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The Biosynthesis of Aristeromycin. Conversion of Neplanocin A to Aristeromycin by a Novel Enzymatic Reduction

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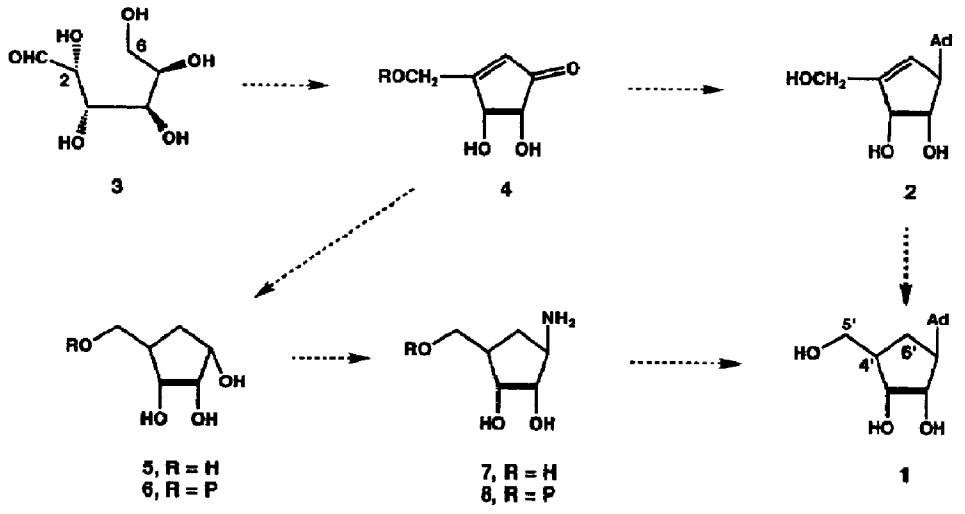
Abstract: Partially purified cell-free extracts of the aristeromycin producer *Streptomyces citricolor* have been shown to catalyze the NADPH-dependent reduction of neplanocin A to aristeromycin. Stereochemical studies revealed that the reduction proceeds with anti geometry and involves transfer of the 4 *pro-R* hydrogen atom of NADPH to the 6'β position of aristeromycin.

The nucleoside antibiotic aristeromycin (**1**) (Scheme I) is a naturally occurring carbocyclic analogue of adenosine produced by *Streptomyces citricolor*.[1,2] The compound exhibits a variety of interesting biological activities,[3,4] such as inhibition of cell division and elongation in rice plants, inhibition of AMP synthesis in mammalian cells, and inhibition of the enzyme S-adenosylhomocysteine hydrolase.[5] Previous investigations have shown that the cyclopentane ring present in **1** is generated by a C-C bond forming reaction between C-2 and C-6 of glucose (**3**) which proceeds in such a manner that C-1 of glucose becomes C-5' of **1** and C-6 of glucose becomes C-6'.[6] These studies also provided

Scheme I



evidence that the cyclization of glucose proceeds by oxidation at C-4 or C-5 of the hexose followed by formation of a cyclopentenone derivative (**4**, R = H or P). This conclusion was supported by the isolation of the potent antitumor agent[7,8] neplanocin A (**2**) from the fermentation broth of *S. citricolor*.[6] The later stages of the pathway were hypothesized to involve conversion of the cyclopentenone **4** to a 1α, 2α, 3α-trihydroxy-4β-hydroxymethylcyclopentane derivative (**5** or **6**) which could then be transformed into aristeromycin via the aminotriols **7** or **8** (Scheme I). Evidence for the presence of **5** or **6** and **7** or **8** in *S. citricolor* was obtained by isotope dilution experiments.[9,10]

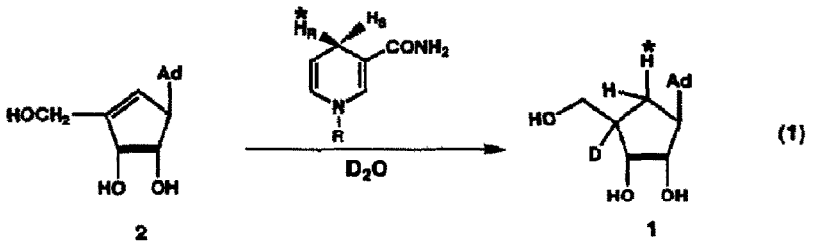
An indication that the biosynthetic pathway to aristeromycin and neplanocin A may be more complex than is implied by Scheme I was provided by the studies of Roberts et al.[11] These investigators prepared *S. citricolor* mutants defective in aristeromycin biosynthesis and examined their cosynthetic behavior. The most interesting finding from these studies was that some aristeromycin non-producing mutants could produce aristeromycin when supplied with neplanocin A. These results suggested that *S. citricolor* may be capable of reducing neplanocin A to aristeromycin (Scheme I). We would now like to report the results of enzymological studies that support this conclusion.

