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Biosynthesis of agglomerin A: stereospecific incorporation of pro-*R*- and pro-*S*-hydrogens at *sn*-C-3 of glycerol into the branched C_3 moiety

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Abstract—The biosynthetic origin of the C_3 branched unit of agglomerin A has been investigated. Feeding of *sn*-(3*R*)- and *sn*-(3*S*)-[3-^{2}H]glycerols to *Enterobacter agglomerans* PB-6042 followed by ^{2}H NMR analysis of the resulting agglomerin A revealed that pro-*R* and pro-*S* hydrogens at *sn*-C-3 of glycerol were incorporated stereospecifically into 5*E* and 5*Z* hydrogens of agglomerin A, respectively. These results imply that the immediate precursor of the C_3 branched unit is not pyruvate, but 1,3-bisphosphoglyceric acid or its biological equivalent.
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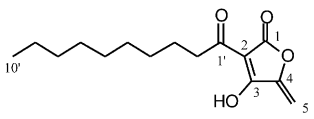
Natural compounds having 2-penten-4-olide and related skeletons have been found in a variety of organisms.[1] This class of secondary metabolites can be classified into two structure types in terms of the oxidation state at C-3, that is, compounds with a 3-OH group (tetronic acid) and compounds with 3-H. We previously investigated the biosynthesis of a 3-H type compound, acaterin (**2**), and showed that glycerol is incorporated into the branched C_3 moiety (C-3,-4 and -5 positions) in such a manner that *sn*-C-1 of glycerol becomes C-3 of **2**.[2] Further, pro-*R* and pro-*S* hydrogens at *sn*-C-3 of glycerol become 5*E* and 5*Z* hydrogens, respectively, at C-5 of 4-dehydroacaterin (**2a**).[2,3] Furthermore, during this transformation hydrogens at *sn*-C-1 of glycerol were lost completely.[4] On the basis of these findings we proposed the immediate precursor of the branched C_3 unit of **2** should be a glyceric acid equivalent, and a tetronic acid type-intermediate would be involved in the formation of 3-H type compounds.[2] Furthermore, it has recently been established that biosynthesis of **2** involves coupling of octanoate and a C_5 unit corresponding to the lactone part.[5]



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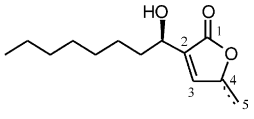

2: 4,5-saturated
2a: 4,5-double bond

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Agglomerins A, B, C and D are structurally simple tetronic acid derivatives, isolated from the fermentation broth of *Enterobacter agglomerans* PB-6042.[6] In order to compare the origin of the branched C-3 moiety of 3-OH type compounds with that of 3-H type compounds, we have now studied the biosynthesis of agglomerin A (**1**).

Feeding of [1-^{13}C]acetate to *E. agglomerans* PB-6042 resulted in the ^{13}C enrichment at C-1′, C-3′, C-5′, C-7′ and C-9′ of **1**,[7] indicating that the branched C_3 unit is not derived from acetate (Fig. 1). Feeding of [1,3-$^{13}C_2$]glycerol afforded **1** enriched at the C-3 and C-5 positions, thus confirming that a glycerol metabolite is a precursor of the C_3 unit (Fig. 2). Feeding of [*sn*-3,3-$^2H_2$]glycerol followed by ^{2}H NMR analysis of the resulting **1** showed that 5*Z* and 5*E* hydrogens of **1** were deuterium-labeled (Fig. 3c), indicating that *sn*-C-3 of glycerol becomes C-5 of **1** whereas *sn*-C-1 becomes C-3. Compound **1** obtained upon feeding [*sn*-1,1-$^2H_2$]glycerol did not exhibit a ^{2}H peak at the positions of 5*Z* and 5*E* hydrogens in ^{2}H NMR analysis. The observed direction of the glycerol incorporation is the same as found in the acaterin biosynthesis.

