Published in final edited form as:

Appl Microbiol Biotechnol. 2019 June ; 103(11): 4337–4345. doi:10.1007/s00253-019-09831-x.

The secondary metabolite pactamycin with potential for pharmaceutical applications: biosynthesis and regulation

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Abstract

The antitumor antibiotic pactamycin is a highly substituted aminocyclopentitol-derived secondary metabolite produced by the soil bacterium *Streptomyces pactum*. It has exhibited potent antibacterial, antitumor, antiviral, and antiprotozoal activities. Despite its outstanding biological activities, the complex chemical structure and broad-spectrum toxicity have hampered its development as a therapeutic, limiting its contribution to biomedical science to a role as a molecular probe for ribosomal function. However, detailed understanding of its biosynthesis and how the biosynthesis is regulated has made it possible to tactically design and produce new pactamycin analogues, some of which have shown improved pharmacological properties. This mini-review describes the biosynthesis, regulation, engineered production, and biological activities of pactamycin and its congeners. It also highlights the suitability of biosynthetic methods as a feasible approach to generate new analogues of complex natural products and underscores the importance of utilizing biosynthetic enzymes as tools for chemoenzymatic production of structurally diverse bioactive compounds.

Keywords

pactamycin; *Streptomyces pactum*; antibiotics; biosynthesis; regulation

Introduction

Since the beginning of the first campaign of drug discovery from microbes in the mid 20th century, thousands of bioactive compounds have been isolated from bacteria and fungi, particularly those from the actinomycetes class of filamentous bacteria. With little to no modifications, many of them have made it to the market and been used in clinics as antibiotics (e.g., tetracycline, erythromycin, and rifamycin), antifungals (e.g., amphotericin

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Conflict of interest

The authors declare that they have no conflicts of interest.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

B), anticancer agents (e.g., bleomycin and doxorubicin), immunosuppressants (e.g., rapamycin and FK506), antidiabetics (e.g., acarbose), etc. Yet, there are numerous bioactive secondary metabolites that were discovered but are underutilized or have been shelved due to their unsuitable chemical and/or pharmacological properties. One example is pactamycin, a structurally unique potent antitumor antibiotic produced by the soil bacterium *Streptomyces pactum*.

Pactamycin was first discovered by scientists at the Upjohn Company over six decades ago. Structurally, it contains a highly decorated aminocyclopentitol ring structure, an aminoacetophenone, a 6-methylsalicylyl, and a dimethyl urea moiety. Other congeners of pactamycin have been isolated from the cultures of *S. pactum*; they differ from pactamycin mainly in the structure of the side chains or the presence of a cyclic carbamate (e.g., pactamycate) or a cyclic urea (e.g., pactalactam) (Figure 1) (Hara et al. 1964, Kondo et al. 1964, Dobashi et al. 1986, Hurley et al. 1986, Iwatsuki et al. 2012). The carbamate and cyclic urea derivatives appear to form non-enzymatically during cultivation and/or isolation processes, and the modifications are likely to be responsible for their diminished growth inhibitory activity (Rinehart et al. 1980). On the other hand, 5''-fluoropactamycin has been produced via directed biosynthesis using 3-amino-5-fluorobenzoic acid as a precursor (Figure 1). However, the product showed no significant differences in antimicrobial and cytotoxic activities compared to pactamycin (Adams and Rinehart 1994).

One of the interesting features of pactamycin is its ability to inhibit protein synthesis in all three phylogenetic domains: eukarya, bacteria, and archaea (Bhuyan et al. 1962, Hara et al. 1964, Dobashi et al. 1986). It has strong antibacterial activity against Gram-(+) and Gram-(-) bacteria, but also exhibits potent cytotoxic activity *in vitro* against several cell lines, including KB human epidermoid carcinoma cell line (IC₅₀ of 0.003 µg/mL) (Bhuyan 1962, Dobashi et al. 1986). *In vivo* studies in mice and hamsters showed that pactamycin can inhibit the growth of solid tumors and increase the survival time of leukemic animals at doses ranging from 0.5 to 2 mg/kg (Bhuyan 1962, Bhuyan et al. 1962). Pactamycin is also active against both drug-resistant and drug-susceptible strains of *Plasmodium falciparum* in low nanomolar concentrations, comparable with those of the currently used antimalarial drug of choice artemisinin (Otoguro et al. 2010). Some pactamycin analogues, such as 7-deoxypactamycin (cranomycin) (IC₅₀ 0.4 nM) and de-6MSA-7-deoxypactamycin (jogyamycin) (IC₅₀ 1.5 nM) were more active than pactamycin and artemisinin (Otoguro et al. 2010, Iwatsuki et al. 2012). Crystallographic studies of pactamycin in complex with the *Thermus thermophilus* 30S ribosomal subunit revealed that the antibiotic binds to the ribosomal E-site, hindering the movement of mRNA-tRNA complex and blocking protein biosynthesis (Brodersen et al. 2000, Dinos et al. 2004).

