The Involvement of C-4 of D-Glucose in the Biosynthesis of the 2-Deoxystreptamine Ring of Neomycin

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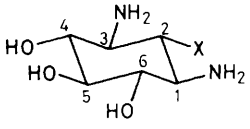
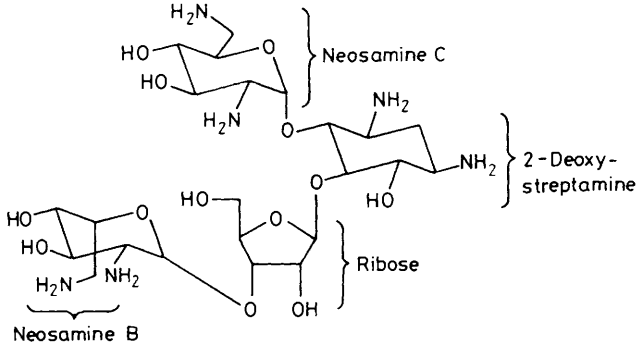
A mechanism, rationalising the loss of the C-4 hydrogen atom of D-glucose in the biosynthesis of the deoxystreptamine ring (**2**) of neomycin (**3**), is considered.

A large group of clinically useful antibiotics, to which streptomycin and neomycin belong, are classified as aminoglycoside-aminocyclitol antibiotics. The name highlights the fact that these antibiotics, in addition to an array of variously modified sugars, contain an unusual cyclohexane derivative which can either be hexasubstituted as the streptamine unit (**1**) of streptomycin or pentasubstituted as the 2-deoxystreptamine ring (**2**) that is present in neomycin (**3**) and more than fifty other related antibiotics. It is now known that the carbon skeletons of both types of aminocyclitols (**1**) and (**2**) arise from D-glucose,[1,2] and the elegant studies of Walker and Walker[3] have established that, in the formation of the streptamine ring system of streptomycin, the key event is the inositol synthase-catalysed cyclisation of glucose 6-phosphate (**4**) into inositol 1-phosphate (**5**) (Scheme 1). The latter, through a multistage process, is then converted into streptidine [this is a diguanidinated derivative of (**1**)]. The knowledge of this biosynthesis provided the stimulus to consider the possibility that a cyclisation process similar to that shown in Scheme 1, followed by the reductive removal of the unwanted hydroxy group, may be involved in the formation of 2-deoxystreptamine (see citations in ref. 4). Gradually however observations were made which questioned this hypothesis and suggested that the two aminocyclitols (**1**) and (**2**) may be synthesised by two entirely different mechanisms. For example, the patterns for the incorporation of the C-skeleton of D-glucose into the two aminocyclitols were found to be different; the C-6 of glucose occupied position 2 in deoxystreptamine[4] but position 6 in the streptamine ring.[5,6] Furthermore, our work on neomycin[7] and the studies of Kakinuma *et al.* on ribostamycin[8] revealed that the 2-deoxystreptamine ring of these antibiotics was formed from D-glucose with the retention of both of its C-6 hydrogen atoms. Since the mechanism of the type shown in Scheme 1 requires the loss of one of the C-6 hydrogen atoms of glucose,[9] the results[7,8] suggested that the pathways for streptamine and 2-deoxystreptamine biosynthesis diverged prior to the cyclisation stage, and led to the proposal of two closely related mechanisms[7,8] (paths A and B, Scheme 2) for the construction of the deoxy centre of the aminocyclitol (**2**). The common feature in both the mechanisms is the involvement of enolic intermediates [(**7**) and (**10**), Scheme 2] which participate in nucleophilic attack at the C-1 carbonyl producing a deoxy carbocyclic ring. The difference in the two mechanisms, however, is that whereas in path A (Scheme 2) the enolic

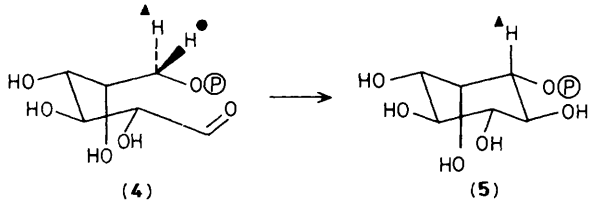


(**1**); X = OH

(**2**); X = H



Neomycin B (**3**)



