Mechanistic Studies on the Biosynthesis of the 2-Deoxystreptamine Ring of Neomycins

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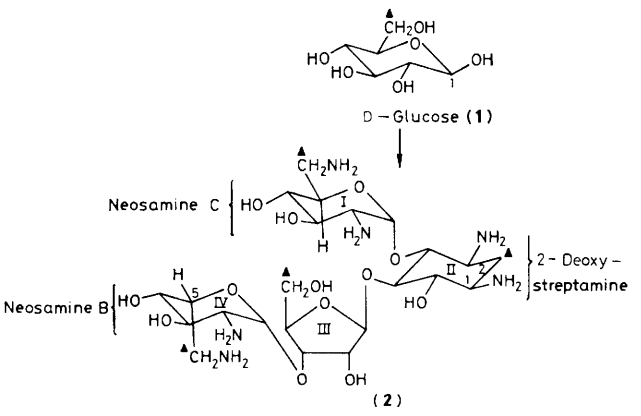
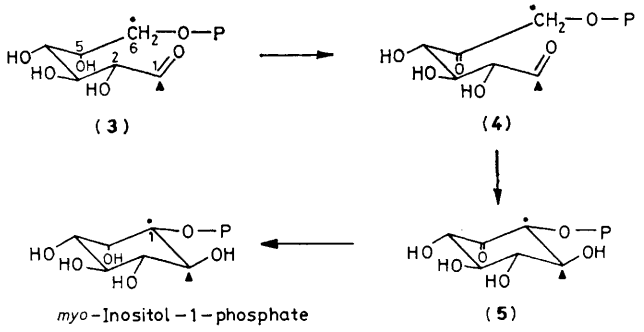
It is shown that both the C-6 hydrogen atoms of D-glucose are retained at C-2 of the 2-deoxystreptamine ring in the biosynthesis of neomycins.

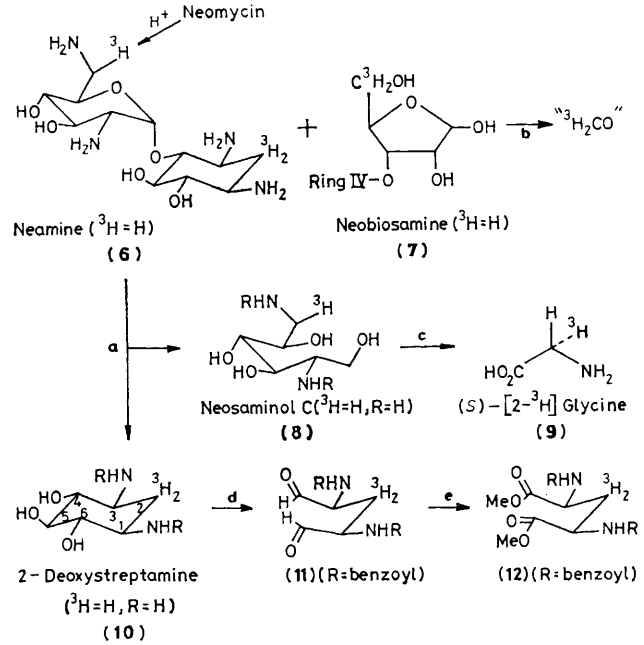
To continue our studies on various aspects of the biosynthesis of neomycins we now extend the approach of the preceding communication[1] to investigate the mechanism of the formation of the 2-deoxystreptamine ring of neomycin B and C (ring II in **2**, Scheme 1). Two types of aminocyclitol rings are present in antibiotics.[2] The hexa-substituted cyclohexane ring of streptamine is found in streptomycin and its 2-deoxycounterpart occurs in a large number of other aminocyclitol antibiotics.[2] With regard to the biosynthesis of 2-deoxystreptamine there is a view that like streptamine[3] this aminocyclitol may also be synthesised *via* the *myo*-inositol-1-phosphate pathway[4] (Scheme 2) necessitating the removal of the unwanted C-2 hydroxy group at a later stage. If this were the case then in a suitable biosynthetic experiment only one of the two hydrogen atoms at C-6 of glucose will be retained at C-2 of 2-deoxystreptamine. In order to shed light on this aspect we have now performed biosynthetic experiments using [6-$^3H_2$,*U*-$^{14}C_6$]-glucose and have determined the status of the two ^3H atoms at three of the four positions derived from C-6 of glucose.[2a] These



Scheme 1. Neomycin B, (**2**). Neomycin C, structure (**2**) with the stereochemistry at C-5 in ring IV that of D-glucose as in ring I. (▲, labelled carbon atom.)



Scheme 2



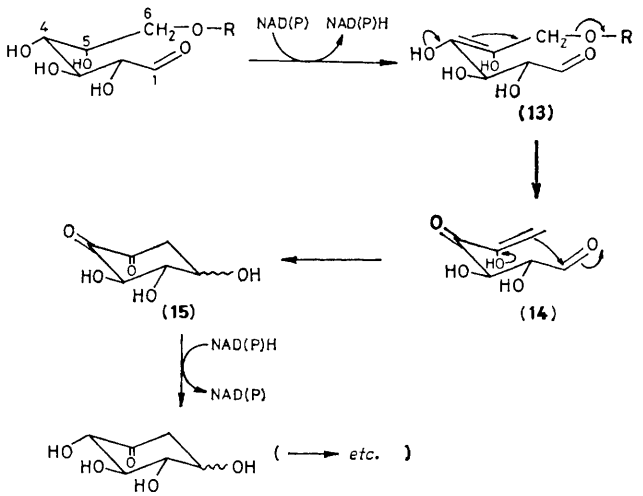
Scheme 3. (a) Acetic anhydride, H^+, $NaBH_4$; (b) acetic anhydride, $NaIO_4$; (c) acetic anhydride, $NaIO_4$–$KMnO_4$, H^+; (d) benzoyl chloride, $NaIO_4$; (e) Br_2–H_2O, CH_2N_2.

positions are C-6 of the neosamine C, C-2 of the 2-deoxystreptamine, and C-5 of the ribose rings of neomycins (rings I, II, and III respectively in **2**, Scheme 1).