The key feeding studies of chirally ^{2}H-labeled glycerols, *sn*-(3*R*)-[3-^{2}H]- and *sn*-(3*S*)-[3-^{2}H]-glycerols[4,8] were then carried out.[9] The ^{2}H NMR spectrum of **1** derived from *sn*-(3*R*)-[3-^{2}H]glycerol showed a ^{2}H signal at δ 5.35 (Fig. 3A), while **1** obtained from *sn*-(3*S*)-[3-^{2}H]glycerol exhibited a ^{2}H signal at δ 4.82 (Fig. 3b).[10] These NMR data clearly indicate that the 5*E* and 5*Z* hydrogens of

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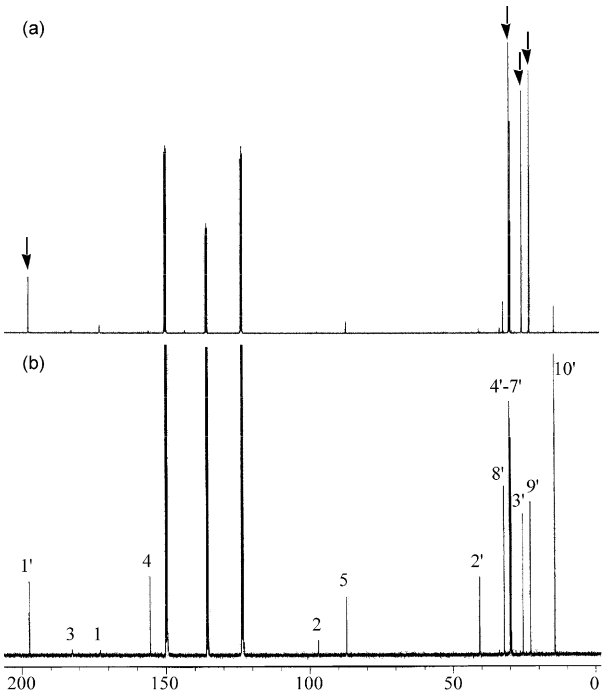


Figure 1. ^{13}C NMR (100 MHz, Py-d_5) spectra of agglomerin A (**1**). a: obtained upon feeding [1-^{13}C]acetate, b: non-labeled sample.

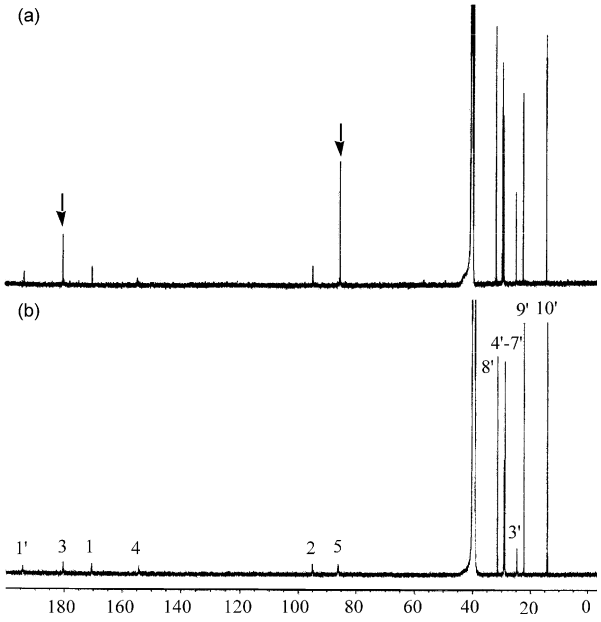


Figure 2. ^{13}C NMR (125 MHz, DMSO-d_6) spectra of agglomerin A (**1**). a: obtained upon feeding of [1,3-$^{13}C_2$]glycerol, b: non-labeled sample.

agglomerin A originate from pro-*R* and pro-*S*-hydrogens, respectively, at *sn*-C-3 of glycerol (Scheme 1). The fate of *sn*-C-3 hydrogens is consistent with that found in acaterin biosynthesis. The stereospecific incorporation of the chirally labeled glycerols ruled out pyruvate as the immediate C_3 precursor, and 1,3-bisphosphoglyceric

