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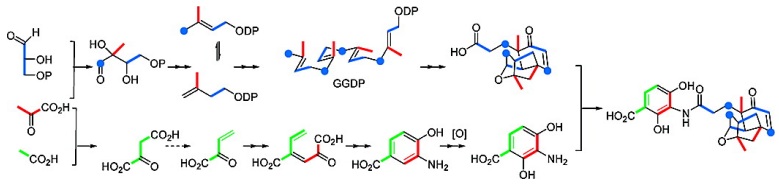

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Biosynthetic Studies of Platensimycin

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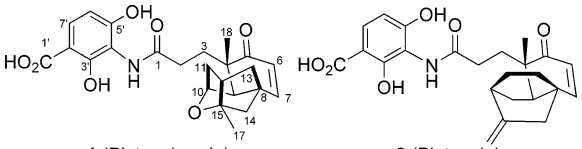
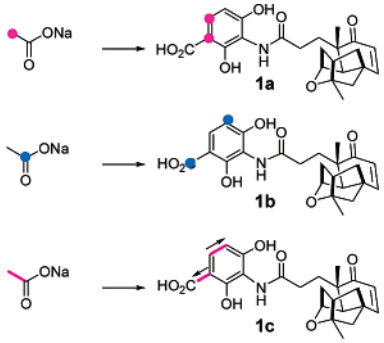
Platensimycin (**1**) and platencin (**2**), two novel antibiotics, were recently isolated from several strains of *Streptomyces platensis.*[1] They impart their potent Gram-positive antibacterial activity including that against drug-resistant organisms (e.g., MRSA, VRE) by uniquely inhibiting acyl enzyme intermediates of the condensing enzymes FabF and FabF/FabH, respectively, vital for fatty acid biosynthesis. Both compounds are highly efficacious in vivo when administered by continuous infusion; however, the efficacy is reduced when administered by conventional routes. This phenomenon is attributed to their poor pharmacokinetic properties which could potentially be improved by chemical modification of the natural product,[2] via total synthesis and through combinatorial biosynthesis. Understanding biosynthetic pathways leading to the biosynthesis of both compounds is a prerequisite for combinatorial biosynthesis and is also helpful for improvement of the titer of these compounds in the producing organism. We report herein the results of biosynthetic studies of platensimycin by stable-isotope precursor incorporation experiments.



1 (Platensimycin) **2** (Platencin)

Platensimycin comprises two structural moieties, a 3-amino-2,4-dihydroxybenzoic acid and a C-17 tetracyclic enone acid (formerly called pentacyclic ketolide),[1a,b] linked by an amide bond. We initiated the investigation by feeding sodium [1-^{13}C]- and [2-^{13}C]-acetate to a culture of *S. platensis* MA7327 with the anticipation that the results would provide hints of the biosynthetic origin of both units of the molecule. The customary production conditions were modified, and a washed-cell incubation procedure was used for precursor feeding. After growing the culture for 4 days in the production medium,[3] when the production of platensimycin had just started (~2 mg/L), the cells were collected and transferred to a 20 mM MES buffer (pH 6.4) containing 5 g/L Stadex-60 as the carbon source. Two aliquots of the precursors (10 mM final concentration in the broth each) were fed at time 0 and at 96 h. The cells were harvested after an additional 74 h of incubation. The fermentation broth was acidified with 3 M HCl and extracted with EtOAc, and the labeled product (titer 25−50 mg/L) was isolated by reversed-phase HPLC. The labeled samples of platensimycin were analyzed by ^{13}C NMR spectroscopy under optimized condition with a 25 s (five times of longest T_1) relaxation delay. The enrichment factor (fold over natural abundance represented as % enrichment) was determined by using external calibration to a natural abundance unlabeled spectrum.



1a **1b** **1c**

Feeding experiments of sodium [2-^{13}C]acetate produced **1a**. The ^{13}C NMR spectrum of **1a** revealed strong (4.5%) enrichment at C-2′ and C-7′. Similar feeding of sodium [1-^{13}C]acetate afforded **1b** with 4.8 and 7.7% incorporations of the label at C-1′ and C-6′, respectively. Feeding of doubly labeled sodium [1,2-^{13}C]-acetate led to **1c** that showed the intact incorporation of the two acetate units labeling C-1′, C-2′ (J = 72 Hz) and C-6′, C-7′ (J = 60 Hz). This labeling pattern indicated that the aminobenzoic acid unit is potentially derived from a four-carbon unit and a three-carbon unit, each likely coming from significantly different in vivo biosynthetic precursor pools. No clear enrichments of the other three aromatic carbons or the carbons in the tetracyclic enone could be discerned. The labeling pattern of the aminobenzoic acid moiety derived from labeled acetate-fed *S. platensis* indicated that the acetate-derived units at C-1′/C-2′ and C-6′/C-7′ were linked in a tail-to-tail manner similar to that described for the biosynthesis of 3-amino-4-hydroxybenzamide.[4] Tail-to-tail acetate coupling is best rationalized by considering the participation of the TCA cycle.

