THE PREPARATION OF 1 D-2,6-DIDEOXY-[1-^{3}H]-STREPTAMINE

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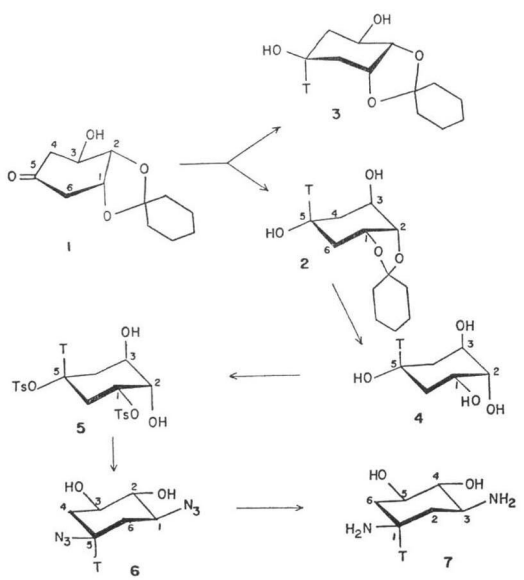
In the course of our studies on the incorporation of 2,6-dideoxystreptamine[1] into novel antibiotics using deoxystreptamine-requiring idiotrophs of Streptomycetes and other microorganisms producing antibiotics, and also for biosynthetic studies, we required a radioactive precursor. Although ^{14}C-deoxystreptamine has been prepared in low yield biosynthetically from labelled precursors[2], and 1-^{14}C-deoxystreptamine has also been synthesized from 6-^{14}C-ethyl 2-acetamido-2,6-dideoxy-6-nitro-1-thio-β-L-idofuranoside[3], we have used an alternative approach using intermediates readily available to us and the relatively inexpensive precursor sodium borotritiide.

This note describes the preparation of the deoxystreptamine analogue, 1-^{3}H-2,6-dideoxystreptamine. The unlabelled 2,6-dideoxystreptamine has recently been described[1] and we have adapted and considerably shortened this route in the later stages to give a higher overall yield. This was desirable because the most convenient step for the introduction of the tritium label is rather early.

Scheme 1.



3 L-3,4-*O*-Cyclohexylidene-3,4/5-trihydroxycyclohexanone (**1**) prepared from quinic acid as previously described[1] was reduced by sodium borotritiide giving two isomeric diols (**2**, **3**). The required tritiated isomer, 1 L-1,2-*O*-cyclohexylidene-1,2,5/3-[5-^{3}H]-cyclohexane-tetrol (**2**) was isolated by addition of a large quantity of unlabelled compound **2** previously prepared[1], followed by fractional recrystallisation to constant melting point and specific activity.

In contrast to the previous method[1], the radioactive 1 L-1,2-*O*-cyclohexylidene-1,2,5/3-[5-^{3}H]-cyclohexanetetrol was then hydrolysed with ethanolic hydrochloric acid and the resulting 1 L-1,2,5/3-[5-^{3}H]-cyclohexanetetrol (**4**) was regiospecifically tosylated to give 1 L-1,5-di-*p*-toluene-sulphonyl-1,2,5/3-[5-^{3}H]-cyclohexanetetrol (**5**) in high yield.

Azidolysis then gave 1 D-1,3,5/2-1,5-diazido-2,3-[5-^{3}H]-cyclohexanediol (**6**), which on catalytic reduction using ADAMS catalyst gave the required **7**, 1 D-2,6-[1-^{3}H]-dideoxystreptamine*, characterised as its sulphate.

Experimental

1 L-1,2-*O*-Cyclohexylidene-1,2,5/3-[5-^{3}H]-cyclohexanetetrol (**2**)

3 L-3,4-*O*-Cyclohexylidene-3,4/5-trihydroxycyclohexanone[1] (**1**, 137 mg, 0.6 mmole) was dissolved in ethanol (2 ml) and sodium borotritiide (5.1 mg, 10 mCi) in water (1 ml) was added and washed with a further 1 ml of water. After 30 minutes, cold sodium borohydride (10 mg) was added, and after a further 30 minutes the mixture was poured into chloroform-saturated sodium chloride solution (30 ml). The chloroform layer was separated and the aqueous layer further extracted with chloroform (5 × 30 ml). The chloroform layer was dried and the solvent removed in a vacuum to give a crystalline mixture of tritiated epimers (**2** and **3**). Pure 1 L-1,2-cyclohexylidene-1,2,5/3-cyclohexane-

* For compounds **1**~**6** the cyclitol nomenclature was used. The name of compound **7** conforms with the nomenclature of the aminocyclitol antibiotics.

tetrol[1] (5 g) was added and the mixture recrystallised repeatedly from ethyl acetate – hexane (1 : 1) (approx. 80 ml) until the specific activity was constant (11 μCi/mmole); yield 4 g, mp 129.5~130°C, $[\alpha]_D$ +6° (*c* 1.31, MeOH).

