

Biosynthesis of Acaterin: Metabolic Fate of *sn*-3 Hydrogens of Glycerol during the Formation of 4-Dehydroacaterin

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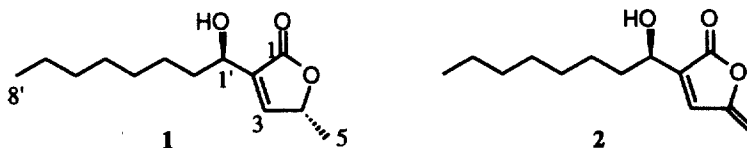
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Abstract: The metabolic fate of the *pro-R* and *pro-S* hydrogens at the *sn*-C-3 position of glycerol during the formation of 4-dehydroacaterin has been investigated using *Pseudomonas* sp. A92. Feeding studies of *sn*-(3*R*)- and *sn*-(3*S*)-(3-²H)glycerols revealed that 5*E* hydrogen of 4-dehydroacaterin originates mainly from *pro-R* hydrogen at *sn*-C-3 of glycerol, while 5*Z* hydrogen comes mainly from *pro-S*. These results ruled out pyruvate and lactate as the immediate precursor of the C₃ branched unit of acaterin, and suggest 1,3-bisphosphoglyceric acid or its biological equivalent as the precursor. © 1999 Elsevier Science Ltd. All rights reserved.

Natural compounds having a 2-penten-4-olide and related skeletons have been found in a variety of organisms.¹⁻² This class of secondary metabolites can be classified into two structure types with respect to the oxidation state of C-3, *i.e.*, compounds with 3-OH group (tetronic acid) and compounds with 3-H. Among these, the biosynthesis of only two compounds, carolic acid (a tetronic acid type) and protoanemonin (a 3-H type) has been studied. It is reported that carolic acid is biosynthesized *via* the condensation of a fatty acid derivative and a C₄ compound of TCA cycle such as succinate.³ α -Ketoglutaric acid is reportedly a biosynthetic origin of protoanemonin.⁴ However, most metabolites of this class appear to be biosynthesized *via* condensation of a polyketide moiety and a three-carbon unit. Indeed, pyruvate has been proposed as an immediate origin of the three-carbon unit although this has not been proved experimentally.⁵⁻¹¹

Acaterin (**1**),¹² isolated from a culture broth of *Pseudomonas* sp. A 92 as an inhibitor of acyl-CoA: cholesterol acyltransferase, and its biosynthetic precursor, 4-dehydroacaterin (**2**)¹³ belong to this class of compounds with 3-H structure. We previously reported that glycerol is efficiently incorporated into the three-carbon unit (C-3, C-4 and C-5 positions) with the *sn*-3 carbon of glycerol becoming C-5 of **1** and **2**, and that two hydrogens at the *sn*-C-1 position are completely lost during the transformation.¹⁴ It is also shown that **2** is efficiently converted into **1**.¹³ On the basis of these results, we proposed that the condensation of a C₁₀ polyketide precursor and a glycerol metabolite with the *sn*-1 carbon being a carboxyl group, such as phosphoglyceric acid, phosphoenolpyruvate or pyruvate, affords a tetronic acid type intermediate.¹⁴



In this paper, we describe the metabolic fate of the pro-*R* and pro-*S* hydrogens at the *sn*-C-3 position of glycerol during the Δ^4 double bond formation of 2, which allowed us to rule out a glycerol metabolite with the *sn*-3 carbon being a methyl group, such as pyruvate and lactate as the immediate precursor of the C₃ unit which condensed with a polyketide precursor.

