Investigations of Aristeromycin Biosynthesis: Evidence for the Intermediacy of a 2α,3α-Dihydroxy-4β-(hydroxymethyl)cyclopentane-1β-amine

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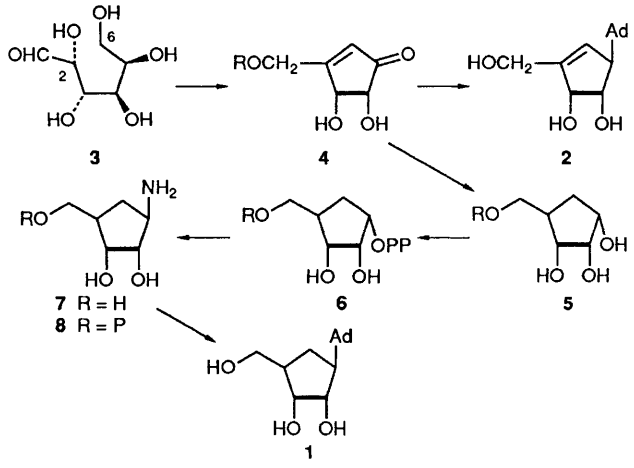
Administration of doubly-labelled forms of D-glucose to the fermentation broth of *Streptomyces citricolor* followed by isotopic trapping has provided evidence for the intermediacy of a 2α,3α-dihydroxy-4β-(hydroxymethyl)cyclopentane-1β-amine **7** or **8** in the biosynthesis of the nucleoside antibiotic aristeromycin **1**.

The naturally occurring carbocyclic nucleosides aristeromycin[1] **1** and neplanocin A[2] **2** (Scheme 1) are of current interest due to their biological activities,[2–6] their biosynthesis[7] and their potential as starting materials for the preparation of useful pharmacological agents. Previous investigations examining the biosynthesis of aristeromycin and neplanocin A in *Streptomyces citricolor* have shown[7] that the carbocyclic ring present in **1** and **2** is created by C–C bond formation between C-2 and C-6 of D-glucose **3** *via* a process that involves oxidation at C-4 or C-5 of a hexose followed by a cyclization that is presumed to lead to a cyclopentenone derivative **4** (R = H or P). The later stages of the pathway are thought to involve conversion of the cyclopentenone **4** to a carbocyclic pyrophosphate **6** (R = H or P) *via* a tetrol **5** (R = H or P). Evidence for the intermediacy of **5** in aristeromycin biosynthesis has recently been obtained by isotope dilution experiments.[8] Once formed, the pyrophosphate **6** could be converted into aristeromycin in two ways. One route would proceed *via* an adaptation of the purine salvage pathway,[9] namely, the reaction of **6** with adenine to produce aristeromycin or aristeromycin 5′-monophosphate. Alternatively, the conversion of **6** to aristeromycin could be accomplished by the stepwise assembly of the adenine ring in a manner analogous to the biosynthesis of the adenine ring of adenosine.[10] Some evidence for the operation of the purine salvage pathway in aristeromycin biosynthesis was obtained in earlier work,[7] but the operation of the salvage route does not preclude the possibility that the adenine ring of aristeromycin can also be assembled in a stepwise fashion. On the basis of the analogy provided by adenosine biosynthesis, the first committed step in the stepwise route to **1** would involve the conversion of the pyrophosphate **6** into the amine triol **7** or **8**. We have now obtained evidence for the intermediacy of compound **7** or **8** in aristeromycin biosynthesis. The results of these studies are reported here.

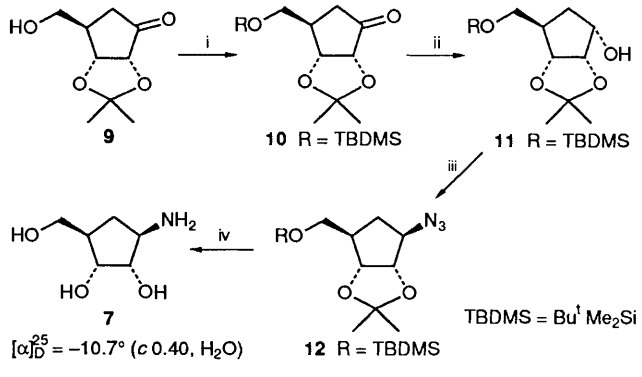
The amine triol **7** was synthesized from the previously prepared[8] cyclopentanone (−)-**9** by the route outlined in Scheme 2. Treatment of **9** with *tert*-butyldimethylsilyl trifluoromethanesulphonate (TBDMSOTf) in the presence of 2,6-lutidine gave the protected alcohol **10** in high yield. Stereoselective reduction of **10** with sodium borohydride then generated the α-alcohol **11**. Mesylation of **11** followed by displacement of the mesylate with sodium azide in hot dimethylformamide (DMF) afforded azide **12**, which has the correct relationship between the four asymmetric centres. Finally, catalytic reduction of the azide functionality with Lindlar's catalyst[11] and acid-catalysed deprotection of the reduction product yielded the desired amine triol (−)-**7**. The physical and spectral properties of **7** agreed with those previously reported.[12]

