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Biosynthetic studies of platencin

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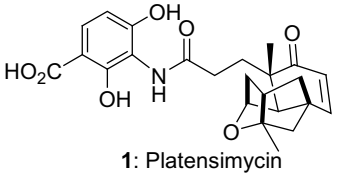
ABSTRACT

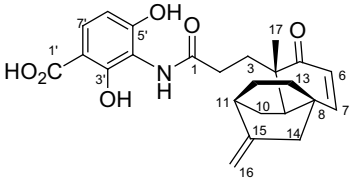
Platensimycin and platencin are two potent and novel antibiotics that exert their antibiotic activities: by inhibiting condensing enzymes of bacterial fatty acid biosynthesis. Both compounds are composed of two structural units; a common 3-amino-2,4-dihydroxybenzoic acid moiety and different C-17 polycyclic enone acid moiety. The latter moiety of each compound is biosynthesized by the non-mevalonate MEP pathway, whereas the former moiety is made from by the TCA cycle and PEP by condensation of 4+3 carbon units. Herein, the details of biosynthesis of platencin are described.

1. Introduction

Platensimycin (**1**) and platencin (**2**) are two recently reported novel antibiotics isolated from several strains of *Streptomyces platensis*.[1–4] These compounds are potent growth inhibitors of Gram-positive bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococci* (VRE). Both compounds impart their antibacterial activities by inhibiting the condensing enzymes called the keto acyl synthases (KAS) of the bacterial fatty acid synthesis pathway. Platensimycin is a specific inhibitor of the elongation condensing enzyme, KAS III, and platencin is a balanced inhibitor of both KAS II and III. The ability to inhibit two enzymes of the same pathway renders platencin more attractive as an antibiotic, due to the lower probability of developing resistance against it. Both compounds are highly efficacious in vivo when administered by continuous infusion but less effective when dosed by subcutaneous or oral routes. The poor in vivo activity is attributed to their poor pharmacokinetic properties, particularly to high rate of clearance. These properties could potentially be improved by chemical modification,[5] via total synthesis[6] and through combinatorial biosynthesis. To perform combinatorial biosynthesis efficiently, a clear understanding of the biosynthetic pathways is a prerequisite. While titer of platensimycin in production medium is good (>52 mg/L),[7] the titer of platencin remains low. An understanding of the underlying biosynthetic pathways should also help in improvement of the titers of these compounds and should allow preparation of new natural analogs by precursor-directed biosynthesis. Recently, we reported the biosynthesis of platensimycin.[8] Herein, we report the results of the biosynthetic studies of platencin by stable-isotope precursor incorporation experiments.



1: Platensimycin



2: Platencin

Similar to platensimycin, platencin is also composed of two structural moieties: a 3-amino-2,4-dihydroxybenzoic acid moiety common to both and a different C-17 tricyclic enone acid (platencinic acid) residue,[2,4] linked by an amide bond. In this way, the two cyclic end pieces are connected with a four-atom linker. While platencin was originally produced by a strain of *S. platensis* that did not produce platensimycin, in the current study platencin was generated as a minor congener of platensimycin by the original producer of platensimycin. The biosynthetic studies were initiated by feeding sodium [1-^{13}C]- and [2-^{13}C]acetate to a culture of *S. platensis* MA7327.[8] Precursor feeding was conducted by the washed-cell incubation method, and the culture was grown for four days in the production medium: Stadex-60 (40 g/L), lactose (40 g/L) and amberex-pH 5.0. The cells were collected by centrifugation immediately after the production of platensimycin was noted (~2 mg/L, after 2 days), and transferred to 20 mM 2-[*N*-morpholino]ethanesulfonic acid (MES) buffer (pH 6.4) containing 5 g/L