[6-$^3H_2$,*U*-$^{14}C_6$]-D-Glucose (100 μmol, 64.1 × 10^6 d.p.m. of ^{14}C) was administered to 10 × 10 ml cultures of *Streptomyces fradiae* wild type ATCC 10745 under the conditions described previously.[1] The biosynthetic antibiotic was isolated by chromatography and was found to have 2.3 × 10^6 d.p.m. of ^{14}C (3.5% incorporation). The biosynthetic material was hydrolysed to obtain[1] neamine (**6**, Scheme 3) and neobiosamine (**7**, Scheme 3). The latter was acetylated and then treated with $NaIO_4$ to release C-5 of the ribosyl unit as formaldehyde, which was isolated as its dimedone derivative. The ^3H:^{14}C ratio of the C-5 of the ribose ring was identical to that of the C-6 of the glucose originally used in the biosynthetic experiment, thus showing that the incorporation of radioactivity into the antibiotic had occurred without the labilisation of the ^3H or the rearrangement of the carbon skeleton of the precursor. With the assurance that the carbon skeleton of glucose was incorporated into the antibiotic intact, the neamine obtained from the double label experiment was further degraded to furnish the ring I of the antibiotic as neosaminol C (**8**, Scheme 3) and the ring II as 2-deoxystreptamine (**10**, Scheme 3). The

Table 1

Compound	^3H:^{14}C Ratio	^3H:^{14}C Atomic ratio
1 Starting [6-$^3H_2$, *U*-$^{14}C_6$]-D-glucose as a glucose-6-phosphate	9.5	2:6
2 C-5 of the ribose ring as formaldehyde dimedone	52.47	1.84 :1
3 2-Deoxystreptamine (**10**)	9.4	1.97 :6
N,*N*′-Dibenzoyl-2-deoxystreptamine (**10**, R = benzoyl)	8.8	1.85 :6
The dialdehyde (**11**)	11.26	1.97 :5
The diester (**12**)	11.07	1.92 :5
4 *N*,*N*′-Diacetylneosaminol C (**8**, R = acetyl)	4.8	1.01 :6
Serine derived from (**8**)	0.6	0.063:3
Glycine derived from (**8**)	13.4	0.94 :2
Glycine after incubation with serine hydroxymethyl transferase	0.7	0.049:2



Scheme 4

^3H:^{14}C ratio of neosaminol C was found to be exactly half that of the starting glucose. This result, when taken in conjunction with the demonstration that in the labelling of the neosamine C ring from its preferred precursor, glucosamine, one of the C-6 hydrogen atoms of glucosamine is lost,[1] suggests that in the present study the loss of ^3H from [6-$^3H_2$]-glucose must have occurred during the elaboration of the aminomethyl group of the neosamine C ring. Furthermore, the ^3H remaining at C-6 of neosaminol C was found to be in the H_S position (see Table 1) as has been previously established for the corresponding experiments performed with [6-$^3H_2$]-glucosamine.[1]

We now turn to the 2-deoxystreptamine ring, which was found to have the same ^3H:^{14}C ratio as the parent glucose, thus suggesting that both the C-6 hydrogen atoms of glucose were retained in 2-deoxystreptamine. A comprehensive degradative protocol to establish that all the ^3H in the biosynthetic 2-deoxystreptamine was located at C-2 was not available; however, this feature was adequately assessed by the following experiments. No loss of ^3H occurred when *N*,*N*′-dibenzoyl-2-deoxystreptamine (**10**, R = benzoyl, Scheme 3) was subjected to the sequence of Scheme 3 to oxidise its C-4, -5, and -6, thus showing that the ^3H was resident in one of the remaining carbon atoms. There is much indirect enzymological evidence to suggest that the amino groups at C-1 and C-3 of 2-deoxystreptamine are introduced *via* the corresponding keto-derivatives.[5] Hence C-1 and C-3 are unlikely to contain the ^3H

originally present at C-6 of the precursor glucose. We therefore conclude that the ^{3}H is located at C-2. Using an elegant ^{2}H n.m.r. approach a similar retention of two ^{2}H atoms from [6-$^{2}H_{2}$]glucose during the biosynthesis of the deoxystreptamine ring of ribostamycin by *Streptomyces ribosdificus* has recently been demonstrated.[6] The approach of the Japanese workers, and that described in the present study, complement each other and rule out the intermediacy of *myo*-inositol-1-phosphate (or its equivalent) produced by the condensation reaction catalysed by inositol synthase in the elaboration of the 2-deoxystreptamine ring in the biosynthesis of the two antibiotics. An alternative cyclisation process leading to the formation of 2-deoxystreptamine and yet broadly patterned on the inositol synthase reaction is shown in Scheme 4. This proposal assumes that a glucosyl intermediate upon oxidation at either C-4 or C-5 followed by prototropic rearrangement furnishes an enediol intermediate (**13**, Scheme 4) which undergoes an elimination reaction producing the intermediate (**14**, Scheme 4) containing the putative deoxy-carbon atom of 2-deoxystreptamine. It is interesting to draw attention to the fact that C-6 in the intermediate of type (**14**) may either be involved in a nucleophilic role reacting with the C-1 of an aldo-hexose (**14** to **15**, Scheme 4) or function as an electrophile for combining with the C-1 of a keto-hexose.

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