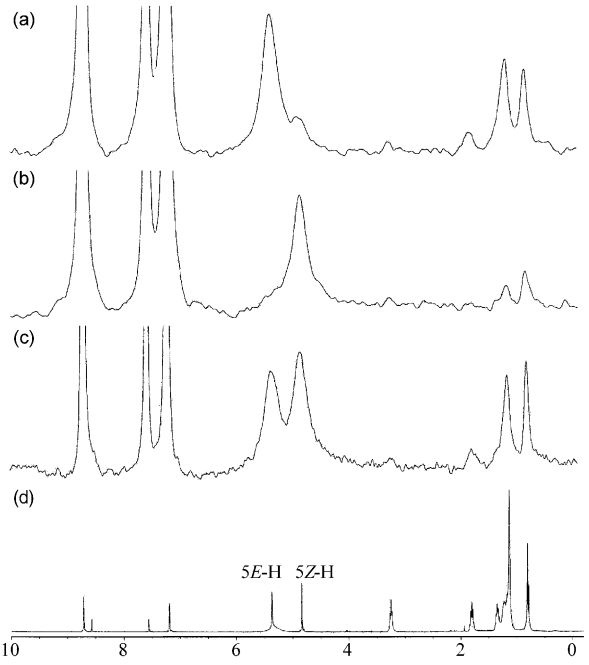
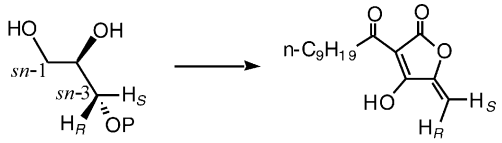


Figure 3. ^2H NMR (61 MHz, Py) spectra of agglomerin A (**1**). a: derived from *sn*-(3*R*)-[3-^2H]glycerol, b: derived from *sn*-(3*S*)-[3-^2H]glycerol, c: derived from [*sn*-3,3-$^2H_2$]glycerol, d: ^1H NMR (400 MHz, Py-d_5) spectrum of **1** (5*E* and 5*Z* hydrogens resonate at δ 5.37 and 4.84, respectively).



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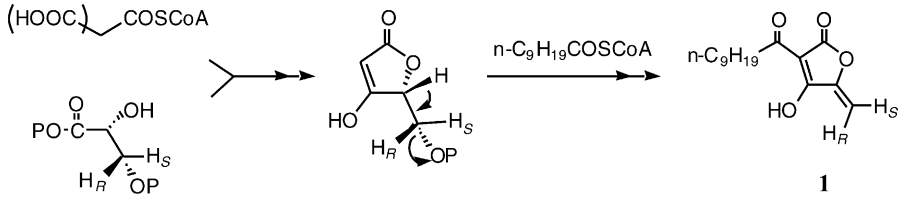
Scheme 1. Metabolic fate of *sn*-3 hydrogens in the conversion to agglomerin A (**1**).

acid or its biological equivalent is proposed as the most likely structure of the C_3 precursor. Attempted feeding of ^{13}C or ^2H-labeled C_{10} and C_{12} fatty acids did not afford useful information on the construction of the agglomerin A carbon skeleton, since none of them was incorporated into **1**.

In conclusion, the present studies have provided evidence that a glyceric acid equivalent serves as the immediate biosynthetic precursor of the branched C_3 unit of **1**, as opposed to pyruvate proposed earlier for this class of compounds. It is highly likely that a common mechanism operate in the construction of the C_5 lactone moiety, irrespective of 3-H and 3-OH type 2-penten-4-olide natural compounds. Scheme 2 represents a postulated biosynthesis of **1**, starting with coupling of 1,3-bisphosphoglyceric acid with acetyl CoA or malonyl CoA. Reaction of a hypothesized C_5 lactone with decanoate and anti-elimination of phosphoric acid would yield **1**.



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Scheme 2. Postulated biosynthesis of agglomerin A (**1**).

Acknowledgements

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7. The relative enrichment of the ^{13}C signals, normalized to C-10′:17 (C-1), 1.2 (C-2), 5.5 (C-3), 0.5 (C-4), 2.3 (C-5), 8.1 (C-1′), 0.7 (C-2′), 19 (C-3′), 1.2 (C-4′), 14 (C-5′), 11 (C-6′ and C-7′), 2.0 (C-8′), 19 (C-9′).
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9. Four 500 mL-baffled Erlenmeyer flasks each containing the ^2H-labeled glycerol (40 mg) and the medium (100 mL) which is composed of 1.0% glucose, 0.5% yeast extract, 0.7% $CaCO_3$, pH 7.2 adjusted by the addition of dil. HCl, were autoclaved. This was innoculated with *E. agglomerans* PB-6042 and the cultures were incubated at 25 °C in the light for 72 h on a rotary shaker at 200 rpm. The fermentation broth was processed as described in ref 6 to give **1** (7 mg) after reversed-phase HPLC purification.
10. The deuterium signals observed at higher region in Figure 3 are probably due to the incorporation of [2-^2H]acetate arising from [3-^2H]pyruvate, a [^2H]glycerol metabolite.