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

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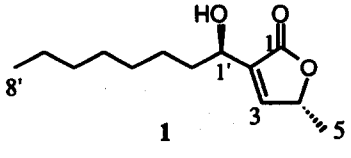
Abstract: The metabolic fate of the pro-*R* and pro-*S* hydrogens at the *sn*-C-3 position of glycerol during the formation of 4-dehydroacaterin has been investigated using *Pseudomonas* sp. A92. Feeding studies of *sn*-(3*R*)- and *sn*-(3*S*)-(3-^{2}H)glycerols revealed that 5*E* hydrogen of 4-dehydroacaterin 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 C_3 branched unit of acaterin, and suggest 1,3-bisphosphoglyceric acid or its biological equivalent as the precursor.

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 C_4 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 C_{10} 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]



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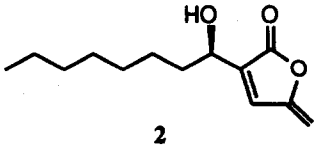

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In this paper, we describe the metabolic fate of the pro-*R* and pro-*S* hydrogens at the *sn*-C-3 position of glycerol during the Δ^4 double bond formation of **2**, which allowed us to rule out a glycerol metabolite with the *sn*-3 carbon being a methyl group, such as pyruvate and lactate as the immediate precursor of the C_3 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 ^{2}H-labeled glycerols were fed to *P.* sp. A 92.[15] The requisite substrates, *sn*-(3*R*)- and *sn*-(3*S*)-(3-^{2}H)glycerols were prepared by the method of Kakinuma *et al.*[16] with a slight modification.[17] The ^{2}H-NMR spectrum of **2** derived from *sn*-(3*R*)-(3-^{2}H)glycerol showed that H-5(*E*) was mainly labeled, whereas *sn*-(3*S*)-(3-^{2}H)glycerol produced 5-H(*Z*)-labeled **2** (Fig. 1).[18]

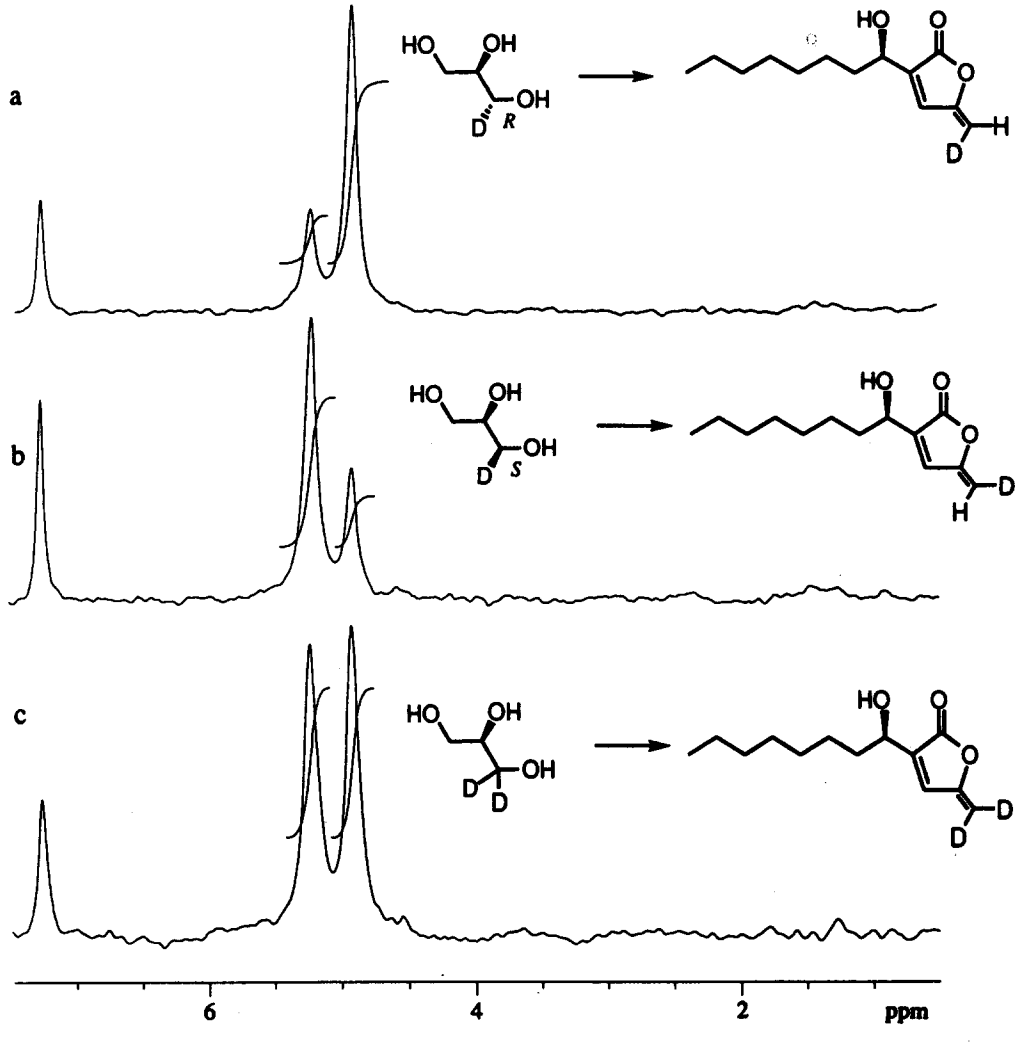
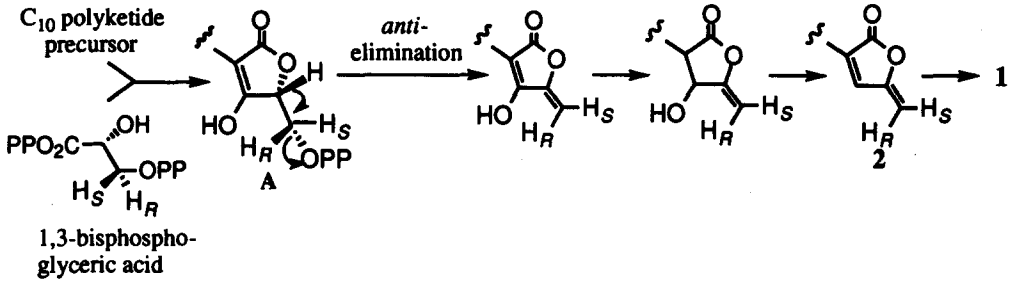


