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The Isolation and Absolute Configuration of (1*S*,2*S*,3*R*)-4-Hydroxymethylcyclopent-4-ene-1,2,3-triol: A Putative Intermediate in the Biosynthesis of Aristeromycin by *Streptomyces citricolor*

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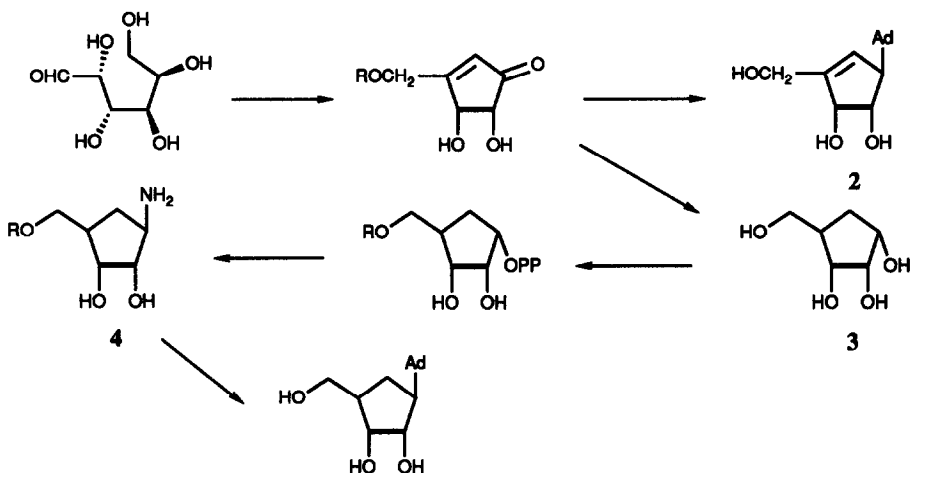
Abstract: The novel tetrol **5** has been isolated from cultures of *Streptomyces citricolor* and shown to have the absolute configuration 1*S*,2*S*,3*R*. Addition of **5** to an aristeromycin non-producing mutant of *S. citricolor* supported production of both aristeromycin **1** and neplanocin A **2**, suggesting that **5** is an intermediate on the biosynthetic pathway.

Aristeromycin **1** and neplanocin A **2** are two naturally occurring carbocyclic nucleosides produced by *Streptomyces citricolor* IFO 13005.[1] Interest in these compounds derives not only from their pronounced biological activity but also their mechanism of biosynthesis and hence the opportunity for harnessing the enzymes involved and utilising them to produce novel chiral intermediates.



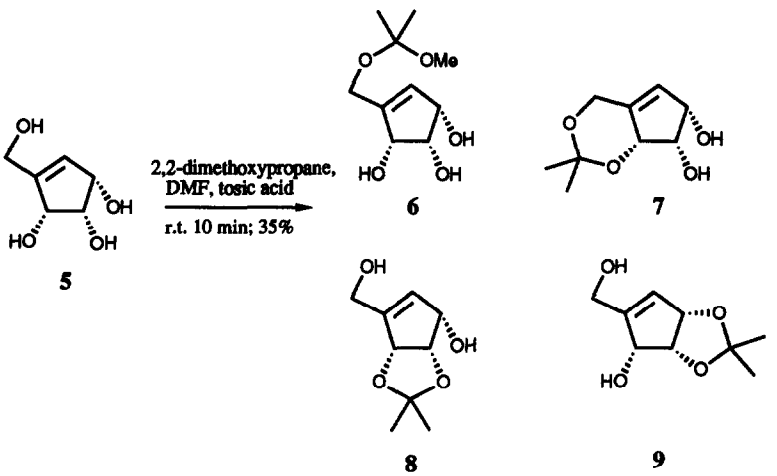
Scheme 1

Most of the biosynthetic work on **1** and **2** has emanated from the laboratories of Parry and Johnson. Their studies have revealed the following ; (i) the cyclopentane ring in aristeromycin **1** is derived from C-C bond formation between C2 and C6 of glucose[2] (ii) the incorporation of adenine from adenosine albeit at low efficiency[2] and (iii) the implied involvement of metabolites **3** and **4**.[3,4] Together these observations have led them to propose the biosynthetic pathway shown in **scheme 1** in which bifurcation of aristeromycin and

neplanocin A biosynthesis occurs at an early stage. We now wish to report the isolation and characterisation of the novel cyclopentenetetrol **5** and present evidence that **5** may be a precursor for *both* **1** and **2**.

A number of mutants of *S. citricolor* defective in aristeromycin biosynthesis were isolated and partially characterised. It was noted that certain combinations of mutants would produce aristeromycin when grown together in mixed culture (cosynthesis). It was further shown that a sterile aqueous concentrate prepared from freeze-dried supernatant of cultures of one mutant, 3978E, would support production of aristeromycin **1** and neplanocin A **2** when added to cultures of a second mutant, 3977E. This suggested the presence of an aristeromycin precursor in the culture supernatant of mutant 3978E. In the first experiments, purification of the active component from 3978E cultures was followed using bioconversion to aristeromycin by 3977E cultures as an assay. Concentrated column eluates and other extraction samples were tested for the presence of the precursor by addition to cultures of 3977E followed by HPLC assay for aristeromycin. Subsequently, once a preliminary identification had been made, GC-MS and HPLC assays were developed and used to aid purification of the active component from a 40 L culture of 3978E.

The active component was extracted and purified[5] from the clarified broth (40 L) of mutant 3978E following concentration under reduced pressure and precipitation of protein and inorganic materials with methanol. Gel filtration (Sephadex G15) and repeated silica chromatography gave the tetrol **5**.[6]

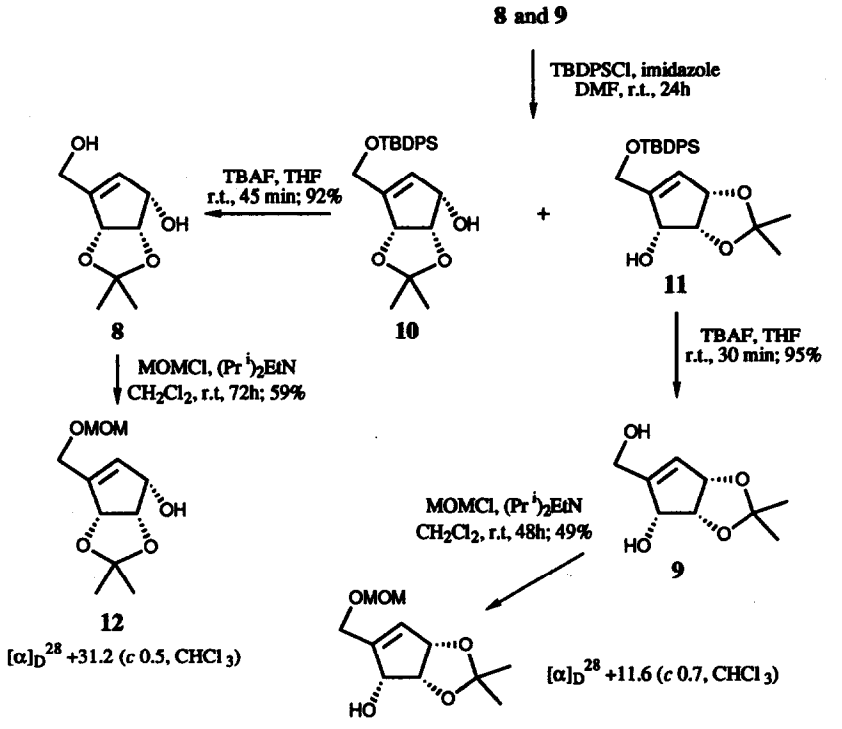


Scheme 2

In order to establish the relative configuration of the hydroxyl groups at C-1, C-2 and C-3 the reaction of **5** with 2,2-dimethoxypropane was investigated, since the number of acetonides formed in the reaction would be a positive indicator as to the stereochemistry of the alcohols. Thus the tetrol **5** was treated with excess 2,2-dimethoxypropane in DMF with acid catalysis giving four products which on the basis of ^{1}H NMR studies were assigned the structures **6-9** shown in **scheme 2**.[7]

The 1,2- and 2,3-acetonides, **9** and **8** respectively, were inseparable by chromatography and were shown to be a 2:1 mixture in favour of the 1,2-acetonide **9**. It was found that if the reaction was worked up under acidic conditions both the 6-membered acetonide **7** and the methyl ketal **6** were unstable to these conditions (resulting in hydrolysis of these products) whilst the two 5-membered acetonides **8** and **9** were stable under these conditions. Besides being a convenient method of solely isolating both the 1,2- and 2,3-acetonides this

result confirmed the ^1H NMR results as to the structures of the four acetals. On the basis of these results it can be concluded that the three secondary alcohols have a *syn* relationship to one another.



Scheme 3

With the relative stereochemistry determined it was necessary to establish the absolute configuration of the tetrol **5** (**scheme 3**). In order to fulfil this aim it was decided to convert the tetrol **5** to the known methoxymethyl (MOM) ether **12** previously prepared by Bestmann as an intermediate towards the enantioselective synthesis of (-) neplanocin A.[8] Unfortunately reaction of the mixture of alcohols **8** and **9** with MOMCl gave two inseparable MOM ethers so a more indirect approach to **12** was undertaken. Thus alcohols **8** and **9** were converted to a mixture of the *tert*-butyldiphenylsilyl ethers **10** and **11** respectively which were now separable by flash chromatography. The separate silyl ethers were then converted back to the corresponding primary alcohols **8** and **9** *via* cleavage of the silyl protecting groups with tetrabutylammonium fluoride. Selective protection of the remaining primary alcohol of **8** with MOM-Cl gave the MOM ether derivative **12** whose ^1H n.m.r. spectrum compared with that reported by Bestmann. The $[\alpha]_D^{28}$ value for **12** +31.2 (lit.[8] $[\alpha]_D^{24}$ +36.8°) established the configuration to be 1*S*,2*S*,3*R*.

