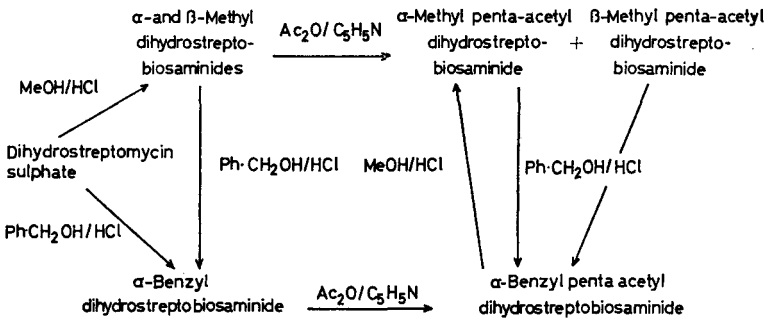
anhydrous ether yielded a hygroscopic precipitate of benzyl dihydrostreptobiosaminide hydrochloride.

The stereochemical homogeneity of the benzyl glycoside was indicated by thin-layer chromatography on silica gel using the organic phase of n-butanol-ethanol-water, 4 : 1 : 5, as solvent with periodate-permanganate (Lemieux & Bauer, 1954) as detecting agent (single spot with R glucosamine value, 3·0). This was confirmed by acetylation of the base (obtained

Scheme I



from the hydrochloride by means of anionic exchange resin) with acetic anhydride in pyridine to a single anomer of benzyl penta-acetyldihydrostreptobiosaminide (V), m.p. 141–142°, $[\alpha]_D^{20}$–138 (*c*,1 in $CHCl_3$). Found: C, 57·2; H, 6·6; N, 2·2. $C_{30}H_{41}NO_{14}$ requires C, 56·7; H, 6·5; N, 2·2%. This product showed solubility properties identical with those of α-methyl penta-acetyldihydrostreptobiosaminide, and, in contrast to the corresponding mixed α- and β-methyl penta-acetates, could not be fractionated by differential solubility in ether.

TABLE 1. DIHYDROSTREPTOBIOSAMINIDES

Glycoside	m.p.	$[\alpha]_D^{20}$	Found			Required as			
			C	H	N	C	H	N	
Benzyl HCl ..	210°	−110°	49·2	6·5	3·0	51·5	6·7	3·0	$C_{20}H_{32}ClNO_9$
2-Bromoethyl HCl	125–130°	−100°	35·3	6·0	3·1	37·3	5·8	2·9	$C_{15}H_{29}BrClNO_9$
Phenyl HCl ..	115°	−131°	51·1	6·7	2·9	50·05	6·5	3·1	$C_{19}H_{30}ClNO_9$
α and β-Methyl (*ca* 90% α)† ..	—	−135°*	—	—	—	—	—	—	

* Fried & Wintersteiner (1947).
† Brink & others (1946).

Assignment of the configuration of benzyl dihydrostreptobiosaminide as α-L is supported by rotational evidence (Table 1). This should be considered together with the specific rotations of α- and β-methyl penta-acetyldihydrostreptobiosaminides $[\alpha]_D^{20}$–117° and −34° respectively (Brink & others, 1946) and of the derived α- and β-methyl *N*-acetyldihydrostreptobiosaminides ($[\alpha]_D^{25}$–160° and −32° respectively) (McGilveray & Rinehart, 1965). This assignment as the α-anomer was confirmed as follows. α-Methyl penta-acetyldihydrostreptobiosaminide was treated (24 hr) with benzyl alcohol-hydrogen chloride. Subsequent acetylation with acetic anhydride in pyridine followed by chromatography

on alumina gave the same benzyl penta-acetyldihydrostreptobiosaminide (crystals from ethanol), m.p. 141–142° (undepressed on admixture with authentic material), $[\alpha]_D^{20}$–138°($CHCl_3$). In the same way methanolysis of benzyl penta-acetyldihydrostreptobiosaminide gave α-methyl penta-acetyldihydrostreptobiosaminide as the sole product, in further support of the α- assignment to the benzyl glycoside.

Although the course of the transglycosidation reaction has not been investigated, a carbonium ion intermediate would be anticipated by analogy with the Fischer glycoside synthesis (Shafizadeh, 1958; Bishop & Cooper, 1962, 1963; Capon, Loveday & Overend, 1962). Thermodynamic considerations should therefore control the stereochemistry of the reaction products. This is consistent with the experimental evidence that the bulkier benzyl group allows formation of only one anomer, namely α-L-benzyl dihydrostreptobiosaminide. Some indirect evidence that this form is sterically favoured comes from the experiments of Bishop & Cooper (1963) on the formation of D-lyxofuranosides in which only the α-D-methyl lyxofuranoside was obtained. Indeed, contrary to previous reports, the streptose-streptidine link in streptomycin itself has now been assigned the α-L configuration (McGilveray & Rinehart, 1965). More directly, we find that when β-methyl penta-acetyldihydrostreptobiosaminide is treated with benzyl alcohol-hydrogen chloride it is the α-benzyl anomer which is produced. This we regard as significant evidence for the greater stability of the α- over the β-anomer.

MICROBIOLOGICAL RESULTS

Phenyl dihydrostreptobiosaminide (VII) and 2-bromoethyl dihydrostreptobiosaminide (VI), tested as their hydrochlorides at a concentration of 250 μg/ml in nutrient broth at 37°, were inactive against *Staphylococcus aureus* 898, *Bacillus subtilis* 814E, *Escherichia coli* 741; and *Pseudomonas pyocyanea* 150E. The same compounds were also inactive against *Mycobacterium tuberculosis* 666 at the same concentration in Dubos medium after two weeks at 37°.

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