The Biosynthesis of Deoxynojirimycin and Deoxymannonojirimycin in *Streptomyces subrutilus*

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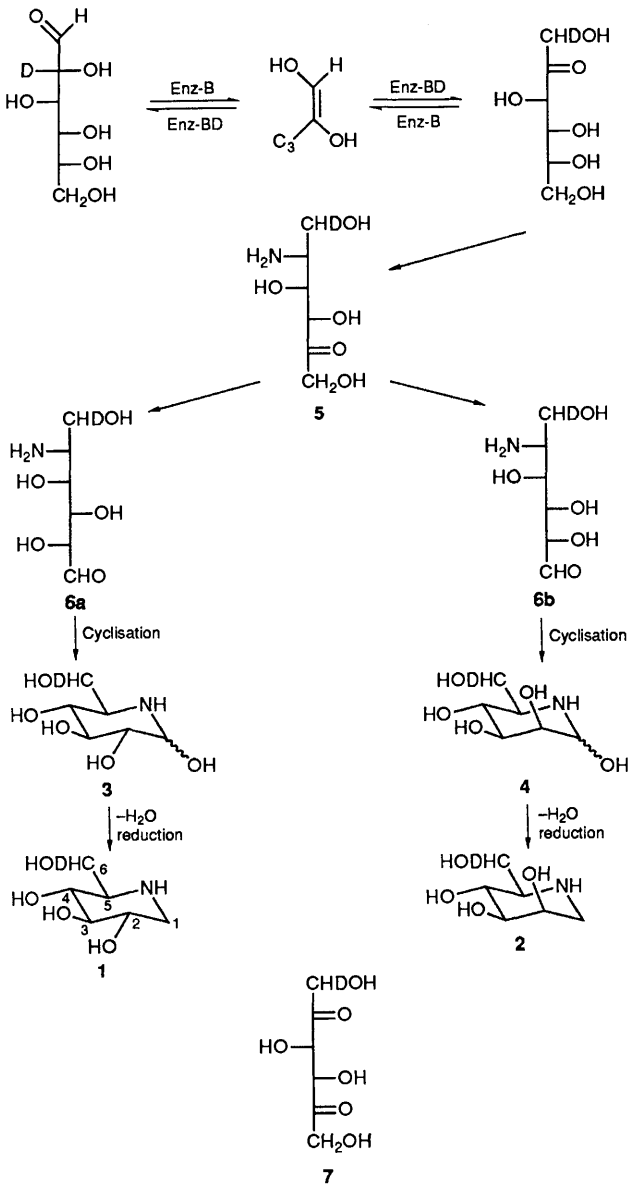
Glucose is the biosynthetic precursor of deoxynojirimycin and deoxymannonojirimycin in *Streptomyces subrutilus*.

The envelope glycoprotein gp120 of HIV-1, the causative agent of AIDS plays a critical role in the infectious cycles of the virus. Anti-HIV alkaloids *e.g.* deoxynojirimycin (DNJ) **1** inhibit the replication of HIV-1 by inhibiting glycosidases involved in the biosynthesis of *N*-linked oligosaccharides of gp120.[1] Several chemical syntheses of DNJ have been published[2] and DNJ together with deoxymannonojirimycin (DMJ) **2** are produced by plants and streptomycetes.[3,4] Little has been published on the biosynthesis of these alkaloids apart from a brief mention that glucose is a precursor to nojirimycin **3** in an unspecified organism (presumably a streptomycete).[5] Full details of this work have never been published, we now wish to report that glucose is a biosynthetic precursor of both DNJ and DMJ. This contrasts with the biosynthesis in *Rhizoctonia leguminicola* of swainsonine, another alkaloid which inhibits glycosidases, where the biosynthetic precursor is pipecolic acid.[6]

Previously, the production of DNJ and DMJ by *Streptomyces lavendulae* ATCC 31434 has been reported.[3,4] We have detected both DNJ and DMJ in culture broths of *Streptomyces subrutilus* ATCC 27467 and fermentation conditions have been developed which result in the production of DNJ and DMJ from glucose as a carbon source allowing us to use [1-^{2}H]- or [2-^{2}H]-D-glucose in biosynthetic experiments. Deuteriated DNJ and DMJ were isolated from the culture media as their peracetylated derivatives which were deacetylated to *N*-acetyl-DNJ and free DMJ for spectroscopic assignments. In peracetylated DNJ and DMJ, deuterium from



Scheme 1

[1-^{2}H]-D-glucose (30% dilution) was retained (28% by mass spectrometry) in the C-6 methylene group only. [2-^{2}H]-D-Glucose (25% dilution) gave a much lower deuterium incorporation into peracetylated DNJ and DMJ (5% incorporation by mass spectrometry) and the label was again found in the C-6 methylene group only in both compounds.

