Tetrahedron Letters, Vol.31, No.52, pp 7549-7552, 1990
Printed in Great Britain

0040-4039/90 $3.00 + .00
Pergamon Press plc

BIOSYNTHESIS OF ARISTEROMYCIN: EVIDENCE FOR THE INTERMEDIACY OF A 4β-HYDROXYMETHYL-1α, 2α, 3α-TRIHYDROXYCYCLOPENTANETRIOL.

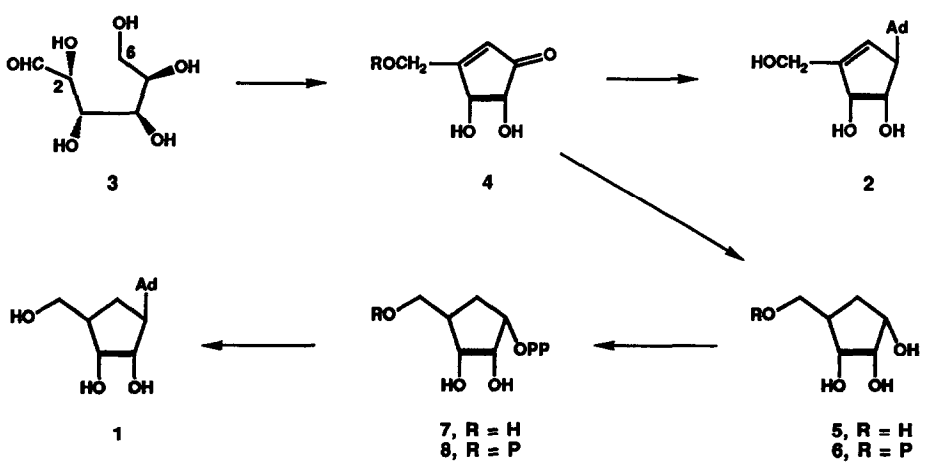
Ronald J. Parry* and Kochat Haridas
Department of Chemistry, Rice University, Houston, TX 77251

Randall De Jong and Carl R. Johnson*
Department of Chemistry, Wayne State University, Detroit, MI 48202

Abstract: Evidence for the intermediacy of a 4β-hydroxymethyl-1α, 2α, 3α-trihydroxycyclopentanetriol (**5** or **6**) in the biosynthesis of the nucleoside antibiotic aristeromycin (**1**) has been obtained by administration of doubly-labeled forms of D-glucose to the fermentation broth of *Streptomyces citricolor* followed by trapping of the tetrol **5** using isotope dilution methods.

The nucleoside antibiotic aristeromycin (**1**) (Scheme I) is a naturally occurring carbocyclic analogue of adenosine produced by *Streptomyces citricolor*.[1] The compound exhibits a variety of interesting biological activities,[2,3] including the inhibition of AMP synthesis in mammalian cells, inhibition of cell division and elongation in rice plants, and inhibition of the enzyme *S*-adenosylhomocysteine hydrolase.[4] Previous investigations in our laboratory have revealed that the cyclopentane ring present in **1** is created by C-C bond formation between C-2 and C-6 of glucose (**3**).[5] These studies also provided evidence that the cyclization of glucose proceeds by