Despite the broad-spectrum activity of pactamycin and its analogues against bacteria, tumors, viruses, and protozoa, their high toxicity profile has restricted their development for clinical use. Pactamycin, 7-deoxypactamycin, and de-6MSA-7-deoxypactamycin are toxic against the human diploid embryonic cell line MRC-5 (IC₅₀ 95, 29.5, and 5.6 nM, respectively) (Iwatsuki et al. 2012). *In vivo* studies of pactamycin in mice, rats and dogs also showed significant toxicity in these animals (Bhuyan et al. 1962). The oral and intravenous LD₅₀ values in mice were 10.7 mg/kg and 15.6 mg/kg, respectively, whereas the IV LD₅₀ in

rats was 1.4 mg/kg. Interestingly, dogs appear to be more sensitive than mice or rats to pactamycin with a lethal dose of only 0.75 mg/kg (Bhuyan et al. 1962). 7-Deoxypactamycin showed higher *in vivo* toxicity than the other analogues in mice with LD₅₀ values of 0.76 mg/kg (intraperitoneal), 0.84 mg/kg (intravenous) and 8.6 mg/kg (oral). The percutaneous LD₅₀ value in pigs was lower than 10 mg/kg (Kondo et al. 1964). Another study showed that the intraperitoneal LD₁₀₀ of 8''-hydroxypactamycin and 7-deoxypactamycin in mice were 25 mg/kg and 1.57 mg/kg, respectively (Dobashi et al. 1986).

The identification of the pactamycin biosynthetic gene clusters (Kudo et al. 2007, Ito et al. 2009) and the availability of genetic tools to manipulate the producing organisms have advanced our knowledge of its biosynthesis and provided alternative means to produce new analogues of pactamycin as lead compounds for therapeutic uses. Detailed understanding of its mode of formation and regulation in the producing bacteria has made it possible to tactically design new compounds using biological systems. So far, numerous new pactamycin analogues have been produced using genetic engineering (Ito et al. 2009, Lu et al. 2011, Abugrain et al. 2016), mutasynthesis (Almabruk et al. 2013), or chemoenzymatic (Abugrain et al. 2017) approaches. In fact, some of them exhibited improved pharmacological properties with outstanding selectivity (Lu et al. 2011, Abugrain et al. 2016). This mini-review focuses on the biosynthesis, gene regulation, engineered production, and biological activities of pactamycin and its analogues.

Biosynthetic building blocks of pactamycin

In *S. pactum*, three major metabolic pathways have been known to contribute to the biosynthesis of pactamycin: the shikimate pathway, the amino sugar pathway, and the polyketide pathway. Earlier work by Rinehart and co-workers using isotopically labeled precursors revealed that glucose, methionine, and acetate are the essential starting units in the biosynthesis of pactamycin (Figure 2) (Weller et al. 1978, Weller and Rinehart 1978, Rinehart et al. 1980, Rinehart et al. 1981). The core aminocyclopentitol moiety is derived from the amino sugar pathway, most likely *via* *N*-acetylglucosamine. The *N*- and *C*-methyl groups on the *N,N*-dimethyl urea and the cyclopentane ring originate from methionine presumably by SAM-dependent methyltransferases (Weller and Rinehart 1978, Kudo et al. 2007, Ito et al. 2009). In addition, the carbons C-6'' and C-2'' of the 3-aminoacetophenone moiety originate from C-6 of glucose while C-8'' is derived from the methyl group of acetate, suggesting a polyketide origin. Moreover, the 6-methylsalicylyl moiety is formed from acetic acid by an iterative type I polyketide synthase (Weller et al. 1978, Ito et al. 2009). Further studies on the building blocks of pactamycin also revealed that the 3-aminoacetophenone (3AAP) moiety is derived from 3-aminobenzoic acid (3ABA) (Rinehart et al. 1981).

The formation of 3-aminobenzoic acid

While 3ABA is commonly used as a starting material in organic synthesis, its occurrence in nature is astonishingly rare. In contrast to its regioisomers, 2-aminobenzoic acid and 4-aminobenzoic acid, which are derived from chorismate, the biosynthesis of 3ABA was unknown. