Communications to the Editor

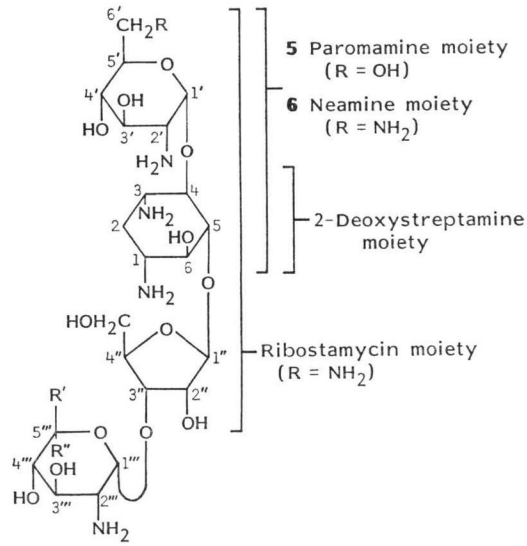
NEOMYCIN BIOSYNTHESIS: THE INVOLVEMENT OF NEAMINE AND PAROMAMINE AS INTERMEDIATES

Sir:

Neomycin (**1** and **2**) is an aminocyclitol antibiotic produced by *Streptomyces fradiae*, and both its structure and biosynthesis have been studied in this laboratory for a number of years[1,2]. Although some features of the biosynthetic route leading to neomycin have been elucidated, there remain a number of questions unanswered. One of these pertains to the stage in the biosynthesis of the aminoglycosyl subunits at which they are attached to their neighbor, and another to the order in which all the subunits are linked. In the latter connection, work on the related gentamicins[3], sisomicins[4], sagamicins[5], ribostamycin[6], and butirosin[7] suggests that the 2-deoxystreptamine unit is glycosylated in the 4-position first and in the 5- or 6-position second. There is also evidence that the neomycin/paromomycin family use a similar order of addition[8].

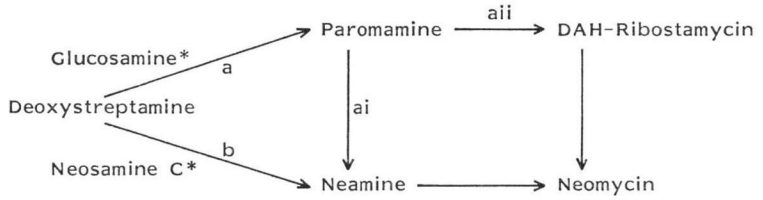
With regard to the former question, experiments on the biosynthesis of gentamicins, sisomicins, sagamicins, ribostamycin, and butirosin suggest that 2-deoxystreptamine is glycosylated with subunits which are incomplete and which are further functionalized after the formation of either a pseudo-di- or trisaccharide. It has been proposed that in each case deoxystreptamine is glycosylated with glucosamine to produce paromamine (**5**), which is then either converted to neamine (**6**) by amination at the 6-position and then glycosylated with a third unit, or is further glycosylated and then aminated in the 6-position. Circumstantial evidence that this occurs in neomycin biosynthesis has been presented by the isolation of paromamine (**5**) and paromomycin (**3** and **4**) from *S. fradiae* cultures[9] which indicates these may be intermediates. In addition, the Roussel Uclaf group[10] have isolated 6‴-deamino-6‴-hydroxy analogs of neomycins from *S. fradiae*. Thus it can be argued that the diamino residue on the 4-position of deoxystreptamine is added as its 2-amino precursor, and that a similar second unit is added as the 2-amino precursor to produce the 6‴-deamino-6‴-hydroxy analogs isolated.

One approach to clarifying some of these possibilities is to examine the incorporation of labeled



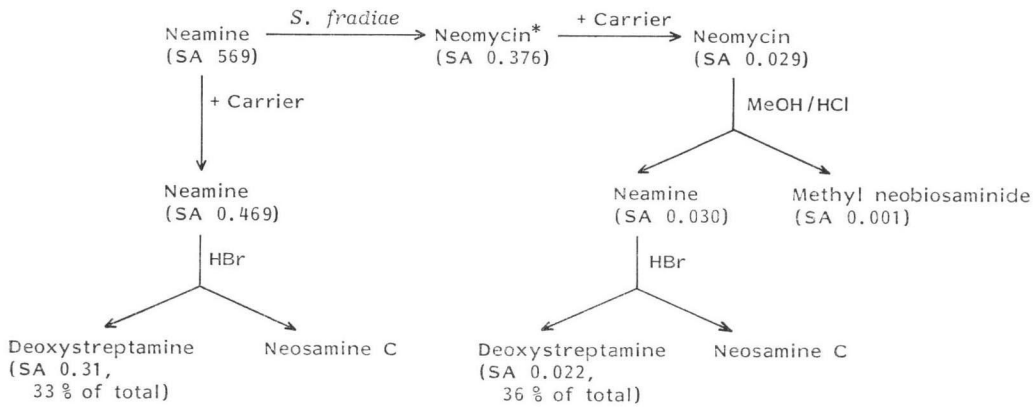
		R	R′	R″
1	Neomycin B	NH_2	H	CH_2NH_2
2	Neomycin C	NH_2	CH_2NH_2	H
3	Paromomycin I	OH	H	CH_2NH_2
4	Paromomycin II	OH	CH_2NH_2	H