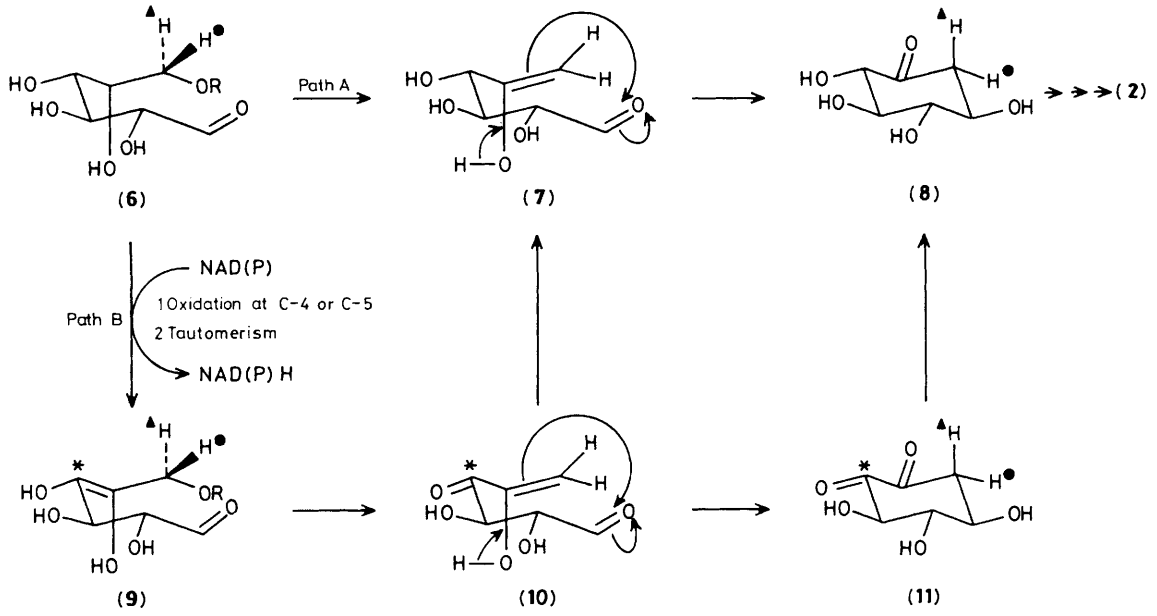
(**4**) (**5**)

Scheme 1

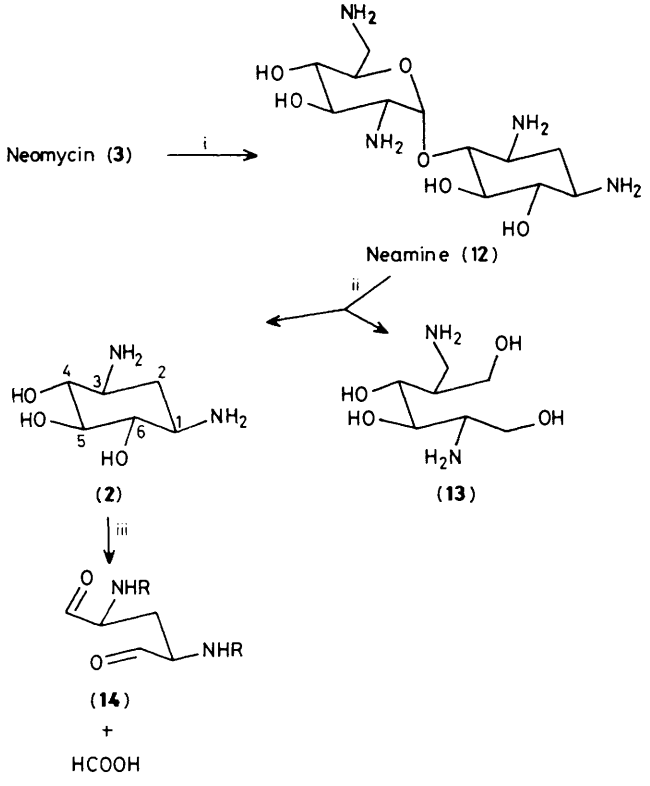
Table 1. ^{3}H : ^{14}C and atomic ratios[a] of the various subunits of neomycin biosynthesised from double-labelled glucose.