Cell-free extracts were prepared from aristeromycin producing cultures of *S. citricolor* by lysozyme treatment. Partial purification of the crude protein extract by DEAE chromatography, ammonium sulfate fractionation, and chromatography on phenyl agarose yielded a cell-free system that appeared to convert neplanocin A into aristeromycin, as judged by HPLC analysis. The production of aristeromycin was dependent upon the addition of NADPH to the incubation mixture, while the addition of NADH was less effective. A combination of FAD and NADPH was no more effective in stimulating the reaction than NADPH alone, and no activity was observed when only $NADP^+$ or NAD^+ was added to the extract. Proof that the product of neplanocin A reduction was in fact aristeromycin was obtained by isolation of the product by preparative HPLC followed by NMR and mass spectral analysis.

In order to gain some insight into the mechanism of the reduction reaction, both the regiochemistry of the reduction and the face selectivity with respect to NADPH were determined. Incubation of neplanocin A with NADPH and the partially purified enzyme in a deuterated buffer yielded a sample of aristeromycin whose ^1H and ^2H NMR spectra indicated that only one deuterium atom had been incorporated into aristeromycin and that this deuterium resided at C-4' ($\delta = 2.33$).[2,12] [4(*R*)-^2H]- and [4(*S*)-^2H]-NADPH were then synthesized,[13] and utilized in the enzymatic reduction. Reduction of neplanocin A to aristeromycin in the presence of [4(*R*)-^2H]-NADPH yielded a sample of antibiotic whose ^1H and ^2H NMR spectra demonstrated that one deuterium atom had been incorporated into the molecule and that this deuterium was present at the 6'β position ($\delta = 1.78$).[6] An incubation with [4(*S*)-^2H]-NADPH carried out under identical conditions yielded aristeromycin in which no deuterium could be detected by ^1H or ^2H NMR analysis. The results of these labeling studies are summarized in equation 1.

Several conclusions can be drawn from these experiments. First, the enzymatic reduction proceeds with transfer of hydrogen from the 4 *pro-R* position of NADPH. In contrast, the three double-bond reductions involved in the conversion of lanosterol to cholesterol by rat liver microsomes have been shown to transfer hydride from the 4 *pro-S* face of NADPH.[14] Second, the stereochemistry of the

double-bond reduction is anti, a conclusion which is in agreement with earlier observations which demonstrated that the 6'α hydrogen atom of aristeromycin is derived from the 6 *pro-R* hydrogen atom of D-glucose.[6] Third, the reduction proceeds with the addition of hydride to the double-bond in an anti-Markovnikov fashion. This regiochemistry is somewhat surprising since the previously reported[14-17]



(1)

examples of the reduction of isolated double-bonds or dienes by NADPH-requiring enzymes exhibit a Markovnikov orientation, i. e. the mechanism of the latter reductions can be rationalized as proceeding by protonation of the double-bond to yield the more stable carbonium ion followed by quenching of the carbonium ion with hydride. The alternate regiochemistry observed for neplanocin A reduction can be rationalized if the reaction proceeds in three stages: 1) oxidation of 2 to the corresponding unsaturated aldehyde, 2) 1,4-reduction of the unsaturated aldehyde with NADPH, and 3) reduction of the aldehyde moiety in the saturated aldehyde back to the alcohol level. This mechanism would be consistent with fact that approximately 43 - 59% of the tritium is lost from C-1 of glucose during its conversion to aristeromycin,[6] provided that the neplanocin A formed from the [1-^{3}H]-D-glucose is non-stereospecifically tritiated at C-5'.[18] However, the fact that no deuterium incorporation into C-5' of aristeromycin was observed in the incubations with either 4 *pro-R* or 4 *pro-S* labeled NADPH suggests that the hypothetical oxidation of the C-5' hydroxyl group may be carried out by another cofactor besides $NADP^+$. Additional studies will clearly be required in order to elucidate the precise nature of the double-bond reduction mechanism. Finally, our results clearly demonstrate that *S. citricolor* can reduce the double-bond in neplanocin A to produce aristeromycin. Since evidence has also been obtained for the presence in *S. citricolor* of saturated carbocyclic compounds apparently related to aristeromycin,[9,10] the role of the latter compounds in aristeromycin biosynthesis will need to be clarified by future investigations.

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18. An alternative oxidation-reduction mechanism that involves oxidation of C-3' of neplanocin A appears less likely in view of the fact that no tritium is lost from C-3 of glucose, which corresponds to C-3' of **1**, during its incorporation into aristeromycin.[6]

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