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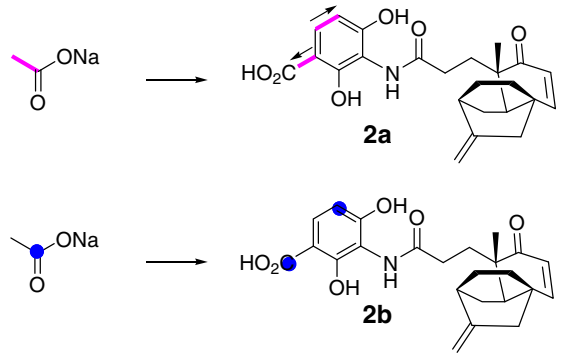
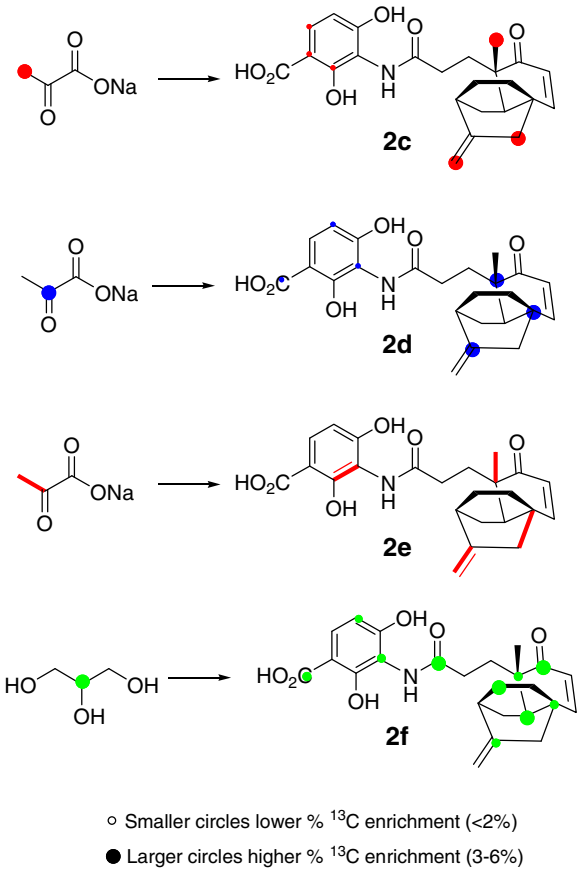
Stadex-60 as the carbon source. In general, two aliquots of each labeled precursors (10 mM final concentration in the broth) were fed at time zero and at 96 h. After incubating for additional 74 h, the fermentation broth was acidified with 3 M HCl to pH 2.0 and extracted with EtOAc and concentrated. The labeled metabolites were isolated by reversed phase HPLC on a Zorbax RX C_8 (21.2 × 250 mm) column eluted by a 37 min gradient from 30% to 90% aqueous CH_3CN +0.1% TFA at a flow rate of 12 mL/min. Labeled platensimycin (titer 25–50 mg/L) eluted at 15 min, and labeled platencin (1–2 mg; titer 1–3 mg/L) eluted at 22 min. The labeled samples of platencin were analyzed by ^{13}C NMR spectroscopy under optimized conditions (relaxation delay = 25 s). The enrichment factor (fold over natural abundance represented as % enrichment) was determined by using external calibration to a natural abundance unlabeled spectrum of platencin.

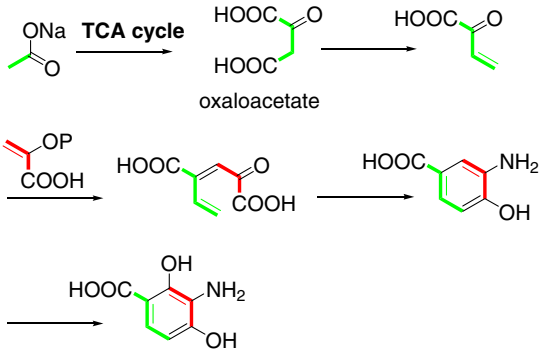




2. Biosynthesis of 3-amino-2,4-dihydroxybenzoic acid

Feeding experiments with [1,2-$^{13}C_2$]CH_3CO_2Na produced labeled platencin **2a**. The ^{13}C NMR analysis of **2a** showed the intact incorporation of the two acetate units. Isotope labels observed at C-1′, C-2′ (J = 72 Hz) and C-6′, C-7′ (J = 62 Hz) indicated that four carbons of the aromatic unit originated from two acetate units. The directionality of the acetate incorporation was established by feeding of [1-^{13}C]CH_3CO_2Na, which afforded labeled platencin **2b** showing labeling at C-1′ and C-6′. This labeling pattern indicated a tail-to-tail incorporation of the two acetate units, and suggested that the aminobenzoic acid is biosynthesized using four carbons from a distinct acetate precursor pool and the remaining three carbons from a separate biosynthetic precursor pool. Similar to what was observed for platensimycin, no clear enrichments of the other three aromatic carbons or the carbons in the tetracyclic enone could be discerned.[8] The tail-to-tail labeling pattern of C-1′/C-2′ and C-6′/C-7′ of the aminobenzoic acid moiety derived from labeled-acetate-fed *S. platensis* indicated TCA cycle participation as described for the biosynthesis of 3-amino-4-hydroxybenzamide.[9]