The structure of the tetrol **5** isolated from mutant 3978E, and its ability to support production of both neplanocin A and aristeromycin by 3977E, suggests that **5** may be a precursor in aristeromycin biosynthesis. It has been reported previously that the related nucleoside neplanocin A **2** is also produced by *S. citricolor*[2] and we have isolated mutants of *S. citricolor* producing neplanocin A in much higher titre with no or trace amounts of aristeromycin. We have further shown that cultures of 3977E and 3978E can produce aristeromycin when

supplied with neplanocin A. This suggests that contrary to **scheme 1** neplanocin A may be a precursor of aristeromycin, which would be consistent with a biosynthetic pathway involving the novel tetrol **5**.[9]

Acknowledgements: We thank the SERC/Glaxo Group Research for the a CASE studentship (A.J.T.).

References and Notes

1. Kusaka, T.; Yamamoto, H.; Shibata, M.; Muroi, M.; Kishi, T.; Mizuno, K.; *J. Antiobiotics*, 1967, **21**, 255.
2. Parry, R.J.; Bornemann, V.; Subramanian, R.; *J. Am. Chem. Soc.*, 1989, **111**, 5819.
3. Parry, R.J.; Haridas, K.; DeJong, R.; Johnson, C.R.; *Tetrahedron Lett.*, 1990, **31**, 7549.
4. Parry, R.J.; Haridas, K.; DeJong, R.; Johnson, C.R.; *J. Chem. Soc., Chem. Commun.*, 1991, 740.
5. A spore suspension of strain 3978E was used to inoculate two 250 ml shake flasks containing 50 ml of a medium comprising arkasoy 60 g/L, glucose 60 g/L, uracil 150 mg/L and MOPS buffer 21 g/L. After incubation with shaking (250 rpm) for 3 days at 28 °C, these flasks were used to inoculate a fermenter containing 4 L of the same medium (without MOPS). After 2 days incubation at 28 °C with stirring (500 rpm) and aeration (3 L/min), 800 ml was used to inoculate 40 L of the same medium in a fermenter and incubated for 5 days at 28 °C with stirring (550 rpm) and aeration (40 L/min). Clarified *Streptomyces citricolor* (3978E) broth (40 L) was concentrated under reduced pressure to 7 L and diluted with 9 volumes of methanol. The supernatant liquors were concentrated and the resulting aqueous suspension was clarified by centrifugation and fractionated on a column of Sephadex G15 (Pharmacia) (22 L) in water. The fractions containing the tetrol **5** were concentrated and fractionated again on the same column to give a best cut, resulting in an oil (50.4 g) on removal of solvent. The oil was leached with 95% ethanol by agitating with a Silverson Mixer/Emulsifier (500 ml, 300 ml:30 min each) and the ethanol extract boiled to dryness to leave 28.5 g of oil. The crude oil was dissolved in methanol (500 ml), distributed on silica (Kieselgel 60, Merck, 150 ml) by removal of the solvent and the silica was added to the top of a silica column made up in 1:1 methanol/chloroform. Development in 1:1 and 4:1 methanol/chloroform mixtures gave a tetrol fraction on removal of solvent (6.1 g). Recycling on a silica column made up in 1:3 methanol/chloroform and batched gradient elution gave only 20-30% recovery hence the silica was extruded and extracted twice with water (750 ml, 1000 ml). The aqueous extracts were distilled to dryness and extracted with methanol to give two oils (0.506 g and 0.591 g) with identical ^{1}H NMR spectra.
6. Nmr data for **5**: δ_H (500 MHz, d_6-DMSO) 3.92 (1H, overlapping dd, J 6 Hz, H-2), 4.01 (1H, d, J 15 Hz, H-6), 4.10 (1H, d, J 15Hz, H-6), 4.15 (1H, d, J 6 Hz, H-3), 4.24 (1H, br d, J 6Hz, H-1), 5.62 (1H, br s, H-5): tetra-acetate of **5**: $[\alpha]_D^{24}$ +89.8 (*c* 0.84, $CHCl_3$); δ_H (250 MHz, $CDCl_3$) 2.03, 2.06, 2.07, 2.08 (12H, 4 x s, Ac), 4.68 (2H, s, H-6), 5.46, (1H, dd, J 5, 6 Hz, H-2), 5.61 (1H, m, H-1), 5.68 (1H, d, J 6 Hz, H-3), 6.05, (1H, m, H-5); δ_C (62.9 MHz, $CDCl_3$) 20.29, 20.53, 20.60, 20.66, (4 x CH_3), 60.08 (CH_2), 69.94, (CH), 72.67 (CH), 73.05 (CH), 129.30 (CH), 142.32 (C), 169.37, 169.72, 170.21, (C=O).
7. All new compounds gave satisfactory spectroscopic and analytical data.
8. Bestmann, H.J.; Roth, D.; *Angew. Chem. Int. Ed. Engl.*, 1990, **29**, 99.
9. Alternative biosynthetic routes involving oxidation of the tetrol **5** at C-1 and subsequent conjugate reduction of the double bond cannot, of course, be ruled out at this stage.

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