Found: C, 63.2; H, 8.75. $C_{12}H_{20}O_4$ requires C, 63.15; H, 8.80.

1 L-1,2,5/3-[5-^{3}H]-Cyclohexanetetrol (**4**)

1 L-1,2-*O*-Cyclohexylidene-1,2,5/3-[5-^{3}H]-cyclohexanetetrol (**2**) 4 g was dissolved in ethanol (40 ml) containing concentrated hydrochloric acid (4 ml) and left overnight before evaporation to dryness in a vacuum. The product **4** was recrystallised from ethanol – ethyl acetate; yield 2.6 g (99%), mp 212~214°C, 11.8 μCi/mmole, $[\alpha]_D$ +6° (*c* 2.2, H_2O).

Found: C, 48.40; H, 8.20. $C_6H_{12}O_4$ requires C, 48.60; H, 8.15.

1 L-1,5-Di-*O*-*p*-toluenesulphonyl-1,2,5/3-[5-^{3}H]-cyclohexanetetrol (**5**)

1 L-1,2,5/3-[5-^{3}H]-Cyclohexanetetrol (**4**, 2.6 g) was dissolved in dry pyridine (15 ml) and cooled to 0°C. Toluenesulphonyl chloride (9 g) in pyridine (20 ml) at 0°C was added rapidly with good magnetic stirring, excluding moisture. The temperature rose slowly and the solution was left for 18 hours at room temperature with continued stirring. The reaction mixture was then poured into chloroform-ice and extracted 3 times with chloroform (50 ml). The chloroform layers were dried and evaporated to dryness in a vacuum. The 1 L-1,5-di-*O*-*p*-toluenesulphonyl-1,2,5/3-[5-^{3}H]-cyclohexanetetrol (**5**) was crystallised from chloroform-cyclohexane, standing at least 3 days in the refrigerator; yield 5.78 g. (72%), mp 121~123°C, $[\alpha]_D$ +10° (*c* 1.0, CH_3OH).

Found: C, 52.85; H, 5.50; S, 14.20. $C_{20}H_{24}O_8S_2$ requires C, 52.65; H, 5.30; S, 14.05.

1 D-2,6-[1-^{3}H]-Dideoxystreptamine (**7**)

1 L-1,5-Di-*O*-*p*-toluenesulphonyl-1,2,5/3-[5-^{3}H]-cyclohexanetetrol (**5**, 5.78 g) was dissolved in dry hexamethylphosphoramide (15 ml) and sodium azide (3.3 g) was added. The mixture was heated at 80°C for 4 hours with magnetic stirring and after cooling it was poured into ice-water and extracted 3 times with chloroform. The chloroform layers were dried and evaporated yielding 2.2 g (88%) of 1 D-1,3,5/2-1,5-diazido-2,3-[5-^{3}H]-cyclohexanediol (**6**), recrystallized from ethylacetate-hexane, mp 62~63°C, $[\alpha]_D$ +2° (*c* 1.0, CH_3OH), 11.6 μCi/mmole.

Found: C, 36.47; H, 5.14; N, 42.34. $C_6H_{10}N_6O_2$ requires C, 36.36; H, 5.09; N, 42.41.

The diazide (**6**, 0.304 g) was dissolved in methanol (5 ml) and hydrogenated over ADAMS catalyst for 2 days. After removal of the catalyst, the solution was acidified with 2 N sulphuric acid, and methanol (4 ml) was added. The precipitate was filtered off and the colorless amorphous sulphate monohydrate salt of 1 D-2,6-[1-^{3}H]-dideoxystreptamine (**7**) was recrystallised from methanol; yield 0.255 g (63%), mp 231~233°C, $[\alpha]_D$ +4.6° (*c* 3.06, H_2O), 11 μCi/mmole.

Found: C, 27.66; H, 6.80; N, 10.55; S, 12.6. $C_6H_{18}N_2O_7$ requires C, 27.50; H, 6.91; N, 10.60; S, 12.6.

References

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