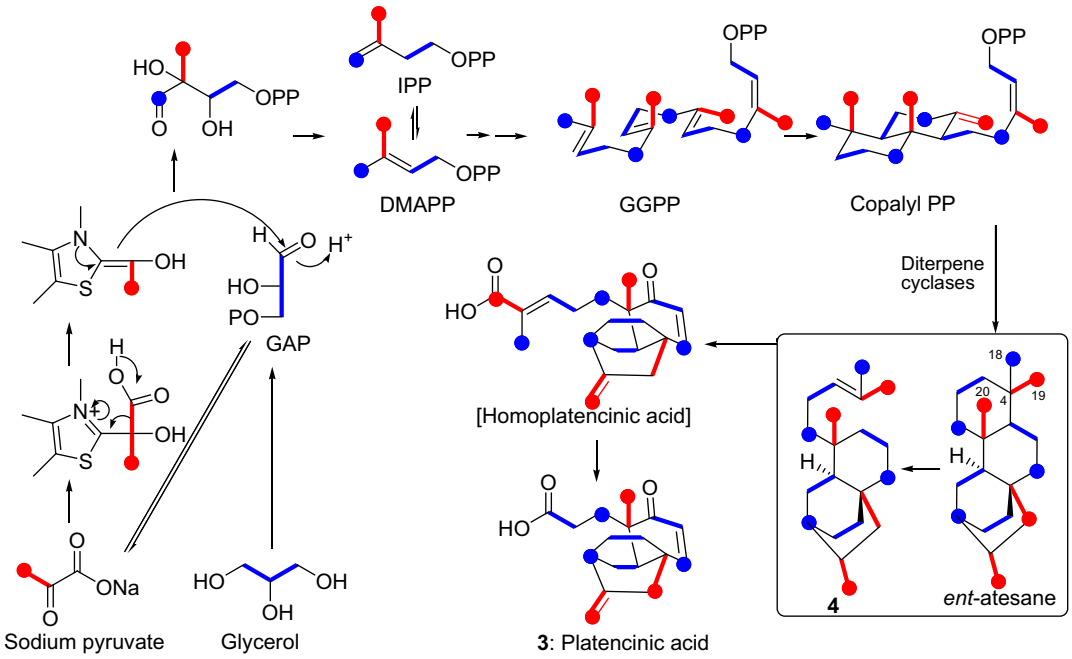
The origin of the remaining three carbon units of the aminobenzoic acid moiety was traced to pyruvate, which was similar to the labeling pattern determined for platensimycin.[8] Feeding sodium [3-^{13}C]pyruvate, sodium [2-^{13}C]pyruvate, and sodium [2,3-$^{13}C_2$]pyruvate produced labeled platencins **2c**, **2d**, and **2e** with enrichments at the same aromatic carbons similar to that was observed for platensimycin. The [2,3-$^{13}C_2$]pyruvate showed incorporation to C-3′/C-4′ (J = 73 Hz) as an intact unit. Similar to that seen for platensimycin, low levels of incorporation were also observed at C-2′, C-3, and C-6′ positions from sodium [3-^{13}C]pyruvate and C-1′, C-4′, and C-6′ from sodium [2-^{13}C]pyruvate. The normal breakdown of the pyruvate into acetyl CoA and conversion into oxaloacetate in the TCA cycle explains the low levels of incorporations and labeling pattern of the aromatic ring. Feeding [2-^{13}C]glycerol produced labeled platencin **2f**. The ^{13}C NMR spectrum of **2f** showed strong enrichments at C-1′ and C-4′ and very weak enrichment at C-6′, similar to observation made with platensimycin. Glycerol is converted to phosphoenolpyruvate (PEP) and pyruvate via the glycolysis pathway. The labeling pattern observed from glycerol further corroborated that the pyruvate was the origin of three carbons. Thus, a biosynthetic scheme for 3-amino-2,4-dihydroxybenzoic acid is proposed in Scheme 1, similar to that proposed for 3-amino-4-hydroxybenzamide.[9]



Scheme 1. Proposed biosynthesis pathway for 3-amino-2,4-dihydroxybenzoic acid.