Fig. 1 ^{2}H-NMR spectra of **2**. a) Derived from *sn*-(3*R*)-(3-^{2}H)glycerol; b) derived from *sn*-(3*S*)-(3-^{2}H)glycerol; c) derived from *sn*-(3,3-$^2H_2$)glycerol.[14]

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.



Scheme 1. Postulated biosynthetic pathway of the lactone moiety of acaterin

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 precursor[19] 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 *Actinomycete* strain DSM 7357 and *Streptmyces* 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

1. Pattenden, G. *Prog. Chem. Org. Nat. Prod.*, **1978**, *35*, 133-198.
2. Zeng, L.; Ye, Q.; Oberlies, N. H.; Shi, G.; Gu, Z. -M.; He, K.; McLaughlin, J. L. *Nat. Prod. Reports*, **1996**, *13*, 275-306.
3. Reffstrup, T.; Boll, P. M. *Acta. Chem. Scand.*, **1980**, *B34*, 653-659.

4. Suga, T.; Hirata, T. *Bull. Chem. Soc. Jpn.*, **1982**, *55*, 1584-1587.
5. Bohlmann, F.; Grenz, M. *Tetrahedron Lett.*, **1971**, *39*, 3623-3626.
6. Takeda, K.; Sakurai, K.; Ishii, H. *Tetrahedron*, **1972**, *28*, 3757-3766.
7. Niwa, M.; Iguchi, M.; Yamamura, S. *Tetrahedron Lett.*, **1975**, 1539-1542.
8. Filho, R. B.; Diaz, P. P.; Gottlieb, O. R. *Phytochemistry*, **1980**, *19*, 455-459.
9. Vanwagenen, B. C.; Cardellina II, J. H. *Tetrahedron*, **1986**, *42*, 1117-1122.
10. Etse, J. T.; Waterman, P. G. *Phytochemistry*, **1986**, *25*, 1903-1905.
11. Hadacek, F.; Greger, H.; Grenz, M.; Bohlmann, F. *Phytochemistry*, **1987**, *26*, 1527-1529.
12. Naganuma, S.; Sakai, K.; Hasumi, K.; Endo, A. *J. Antibiotics*, **1992**, *45*, 1216-1221.
13. Sekiyama, Y.; Araya, H.; Hasumi. K; Endo, A.; Fujimoto, Y. *Nat. Prod. Lett.*, **1997**, *11*, 61-66.
14. Sekiyama, Y.; Araya, H.; Hasumi. K; Endo, A.; Fujimoto, Y. *Tetrahedron Lett.*, **1998**, *39*, 6233-6236.
15. A 500-mL flask containing ^{2}H-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**.
16. Kakinuma, K.; Iihama, Y.; Takagi, I.; Ozawa, K.; Yamauchi, N.; Imamura, N.; Esumi, Y.; Uramoto, M. *Tetrahedron*, **1992**, *48*, 3763-3774.
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'-^{2}H)-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.
20. Roggo, B. E.; Hug, P.; Moss, S.; Raschdorf, F.; Peter, H. H. *J. Antibiotics*, **1994**, *47*, 143-147.
21. Hamaguchi, T.; Sudo, T.; Osada, H. *FEBS Lett.*, **1995**, *372*, 54-58.
22. Sodeoka, M.; Sampe, R.; Kagamizono, T.; Osada, H. *Tetrahedron Lett.*, **1996**, *37*, 8775-8778.