The three-carbon unit is in fact derived from pyruvate. Feeding of sodium [3-^{13}C]pyruvate produced **1d** which showed enrichment at C-2′, C-3′, and C-7′ aromatic carbons {C-2′ (3.2%), C-3′-(1.6%),[5] C-7′ (2.6%)}, and **1e** from the sodium [2-^{13}C]pyruvate experiment exhibited enrichment at C-1′ (2.3%), C-6′ (1.8%), and C-4′ (2%). These results were corroborated by the feeding of sodium [2,3-^{13}C]pyruvate, leading to labeled product **1f** with intact incorporation of pyruvate at C-3′ and C-4′ (J = 70.5 Hz) with ~5% enrichments. The product also exhibited ~4.5 and ~3.5% enrichments at C-1′/C-2′ (J = 72.2 Hz) and C-6′/C-7′ (J = 60 Hz), respectively. The labeling of the latter four carbons likely comes from the breakdown of the pyruvate into acetyl CoA and conversion into oxaloacetate through the TCA cycle. This observation was further supported by strong (6%) enrichment at C-4′ and the low-level enrichment at the C-1′ and C-6′ position of **1g** derived from the feeding of [2-^{13}C]glycerol. Glycerol is converted to phosphoenolpyruvate (PEP) and pyruvate via the glycolytic pathway. Thus, a biosynthetic scheme for 3-amino-2,4-dihydroxybenzoic acid is

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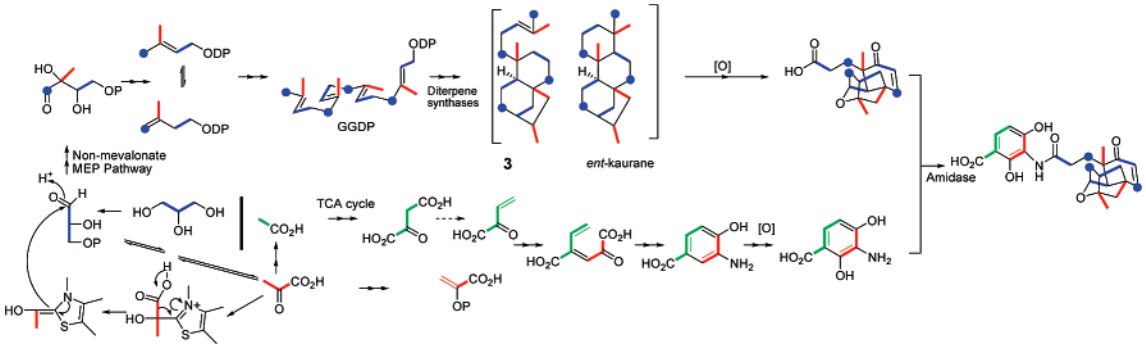
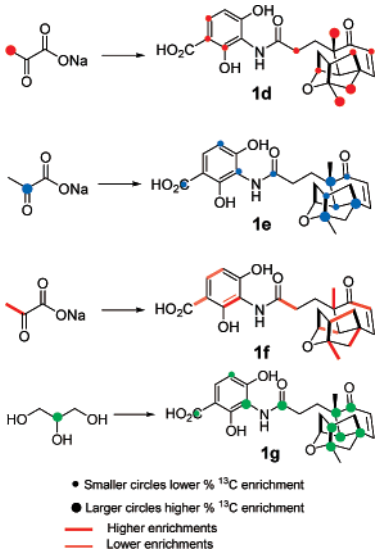
Scheme 1. Proposed Biosynthesis of Platensimycin



proposed as shown in Scheme 1, similar to that proposed for 3-amino-4-hydroxybenzamide.[4]



Feeding experiments with labeled acetate or mevalonate did not lead to significant enrichment of any of the tetracyclic enone carbons. In contrast, feeding of sodium [3-^{13}C]pyruvate led to **1d** with strong labeling at the three tetracyclic enone carbons C-14 (6.4%), C-17 (6.2%), and C-18 (5.3%) and weak enrichment at C-2 (1.9%), C-6 (2.2%), C-10 (2.3%), and C-13 (2.6%); sodium [2-^{13}C]pyruvate led to **1e** with high-level enrichments at C-4 (7.2%), C-8 (8.1%), and C-15 (7.4%) and low enrichments at C-1 (2%), C-5 (2.4%), C-9 (2.6%), and C-12 (2.3%). These labeling patterns were confirmed by feeding sodium [2,3-^{13}C]pyruvate that led to **1f** which showed high levels (>10−13%) of intact incorporations of pyruvate at C-4/C-18 (J = 31.8 Hz), C-8/C-14 (J = 31.8 Hz), and C-15/C-17 (J = 42.4 Hz) and lower level (3−5%) of intact incorporations at C-1/C-2 (J = 49.4 Hz), C-5/C-6 (J = 51 Hz), and C-12/C-13 (J = 32 Hz), indicating that the tetracyclic enone core is derived from the non-mevalonate terpenoid pathway. This postulation was further supported by the 5−6% enrichments observed at C-1, C-4, C-5, C-8, C-9, C-12, and C-15 positions from feedings of [2-^{13}C]glycerol. The observed tetracyclic enone isotope labeling pattern is consistent with the biosynthesis of the tetracycle via the non-mevalonate terpenoid pathway proposed by Rohmer et al.[6] and Arigoni and co-workers.[7] This pathway involves condensation of a thiamine-activated acetyl group arising from the decarboxylation of pyruvate and glyceraldehye-3-phosphate followed by a transposition step. Since both pyruvate and glyceraldehyde-3-phosphate (also glycerol) are part of the glycolytic pathway, varying levels of incorporations are expected.