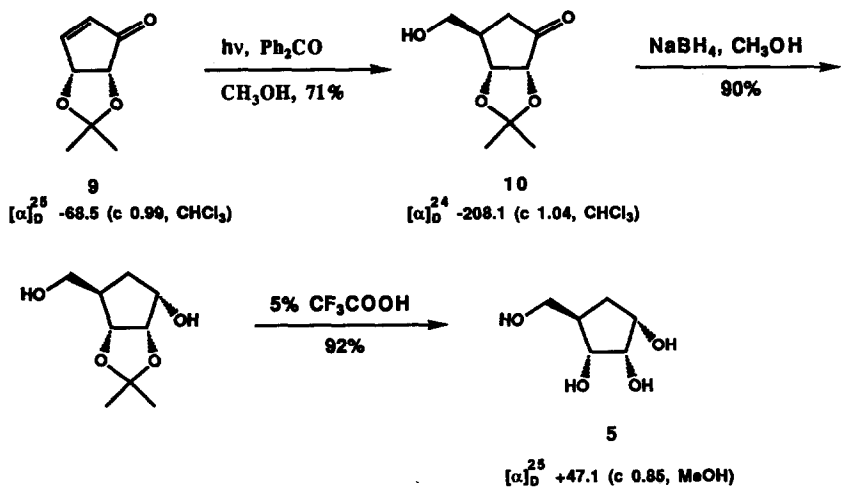
Scheme I



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 also supported by the isolation of the potent antitumor agent neplanocin A (**2**)[6] from the fermentation broth of *S. citricolor*. The later stages of the pathway were postulated to involve conversion of the cyclopentenone **4** to a 4β-hydroxymethyl-1α, 2α, 3α-trihydroxycyclopentanetriol derivative (**5** or **6**) which could then be transformed into aristeromycin via the pyrophosphate **7** or **8**. We would now like to report the results of isotope dilution experiments that support the intermediacy of the cyclopentanetetrol **5** in aristeromycin biosynthesis.

The cyclopentanetetrol (+)-**5** was synthesized from the known[7] cyclopentenone derivative (-)-**9** by the route shown in Scheme II. The photochemical addition of methanol[9] to **9** gave the adduct (-)-**10** which upon borohydride reduction and exposure to aqueous trifluoroacetic acid provided (+)-**5**.[10] Racemic **5** was prepared from the MOM derivative[8] of racemic **10** by DIBAL reduction followed by acid-catalyzed deprotection. Independent syntheses of **5** have been reported.[11] Once the tetrol **5** was in hand, isotope dilution experiments were carried out. For these studies, radiolabeled glucose was pulse-fed at 48 h and 60 h to cultures of *S. citricolor* growing in the normal production medium.[5] After 72 h, the mycelium from 65 mL of fermentation broth was harvested by centrifugation, washed twice with distilled water, resuspended in distilled water, and disrupted by sonication. The tetrol **5** was then added as carrier and the cellular debris removed by centrifugation. The sedimented debris was washed twice with distilled water, and the centrifuged washings were combined with the original supernatant. The total aqueous supernatant was then

Scheme II



lyophilized and the residue was redissolved in 10 mL of dephosphorylation buffer (1 mM $ZnCl_2$, 1 mM $MgCl_2$, 5 mM acetate, pH 4.8) and treated with acid phosphatase (15 U) at 37° for 1 h. In this way, it was hoped that phosphorylated forms of **5** present in the extract would be hydrolyzed to give

the free tetrol. After dephosphorylation, the extract was acidified to pH 3, the precipitated proteins were removed by centrifugation, and the supernatant was lyophilized. The residue from lyophilization was dissolved in dry pyridine and treated with excess benzoyl chloride to give the tetra-*O*-benzoate of the tetrol **5**. The tetrabenzoate was rigorously purified by preparative tlc to give pure tetrabenzoate as a colorless oil. The tetrabenzoate was then hydrolyzed back to the free tetrol by treatment with methanolic NaOH. The recovered tetrol was next converted to its tetra-N-phenylurethan by reaction with phenyl isocyanate. The urethan obtained was recrystallized to constant radioactivity and constant isotopic ratio.

Preliminary incorporation experiments with [1-^{3}H]-D-glucose were carried out to develop the protocol just outlined and to determine if the tetrol **5** could be successfully trapped. These experiments demonstrated that radioactivity from [1-^{3}H]-D-glucose was indeed incorporated into the recovered and purified tetrol (Table 1, expt. 1). In order to verify that intact incorporation of glucose had taken place, [3-^{3}H, 1-^{14}C]-D-glucose was then utilized as a precursor. Since this form of doubly-labeled glucose is incorporated into aristeromycin without tritium loss,[5] the intact

Table 1. Incorporation of Labeled D-Glucose into Tetrol **5** by *Streptomyces citricolor*.

Expt. No.	Precursor (^{3}H/^{14}C)	% Incorpn.	^{3}H/^{14}C	% ^{3}H Retention
1	[1-^{3}H]-D-Glucose	0.05	--	--
2	[3-^{3}H, 1-^{14}C]-D-Glucose (4.98)	0.50[a]	4.57	91.6
3	(6*RS*)-[6-^{3}H, 6-^{14}C]-D-Glucose (5.84)	0.05	3.22	55.1

[a] Urethan prepared directly from crude, dephosphorylated tetrol.