In order to establish the stereochemical correlation between the prochiral *sn*-C-3 carbon of glycerol and exomethylene carbon (C-5) of 2, chirally ²H-labeled glycerols were fed to *P. sp. A 92*.¹⁵ The requisite substrates, *sn*-(3*R*)- and *sn*-(3*S*)-(3-²H)glycerols were prepared by the method of Kakinuma *et al.*¹⁶ with a slight modification.¹⁷ The ²H-NMR spectrum of 2 derived from *sn*-(3*R*)-(3-²H)glycerol showed that H-5(*E*) was mainly labeled, whereas *sn*-(3*S*)-(3-²H)glycerol produced 5-H(*Z*)-labeled 2 (Fig. 1).¹⁸

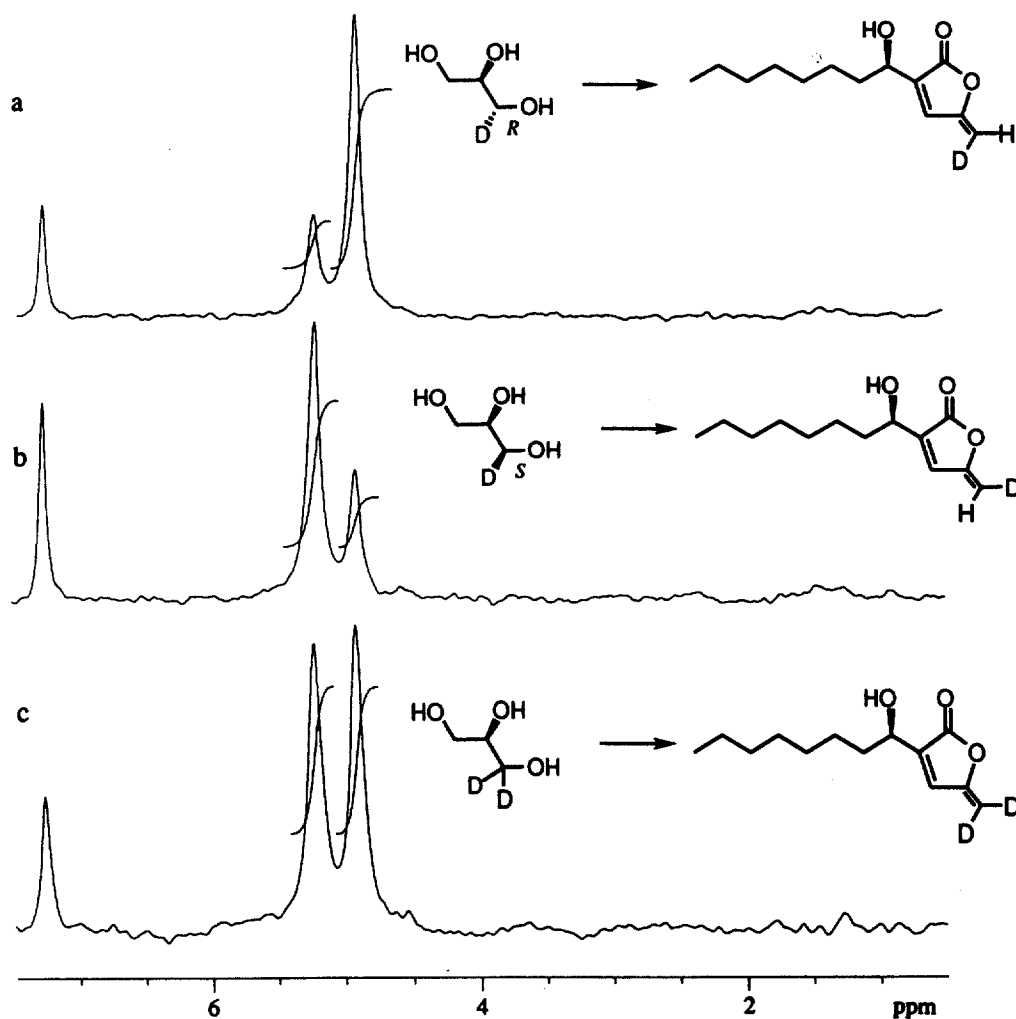


Fig. 1 ²H-NMR spectra of 2. a) Derived from *sn*-(3*R*)-(3-²H)glycerol; b) derived from *sn*-(3*S*)-(3-²H)glycerol; c) derived from *sn*-(3,3-²H₂)glycerol.¹⁴

These results rule out pyruvate and lactate as the immediate C_3 precursor. If the two acids function as the precursor, compound **2** labeled equally at (5*E*)- and (5*Z*)-positions would be produced. On the basis of the well-known glycerol metabolism, together with the finding that *sn*-C-1 hydrogens are lost, candidates of the C_3 precursor can be narrowed down to phosphoglyceric acid or phosphoenolpyruvate. However, the structure of phosphoenolpyruvate seems to be unsuitable for the coupling with a putative C_{10} polyketide in the form of a thioester. We, therefore, propose 1,3-bisphosphoglyceric acid or its biological equivalent (for example, thioester of 3-phosphoglyceric acid) as the most likely structure of the C_3 precursor.