Thus, the terpenoid building blocks, dimethylallyl diphosphate and isopentenyl diphosphate, synthesized by the non-mevalonate pathway utilizing pyruvate and glyceraldehyde-3-phosphate, condense to form the diterpenoid precursor geranylgeranyl diphosphate that cyclizes to intermediate **3** which is related to (or derived from) *ent*-kaurene.[8] Oxidative cleavage of the double bond of intermediate **3** (Scheme 1, see also Supporting Information) would result in the loss of the terminal three carbons producing the C-17 tetracyclic enone acid unit. An *N*-acyltransferase reaction of tetracyclic enone and aminobenzoic acid would lead to platensimycin (**1**).

In summary, we have described here the biosynthesis of platensimycin, which utilizes a non-mevalonate terpenoid pathway—a pathway common to biosynthesis of terpenoids in actinomycetes and higher plants[7]—via an *ent*-kaurene type intermediate (**3**) leading to the tetracyclic enone acid core and a 4 + 3 carbon unit biosynthesis involving a TCA cycle intermediate and PEP for the biosynthesis of the anilide unit.

Acknowledgment. The authors thank Dr. Neal Connors for helpful discussions.

Supporting Information Available: Detailed biosynthetic schemes and copies of ^{13}C NMR spectra of the labeled compounds **1a**−**1g**, natural abundance ^{13}C NMR spectrum of platensimycin, and complete author list of ref 1a. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Wang, J.; et al. *Nature* **2006**, *441*, 358−361. (b) Singh, S. B.; Jayasuriya, H.; Ondeyka, J. G.; Herath, K. B.; Zhang, C.; Zink, D. L.; Tsou, N. N.; Ball, R. G.; Basilio, A.; Genilloud, O.; Diez, M. T.; Vincente, F.; Palaez, F.; Young, K. *J. Am. Chem. Soc.* **2006**, *128*, 11916−11920 and 15547. (c) Wang, J.; et al. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 7612−7616. (d) Jayasuriya, H.; Herath, K. B.; Zhang, C.; Zink, D. L.; Basilio, A.; Genilloud, O.; Diez, M. T.; Vicente, F.; Gonzalez, I.; Salazar, O.; Pelaez, F.; Cummings, R.; Ha, S.; Wang, J.; Singh, S. B. *Angew. Chem., Int. Ed.* **2007**, *46*, 4684−4688.

(2) Singh, S. B.; Herath, K. B.; Wang, J.; Tsou, N. N.; Ball, R. G. *Tetrahedron Lett.* **2007**, *48*, 5429−5433.

(3) Production media: Stadex-60 (40 g/L), lactose (40 g/L), amberex pH 5.0.

(4) Gould, S. J.; Melville, C. R.; Cone, M. C. *J. Am. Chem. Soc.* **1996**, *118*, 9228−9232.

(5) The original ^{13}C NMR assignments of C-3′ and C-5′ are reversed. New assignments are C-3′ (δ_C 159.1) and C-5′ (δ_C 158.5).

(6) (a) Rohmer, M.; Knani, M.; Simonin, P.; Sutter, B.; Sahm, H. *Biochem. J.* **1993**, *295* (Pt 2), 517−524. (b) Rohmer, M.; Seemann, M.; Horbach, S.; Bringer-Meyer, S.; Sahm, H. *J. Am. Chem. Soc.* **1996**, *118*, 2564−2566.

(7) Eisenreich, W.; Bacher, A.; Arigoni, D.; Rohdich, F. *Cell. Mol. Life Sci.* **2004**, *61*, 1401−1426.

(8) (a) For a review on terpenoid biosynthesis by actinomycetes, see: Dairi, T. *J. Antibiot.* **2005**, *58*, 227−243. (b) Ruzika, L. *Experientia* **1953**, *9*, 357−367. (c) Wenkert, E. *Chem. Ind.* **1955**, 282−284. (d) Graebe, J. E.; Dennis, D. T.; Upper, C. D.; West, C. A. *J. Biol. Chem.* **1965**, *240*, 1847−1854. (e) Hanson, J. R.; White, A. F. *J. Chem. Soc. (C)* **1969**, 981−985. (f) Habich, D.; von Nussbaum, F. *ChemMedChem* **2006**, *1*, 951−954.

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