Scheme 1. Possible biosynthetic routes to neomycin.



* Activated, perhaps as a nucleotide diphosphate.

Scheme 2. Biosynthesis and degradation of [^{14}C]neomycin from [^{14}C]neamine.



SA=Specific activity (μCi/mM).

* Separated from recovered neamine on Dowex 1X2 and monitored by TLC (Polygram SiLG/UV254 silica gel; $CHCl_3$ - MeOH - concd.NH_4OH - H_2O, 20: 80: 40: 20 and 1-BuOH - EtOH - concd. NH_4OH - $CHCl_3$, 40: 50: 50: 20).

neamine and paromamine into the neomycins. In principle, there are three possible results, as shown in Scheme 1: either neamine or paromamine or both may be incorporated into neomycins, the simple explanation being that neamine or paromamine or both are precursors. This paper presents data which support route b of Scheme 1.

[*G*-^{14}C]Neamine and [*G*-^{14}C]paromamine were produced by methanolysis[1] of [*G*-^{14}C]neomycin and [*G*-^{14}C]paromomycin*, respectively. *S. fradiae* (3535x) was grown in seed medium[11] for 2 days and 0.25 ml was then used to inoculate 50 ml of production medium[11]. Cultures were incubated at 30°C on a rotary shaker. Radioactive precursors (neamine or paromamine) were added at various times and neomycins isolated after 5 days incubation by passing the supernatant from centrifugation through Amberlite IRC-50 resin in the NH_4^+ form, followed by elution with 2 M NH_3 solution. The crude extract was further purified by chromatography over Dowex 1X2 (OH^- form), eluting with water, which clearly separated neamine from neomycin.

The results show that neamine is incorporated into neomycin to the extent of 44% (1500-fold dilution) when added to a 2-day-old culture. Lower incorporations into neomycin were observed when radioactive neamine was added at the start of fermentation and after 1, 3 and 4 days. The remainder of the radioactivity in each case was found in recovered neamine. Minimal activity was found in neomycin when neamine was added at day 5. To determine that the neamine was being incorporated into neomycin without extensive degradation and resynthesis, the following experiment (Scheme 2) was performed: 5 μCi of [*G*-^{14}C]neamine, specific activity 569 μCi/mM, was added to *S. fradiae* 48-hour-old cultures (2×50 ml). After a further 3 incubation days, the neomycin was isolated, purified, and the specific activity determined. Unlabeled neomycin carrier was added and the neomycin degraded using methanolic hydrochloric acid to give neamine and methyl neobiosaminide, which were purified using silica gel column chromatography ($CHCl_3$ - CH_3OH - NH_3 - H_2O, 20: 80: 40: 20). Neamine was further degraded to give deoxystreptamine using 48% hydrobromic acid. The specific activity of each of the products is given in Scheme 2. From these results it can be seen that the neamine appears to be converted into neomycin without any detectable degradation.

When [*G*-^{14}C]paromamine was added to *S. fradiae* cultures and the neomycin isolated after 5 days and purified, negligible incorporation of label was detected.

Taken together, these results support the hypothesis that neamine is a direct precursor for neomycin biosynthesis, whereas paromamine is not,

* Produced by growing *S. fradiae* and *Streptomyces rimosus* forma *paromomycinus*, respectively, in the presence of D-[*U*-^{14}C]glucose.

excluding pathways ai and aii of Scheme 1. If this conclusion is correct, then it suggests a fundamental difference between neomycin synthesis and that of gentamicin, sisomicin, sagamicin, ribostamycin, and butirosin, again assuming those prior conclusions to be correct. Thus one can speculate that deoxystreptamine is glycosylated with a preformed subunit, 2,6-diamino-2,6-dideoxyglucose, possibly added *via* a nucleoside diphosphate intermediate. Finally, in view of the differing procedures employed in this study of neomycin and earlier studies of other aminocyclitols it may be interesting to reinvestigate the biosynthesis of the other aminocyclitol antibiotics using radiolabeled precursors to confirm the various biotransformations.

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