incorporation of glucose into the tetrol **5** should also proceed without tritium loss. The results of this experiment clearly support the intact incorporation of glucose since the rigorously purified tetrol retained over 90% of the tritium label (Table 1, expt. 2). Finally, additional proof for the intact incorporation of glucose into **5** was obtained by carrying out isotopic trapping after administration of 6(*RS*)-[6-^{3}H, 6-^{14}C]-D-glucose. The purified tetrol obtained from this experiment retained *ca.* 55% of the tritium label (Table 1, expt. 3). This result is completely consistent with the fact that 6(*RS*)-[6-^{3}H, 6-^{14}C]-D-glucose is incorporated into aristeromycin with *ca.* 50% tritium loss,[5] and it provides evidence that a cyclopentenone intermediate *precedes* the tetrol **5** on the biosynthetic pathway. The goals of future studies will be to determine whether **5** or its 5-phospho derivative **6** is the true biosynthetic intermediate and to examine the formation of **5** or **6** at the cell-free level.

Acknowledgment. We are pleased to acknowledge the support of this work by the National Institutes of Health (Grant No. GM26569 to R.J.P.) (Grant No. CA37806 to C.R.J.) and by The Robert A. Welch Foundation (Grant No. C-729 to R.J.P.).

References

1. Kusaka, T.; Yamamoto, H.; Shibata, M.; Muroi, M.; Kishi, T.; Mizuno, K. J. *Antibiot.* **1967**, *21*, 255; Kishi, T.; Muroi, M.; Kusaka, T.; Nishikawa, M.; Kamiya, K.; Mizuno, K.; *Chem. Pharm. Bull.* **1972**, *20*, 940.
2. Suhadolnik, R. J. *Nucleoside Antibiotics*; Wiley-Interscience: New York, 1970; p. 236.
3. Suhadolnik, R. J. *Nucleosides as Biological Probes*; Wiley: New York, 1979; p. 147.
4. Guranowski, A.; Montgomery, J. A.; Cantoni, G. L.; Chiang, P. K. *Biochemistry* **1981**, *20*, 110.
5. Parry, R. J.; Bornemann, V.; Subramanian, R. J. *Am. Chem. Soc.* **1989**, *111*, 5819.
6. Yaginuma, S.; Muto, N.; Tsujino, M.; Sudate, Y.; Hayashi, M.; Otani, M. J. *Antibiot.* **1981**, *34*, 359; Hayashi, M.; Yaginuma, S.; Yoshioka, H.; Nakatsu, K. *Ibid.* **1981**, *34*, 675.
7. Johnson, C. R.; Penning, T. D. *J. Am. Chem. Soc.* **1988**, *110*, 4726.
8. Johnson, C. R.; Medich, J. R. *J. Org. Chem.* **1988**, *53*, 4131.
9. For prior examples of the photochemical addition of methanol to enones see: Schenck, G.O.; Koltzenberg, G. Grossman, H. *Angew. Chem.* **1957**, *69*, 177; Bundy, G. L. *Tetrahedron Lett.* **1975**, *16*, 1957; Guyman, A.; Muchowski, J. M. *Tetrahedron Lett.* **1975**, *16*, 2053; Frazier-Reid, B.; Holden, N. L.; Hicks, D. R.; Walker, D. L. *Can. J. Chem.* **1977**, *55*, 3978, 3986.
10. (+)-Tetrol **5** prepared in two different runs had $[\alpha]_D$ + 46.1 (c 0.80, MeOH, 25° C) and $[\alpha]_D$ + 47.1 (c 0.80, MeOH, 25° C); lit. $[\alpha]_D$ + 33.3 (c 0.4, MeOH) (ref. 11a). The latter was converted to the tetracetate $[\alpha]_D$ + 40.4 (c 0.93, $CHCl_3$, 25° C); lit. $[\alpha]_D$ + 38.5 (c 1.05, $CHCl_3$, 26° C) (ref. 11b). Tetrol 5 is highly hygroscopic and the lower rotational values may be due to the presence of water. When completely free of solvent, (+)-tetrol **5** is crystalline. Tetrol **5**: ^{13}C NMR (CD_3OD) δ 75.41, 74.85, 72.83, 64.67, 46.90, 33.64; HRMS calcd for $C_6H_{13}O_4$ (M + 1) 149.0814, found 149.0815.
11. (a) Marschner, C.; Penn, G.; Griengl, H. *Tetrahedron Lett.* **1990**, *31*, 2873; (b) Tadano, K.; Hashino, M.; Ogawa, S.; Suami, T. *J. Org. Chem.* **1988**, *53*, 1427.

(Received in USA 20 September 1990)