3. Biosynthesis of platencinic acid

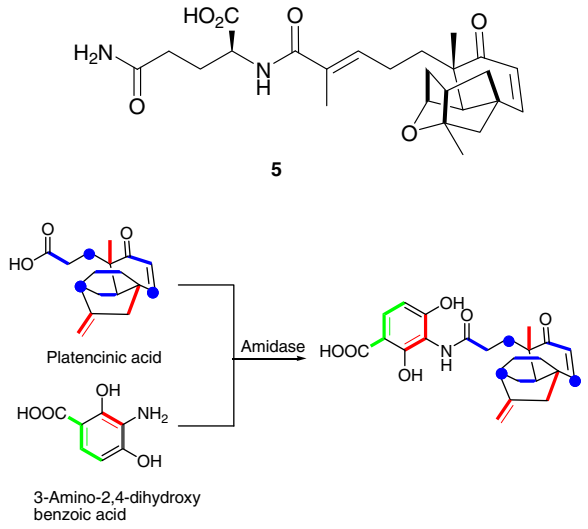
Similar to that seen for platensimycin, no detectable incorporations were observed in platencin upon feeding labeled acetate or mevalonate suggesting the operation of an alternative biosynthetic



Scheme 2. Proposed biosynthesis of platencinic acid.

pathway.[8] Feeding of sodium [3-^{13}C]pyruvate led to **2c**, which showed unambiguous and strong enrichments at C-14, C-16, and C-17 of the platencinic acid. Some of the weaker enrichments observed in platensimycin were not easily discerned in labeled platencin spectrum because of the poor signal to noise ratio due to lower amounts of platencin. Thus, the results were not used to make any conclusions. Labeled platencin **2d** derived from the feeding of sodium [2-^{13}C]pyruvate showed high level of enrichments at C-4, C-8, and C-15 positions. The labeling patterns of platencin observed from the feeding of [2-^{13}C] and [3-^{13}] pyruvates were confirmed by the intact incorporations of sodium [2,3-^{13}C]pyruvate at the corresponding carbons in **2e,** which revealed strong intact incorporations at C-4/C-17 (J = 32 Hz), C-8/C-14 (J = 33 Hz), and C-15/C-16 (J = 67 Hz) and low levels of intact incorporations at C-1/C-2 (J = 50 Hz), C-5/C-6 (J = 50 Hz), and C-12/C-13 (J = 33 Hz) indicating that the platencinic acid portion of the molecule is derived from the non-mevalonate terpenoid pathway, just like platensic acid of platensimycin. This postulation was further corroborated by strong enrichments observed at C-1, C-5, C-9, and C-12 and weak incorporations at C-4, C-8, and C-15 from feeding of [2-^{13}C]glycerol.

The labeling pattern of platencinic acid (**3**) observed from the feeding experiments of pyruvates and glycerol is consistent with the biosynthesis of this unit by the non-mevalonate terpenoid pathway similar to that elucidated for platensimycin and originally proposed by Rohmer et al.[10] and Arigoni and co-workers for the alternate terpenoid biosynthesis pathway.[11] It has been now well established that this pathway utilizes pyruvate and glyceraldehyde-3-phosphate (GAP) in which pyruvate condenses with thiamine. After decarboxylation, thiamine-activated acetyl group is generated which condenses with GAP and undergoes a transposition step to produce first C-5 unit (Scheme 2). This C-5 unit undergoes several reductive and dehydrative transformations to produce the minimal terpenoid building blocks, dimethylallyl diphosphate (DMAPP) and isopentenyl diphosphate (IPP). These isoprenoid precursors produce FPP and GGPP, which undergoes cyclizations by terpene cyclases to generate copallyl diphosphate, the bicyclic precursor. This precursor undergoes further cyclizations by terpenoid



Scheme 3. Proposed biosynthetic coupling of two precursors of platencin.

cyclases to produce first tetracyclic common precursor *ent*-stachane, which undergoes rearrangements to produce either *ent*-kaurane (advanced precursor for platensimycin) or *ent*-atesane.[12] The oxidative cleavage of C4–C5 bond of the A-ring of *ent*-atesane would lead to a tricyclic precursor **4,** which upon further oxidation could produce corresponding C-20 carboxy acid intermediate like that present in homoplatensimide A (**5**).[13] The tricyclic precursor upon further oxidation and excision of the three carbons (formally C-4, C-18, and C-19) could produce platencinic acid (**3**). Amidation of **3** with 3-amino-2,4-dihydroxybenzoic acid would lead to the biosynthesis of platencin (Scheme 3). Varying levels of incorporation of ^{13}C labels from pyruvate and glycerol are expected because of their direct involvement in the glycolytic pathway.

In summary, biosynthesis of platencin involves a mixed mode and utilizes a non-mevalonate terpenoid pathway for the synthesis of the C-17 tricyclic enone acid portion and TCA cycle and PEP for

the synthesis of 3-amino-2,4-dihydroxybenzoic acid each donating 4+3 carbon units.

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