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Biosynthesis of Acaterin: Incorporation of Glycerol into the C_3 Branched Unit

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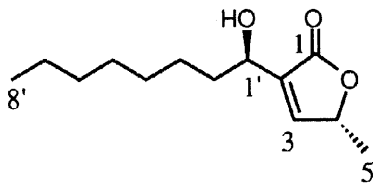
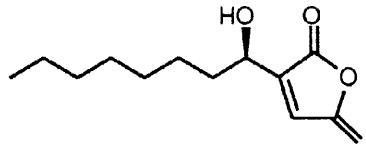
Abstract: The biosynthesis of the lactone moiety of acaterin was studied using *Pseudomonas* sp. A92. Feeding experiment with (2*R S*)-[1-^{13}C]glycerol revealed that glycerol is efficiently incorporated into the branched three-carbon unit (C-3, C-4 and C-5 positions) of acaterin and its precursor, 4,5-didehydro-acaterin. Further feeding studies of (2*R S*)-[1,1-$^{2}H_2$]- and *sn*-[3,3-$^{2}H_2$]-glycerols showed that two hydrogens at the *sn*-C-3 position of glycerol are incorporated into the C-5 position of the two compounds, whereas those at the *sn*-C-1 position are completely lost during the transformation. These results suggest that acaterin is biosynthesized *via* a tetronic acid type intermediate.

Natural compounds having a 2-penten-4-olide and related skeletons have been found in a variety of organisms.[1] This class of secondary metabolites can be classified into two types with respect to the oxidation state of C-3, *i.e.*, compounds with a 3-OH group (tetronic acid) and compounds with 3-H. It is reported that the biosynthetic pathway of carolic acid (a tetronic acid type) involves the condensation of a fatty acid derivative and a C_4 compound of TCA cycle such as succinate.[2] Another example of this family is protoanemonin (a 3-H type compound) whose biosynthetic origin was reported to be α-ketoglutaric acid.[3] However, most metabolites of this class appear to be biosynthesized *via* condensation of a fatty acid moiety and a three-carbon unit. Indeed, pyruvate has been proposed as the origin of the three-carbon unit although this has not been proved experimentally.[4]



acaterin (**1**)



4,5-didehydroacaterin (**2**)

Acaterin (**1**), isolated from a culture broth of *Pseudomonas* sp. A92 as an inhibitor of acyl-CoA: cholesterol acyltransferase,[5] is a 3-H type of compound. We have recently reported the isolation of 4,5-didehydroacaterin (**2**) and its conversion into **1** in *P.* sp. A92.[6] In this paper we describe the results of biosynthetic studies on the origin of the branched three-carbon unit of **1** and **2**.

Although feeding of ^{13}C-acetate to *P.* sp. A92 resulted in negative incorporation, a longer chain fatty acid,

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[1-^{13}C]dodecanoic acid, was positively incorporated into **1**. The ^{13}C-NMR spectrum of the resulting **1** showed enrichment at C-1, -1', -3', -5' and -7', indicating that the C_{10} polyketide moiety was derived from acetate arising from the C_{12} acid *via* β-oxidation. Negligible incorporation of the ^{13}C-label was found into the branched three-carbon unit, indicating this unit should be derived from another source.

[1-^{13}C]Succinic acid was then fed to the organism in order to compare with the result reported for carolic acid.[2] However, no significant incorporation was observed into **1** and **2**. By contrast, (2*RS*)-[1-^{13}C]glycerol was efficiently incorporated into **1** and **2**.[7] It can be seen from the ^{13}C-NMR spectrum of **1** (Fig. 1) that the signals of C-3 (δ 149.3) and C-5 (δ 18.9) were enriched. Similarly, C-3 (δ 136.3) and C-5 (δ 97.6) of **2** were enriched. These results indicate that glycerol was incorporated into the branched three-carbon units of **1** and **2** without prior degradation.