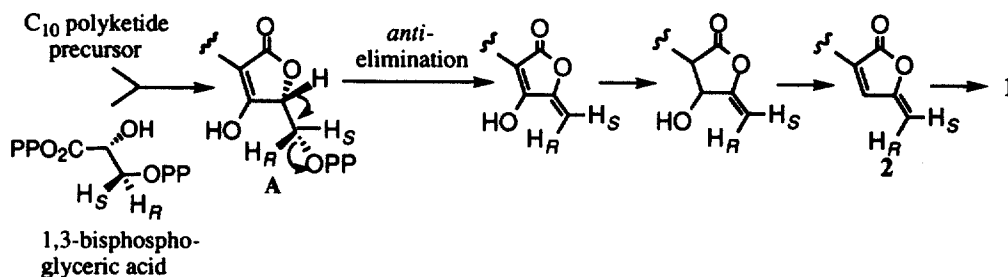


Figure 1 also showed the formation of a lesser amount of **2** with the reversed labeling pattern. This can be interpreted by assuming regeneration of 1,3-bisphosphoglyceric acid *via* gluconeogenesis from the once-formed pyruvate. Partial scrambling of the $^2H/H$ label at the C-3 position of 1,3-bisphosphoglyceric acid would afford the observed labeling pattern at the C-5 of **2**. Indeed, feeding of ($1-^{13}C$)pyruvate under a similar condition to that of ^{13}C -glycerol resulted in a positive incorporation into the C_3 branched unit (C-3 was labeled) of **1** and **2**, although pyruvate was incorporated less efficiently than glycerol. The condensation of (2*R*)-1,3-bisphosphoglyceric acid with a C_{10} polyketide precursor¹⁹ would furnish an intermediate with (4*R*)-configuration (A in Scheme 1). *Anti*-1,2-elimination of A would give a Δ^4 -olefinic product whose C-5 geometry is consistent with the observation in the present study (Scheme 1). Subsequent reduction at C-3 would furnish **2**. It is noted that RK-682 and its homologues, isolated from cultures of *Actinomyces* strain DSM 7357 and *Streptomyces* sp. 88-682,²⁰⁻²² have the structures arising from dephosphorylation of A.

In conclusion, the present studies have provided for the first time evidence that pyruvate is not the immediate precursor of the C_3 branched unit of acaterin.

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References and Notes

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15. A 500-mL flask containing ^2H -labeled glycerol (50 mg) and the medium (100 mL) which is composed of glucose 0.1%, soybean meal 1%, peptone 0.5%, CaCO_3 0.2%, lauric acid 1% was autoclaved. After inoculation, the flask was incubated on a rotary shaker at 25°C and 190 rpm for 2 days in the dark. Typical yield of the products were 20 mg for **2** and 10 mg for **1**.
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17. Coupling of 1,2:5,6-di-*O*-isopropylidene- α -D-ribo-3-hexulofuranose with lithium TMS acetylide in THF gave ethynyl alcohol (80%). Treatment of this with TBAF in D_2O /THF gave (2'- ^2H)-3-C-ethynyl-1,2:5,6-di-*O*-isopropylidene- α -D-allofuranose. Desilylation of the ethynyl alcohol in H_2O /THF gave the corresponding non-labeled compound.
18. 5(*E*)- and 5(*Z*)-protons of non-labeled **2** resonated at δ : 4.92 and 5.23, respectively.
19. C_{10} Polyketide is a hypothetical precursor. At present, we have no information on the possibility of the alternative sequence of condensation: [C_3 unit + acetate (C_2)] + C_8 polyketide.
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