	Subunits			
Precursor	Neosaminol C (**13**)	2-Deoxystreptamine (**2**)	Dialdehyde (**14**)	Formic acid [C-5 of (**2**)]
[U-^{14}C; 3-^{3}H, 4-^{3}H]glucose 7.7 (2 : 6)	7.5 (1.94 : 6)	3.7 (0.97 : 6)	0 (0 : 5)	20.4 (0.88 : 1)
[U-^{14}C; 3-^{3}H]glucose 7.8 (1 : 6)	7.9 (1.01 : 6)	7.9 (1.01 : 6)	0 (0 : 5)	37 (0.80 : 1)

[a] In parentheses.



Scheme 2



Scheme 3. *Reagents and conditions:* i, HCl (1.5 M); ii, Ac_2O, HCl (4 M), $NaBH_4$; iii, $NaIO_4$.

species is produced *via* a direct elimination reaction necessitating the removal of a non-activated C-5 hydrogen, this feature is avoided in the alternative mechanism (path B) where hydrogen removal is facilitated by the generation of a carbonyl function. The two proposals are thus differentiated by the fact that, whereas in path A the C-4 of glucose is not involved, removal of a hydrogen atom from this position is an important requirement of the alternative mechanism (path B).

In this communication we have evaluated these mechanisms through the incorporation of variously labelled D-glucose samples into neomycin and the degradation of biosynthetic antibiotic, to locate the position(s) of the labelled hydrogen. The extent of ^3H retention or loss in the biosynthesis was quantified by using a double labelled approach in which [U-^{14}C]D-glucose served as an internal reference.

Samples of [U-^{14}C; 3-^{3}H, 4-^{3}H]D-glucose or [U-^{14}C; 3-^{3}H]D-glucose were fed to growing cultures of *Streptomyces fradiae* (wild type ATCC 10745), and neomycin was isolated and purified, as described elsewhere.[10]

Based upon the ^{14}C radioactivity initially used, the incorporation of D-glucose into the antibotic in these experiments was about 0.9%. The samples of the labelled neomycin were processed to obtain the rings I and II of the antibiotic as neamine (**12**) (Scheme 3) which was converted into the tetra-*N*-acetyl derivative and subjected to acid hydrolysis. Following treatment with $NaBH_4$ the mixture was separated by chromatography on CG50 (NH_4^+) resin to obtain the two rings of the antibiotic as neosaminol C (**13**) and deoxystreptamine (**2**).[7,10]

Table 1 shows that neosaminol C originating from both the incorporation experiments had the same ^3H : ^{14}C ratio as the sample of the parent glucose. These data provide the assurance that, under the incubation conditions used, the carbon skeleton of glucose was incorporated into the antibiotic intact and without prior labilisation of its labelled hydrogen atoms.

We now turn to 2-deoxystreptamine and note that the ^3H : ^{14}C ratio of this unit was half that of glucose when the latter contained ^3H at C-3 as well as C-4 thus suggesting that in the biosynthesis one of the hydrogen atoms of the precursor, [U-^{14}C; 3-^{3}H, 4-^{3}H]D-glucose, was removed. Since the sample of [U-^{14}C; 3-^{3}H]D-glucose was incorporated into 2-deoxystreptamine with complete maintenance of its ^3H : ^{14}C ratio, this proves that it must be the C-4 tritium that is labilised in the biosynthesis from the 3,4-tritiated glucose. The fact that in both cases the tritium remaining in 2-deoxystreptamine was resident at its C-5 (which corresponds to C-3 of glucose) was

shown by the isolation of C-5 as formate containing all the ^3H radioactivity of the ring, while the aldehyde (**14**) (Scheme 3) containing the remaining carbon skeleton (C-1, C-2, C-3, C-4 and C-6) of 2-deoxystreptamine was completely devoid of any ^3H. These results unambiguously prove that the biosynthesis of the 2-deoxystreptamine ring of neomycin is attended by the loss of the C-4 hydrogen atom of the precursor glucose. This finding is consistent with the basic tenets of the mechanism involving redox changes at the starred carbon atom (*) as suggested in the mechanism of path B (Scheme 2). The stage at which the carbonyl function at C* is reduced to produce the corresponding hydroxy group of 2-deoxystreptamine is not yet predictable. An alternative explanation of the results, invoking the possibility that the C-4 hydrogen atom of glucose may have been lost not in the cyclisation process, but in post-cyclisation modification, though in our view unlikely, cannot be ruled out at this stage.

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