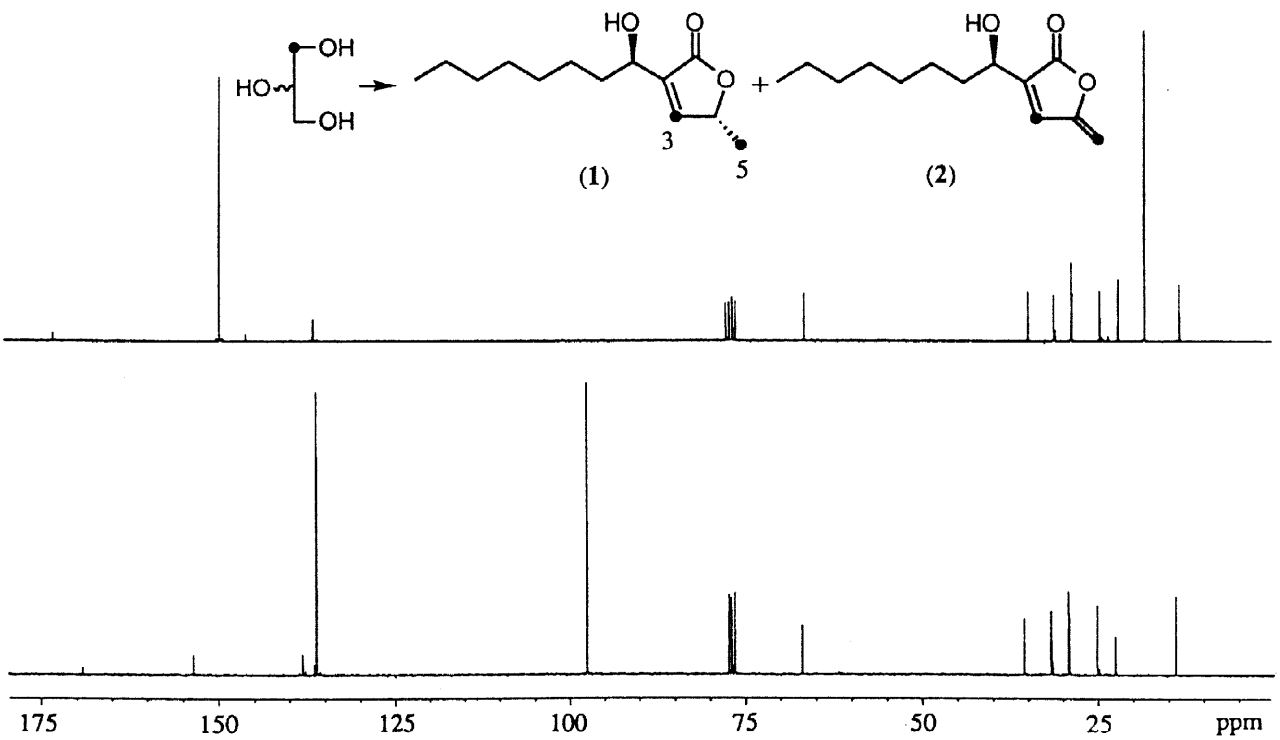


Fig. 1 ^{13}C-NMR spectra ($CDCl_3$, 75 MHz) of **1** (top) and **2** (bottom) derived from (2*RS*)-[1-^{13}C]glycerol

In order to obtain further information on the glycerol metabolite which should be the immediate biosynthetic precursor of **1** and **2**, feeding studies of (2*RS*)-[1,1-$^{2}H_2$]- and *sn*-[3,3-$^{2}H_2$]-glycerols were carried out.[7] The ^{2}H-NMR spectra (Fig. 2) of **1** and **2** derived from (2*RS*)-[1,1-$^{2}H_2$]glycerol showed that deuterium is located at C-5 (δ 1.44 for **1** and δ 4.91 and 5.22 for **2**) of the two compounds, but not at C-3 (H-3 of **1**, δ 7.20 and H-3 of **2**, δ 7.22). The ^{2}H-NMR spectra of **1** and **2** derived from *sn*-[3,3-$^{2}H_2$]glycerol were essentially identical to those from (2*RS*)-[1,1-$^{2}H_2$]glycerol. These results indicated that the C-5 carbon of **1** and **2** comes from *sn*-C-3 of glycerol whereas the C-3 carbon originates from *sn*-C-1 of glycerol. The fact that no deuterium was observed at the C-3 position of **1** and **2** indicates that the *sn*-C-1 carbon of glycerol has to be oxidized to satisfy this requirement during the biosynthesis.[8] The oxidation of *sn*-C-1 of glycerol is in agreement with well-known glycerol metabolism.

On the basis of these data, we would like to propose that the condensation of a C_{10} polyketide precursor

and a glycerol metabolite having a carboxyl group at the *sn*-C-1 position, such as phosphoglyceric acid, phosphoenolpyruvic acid or pyruvic acid, affords a tetronic acid type intermediate. The intermediate can furnish 3-H type acaterin *via* reduction followed by dehydration (Scheme 1). It is noted that a compound corresponding to the stage of the above reduction was previously reported.[9] An alternative mechanism whereby condensation of an C_3 aldehyde, *i.e.*, glyceraldehyde, takes place and then the resulting C-3 alcohol is oxidized to form the tetronic acid can not be ruled out at present. In either case, a tetronic acid type intermediate seems to be involved in the formation of acaterin.