The amine triol **7** having been prepared, isotope dilution studies were carried out. Radiolabelled D-glucose was pulse fed at 60 h and 80 h to cultures of *S. citricolor* growing in the normal production medium. After 90 h, the mycelium from 65 ml of broth was harvested by centrifugation, washed twice with distilled water, and, after resuspending in distilled water, disrupted by sonication. The amine triol **7** was then added as a carrier and the cellular debris removed by centrifugation. The supernatant was lyophilized and the lyophilized residue treated with acid phosphatase to dephosphorylate any phosphorylated forms of **7** that might be present in the extract. After precipitation of the proteins by acidification, the supernatant was lyophilized. The lyophilized residue was dissolved in dry pyridine and treated with an excess of benzoyl chloride to produce the *N*-benzoyl-*O*-tribenzoate derivative of **7**. This derivative was rigorously purified by chromatography and then selectively hydrolysed with methanolic potassium carbonate to yield the *N*-benzoyl derivative of **7**, which was also purified chromatographically. Finally, the *N*-benzoyl compound was derivatized with a slight excess of phenyl isocyanate to give the *N*-benzoyl-tri-*N*-phenylurethane. After chromatographic purification, the urethane derivative was recrystallized until it exhibited constant radioactivity and constant isotopic ratio.

Using the above protocol, [1-^{3}H]-D-glucose was administered to *S. citricolor* to ascertain if the amine triol **7** could be trapped. Since the results of this experiment (Table 1, expt. 1)



Scheme 1 Hypothetical pathway to aristeromycin **1** and neplanocin A **2**



Scheme 2 *Reagents and Conditions*: i, TBDMSOTf, 2,6-lutidine, 92%; ii, $NaBH_4$, 99%; iii, $MeSO_2Cl$, Et_3N, 98% then NaN_3, DMF, 73%; iv, H_2, Lindlar's catalyst, 88% then CF_3CO_2H then Dowex 50 × 8, 98%

Table 1 Incorporation of labelled D-glucose into amine triol **7** by *S. citricolor*

Expt. no.	Precursor ($^3H/^{14}C$)	% Incorpn.[a]	$^3H/^{14}C$	% ^3H Retn.
1	[1-^{3}H]-D-Glucose	0.10	—	—
2	[3-^{3}H, 1-^{14}C]-D-Glucose (4.98)	0.13	4.73	94.9
3	(6*RS*)-[6-^{3}H, 6-^{14}C]-D-Glucose (4.73)	0.07	2.27	47.9

[a] For expts 2 and 3, the % incorpn. is based on ^{14}C.

indicated that radioactivity from the labelled glucose is incorporated into the *N*-benzoyl-tri-*N*-phenylurethane derivative of **7**, it was then necessary to verify that the labelled glucose had been incorporated intact. This was accomplished by carrying out precursor incorporation experiments with doubly-labelled forms of D-glucose. From prior investigations[7] it was known that [3-^{3}H, 1-^{14}C]-D-glucose is incorporated into aristeromycin without tritium loss. Consequently, the intact incorporation of [3-^{3}H, 1-^{14}C]glucose into the amine triol **7** should also proceed with complete retention of tritium. The results of the isotopic trapping experiment with this precursor (Table 1, expt. 2) clearly demonstrate that glucose is incorporated into **7** intact. Additional proof for the intact incorporation of glucose into **7** was then obtained by using (6*RS*)-[6-^{3}H, 6-^{14}C]-D-glucose as a precursor. The purified amine triol derivative isolated from this experiment retained *ca.* 48% of the tritium label (Table 1, expt. 3), a result that is completely consistent with the fact that (6*RS*)-[6-^{3}H, 6-^{14}C]glucose is incorporated into aristeromycin with *ca.* 50% tritium loss.[7] These observations indicate that **7** or its 5-phospho derivative **8** lie on the biosynthetic pathway to aristeromycin. Future studies will attempt to determine whether **7** or **8** is the true biosynthetic intermediate and to examine the formation of **7** or **8** from **6** in cell-free extracts of *S. citricolor*.

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