We assume that the assignment[7] of the signals in the ^{1}H NMR spectrum of glucose due to the pro-*R* (δ 3.61, *J* 12.3 and 5.7 Hz) and pro-*S* (δ 3.78, *J* 12.3 and 2.2 Hz) protons at C-6 can be applied to the protons at C-6 in DNJ and DMJ derivatives. The ^{1}H NMR spectrum of *N*-acetyl-DNJ confirms that this compound adopts a very similar chair conformation to glucose and the C-6 protons have very similar chemical shifts and coupling constants to glucose, pro-*R* (δ 3.69, *J* 12.4 and 5.6 Hz), pro-*S* (δ 3.82, *J* 12.4 and 3.0 Hz). The ^{1}H NMR spectra of deuteriated mono-*N*-acetyl-DNJ and deuteriated free DMJ derived from [1-^{2}H]-D-glucose, showed that the signals due to the pro-*S* proton at C-6 at δ 3.82 (*N*-acetyl-DNJ) and 3.85 (DMJ) were diminished in intensity compared with the unlabelled analogues. Corresponding signals at δ 3.79 and 3.86, respectively were present in the ^{2}H NMR spectra of deuteriated *N*-acetyl-DNJ and deuteriated free DMJ. The intensity of the signals due to pro-*R* proton at C-6 which appear at δ 3.69 (*N*-acetyl-DNJ) and 3.75 (DMJ) were unaltered in comparison with the unlabelled analogues indicating no replacement of the pro-*R* proton by deuterium. When [2-^{2}H]-D-glucose was the carbon source in the biosynthetic experiments, the pro-*R* proton at δ 4.63 (peracetylated DNJ) and 4.81 (peracetylated DMJ) was labelled.

Our evidence suggests a biosynthetic pathway in which glucose is isomerised to fructose *via* an enediol (Scheme 1),[8] a basic group in the active site of the enzyme removing the proton at C-2 and adding it to the carbonyl group at C-1. During this isomerisation, exchange of this proton with solvent can occur accounting for the low incorporation of deuterium from [2-^{2}H]-D-glucose into DNJ and DMJ. However, little loss of label can occur from C-1 when [1-^{2}H-]-D-glucose is the precursor. Further evidence for a glucose–fructose isomerisation is provided by the incorporation of deuterium at the pro-*S* proton at C-6 in DNJ and DMJ from [1-^{2}H]-D-glucose. On the other hand, incorporation of deuterium from [2-^{2}H]-D-glucose occurs at the pro-*R* proton at C-6 in DNJ and DMJ. This labelling pattern has previously been observed in the isomerisation of [1-^{2}H]- and [^{2}H-]-D-glucose catalysed by glucose isomerase.[9]

Amination of the fructose at C-2 gives an aminosugar or its enzyme-bound derivative. Oxidation of the aminosugar leads to **5** which can undergo a ketose–aldose isomerisation between C-5 and C-6 to produce the epimers **6a** and **6b**. Cyclisation of which to nojirimycin **3** or mannonjirimycin **4** followed by dehydration and reduction leads to DNJ and DMJ, respectively. Thus a C-1 to C-6 inversion has occurred during this biosynthesis with C-1 of glucose becoming the exocyclic C-6 in DNJ and DMJ as is confirmed by the labelling experiments.

Alternative biosynthetic intermediates are possible including 5-ketofructose **7** which could be formed at an early stage in the pathway by oxidation of fructose. However, **7** is a symmetrical compound and if it were to be an intermediate which could dissociate freely from enzymes in the pathway, deuterium labelling at C-1 and C-6 would be expected which is contrary to experimental observations. If **7** were to remain enzyme-bound, *e.g.* as a Schiff base, during the rest of the biosynthetic pathway then dissymmetric labelling of the DNJ and DMJ would be expected.

Further studies are in progress to elucidate the precise biosynthetic pathway to DNJ and DMJ in streptomycetes.

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