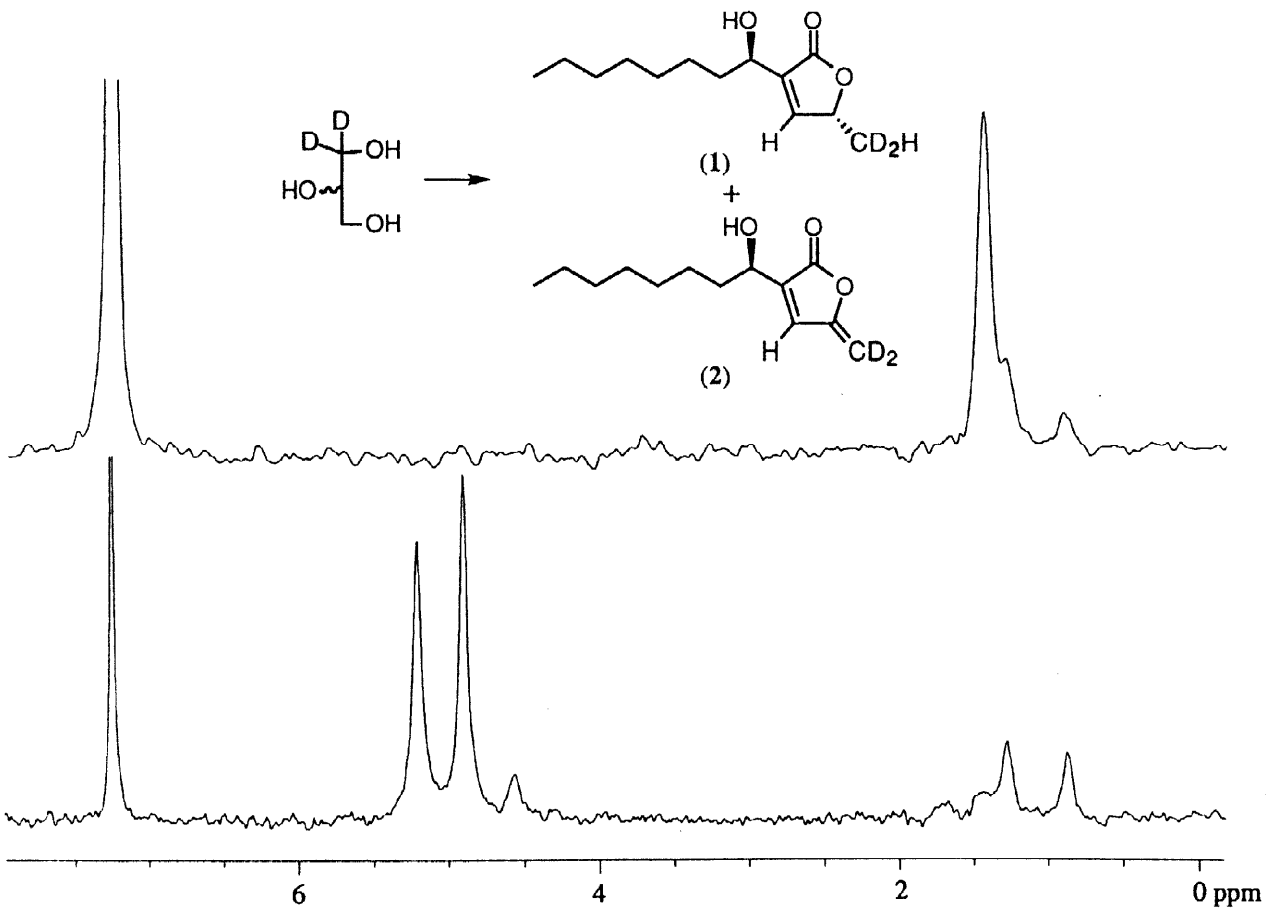
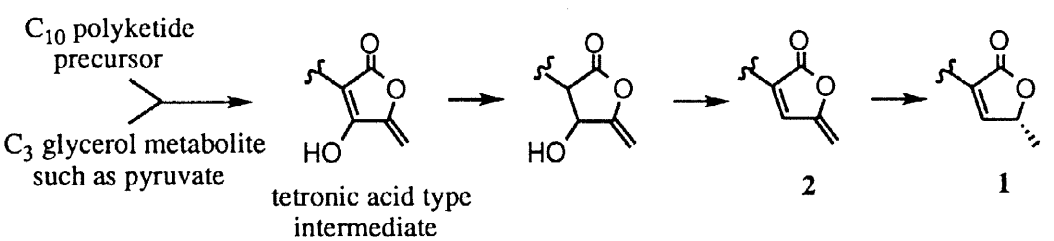


Fig. 2 ^{2}H-NMR spectra ($CHCl_3$, 61.5 MHz) of **1** (top) and **2** (bottom) derived from (2*RS*)-[1,1-$^2H_2$]glycerol

In conclusion, the present studies showed that a glycerol metabolite is the precursor of acaterin, and a tetronic acid derivative would be the biosynthetic intermediate. Further studies on the exact structure of the C_3 precursor are in progress in our laboratory.



Scheme 1 Postulated biosynthetic pathway of the lactone moiety of acaterin

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7. A 500-mL flask containing (2*RS*)-[1-^{13}C]glycerol (30 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. For the ^{2}H-labeled glycerols, 50 mg of each substrate was used. (2*RS*)-[1,1-$^2H_2$]- and *sn*-[3,3-$^2H_2$]-glycerols were prepared from racemic and (2*S*)-forms of methyl 2,3-isopropylidene glycerate, respectively, in two steps (reduction with $LiAlD_4$ and acidic deprotection).
8. The possibility of the specific formation of (*S*)-[3,3-$^2H_2$]glyceraldehyde from (2*RS*)-[1,1-$^2H_2$]glycerol due to a deuterium isotope effect can be ruled out by the finding that feeding of (1*RS*, 2*RS*)-[1-^{2}H]glycerol afforded the same results as shown in Fig. 2.
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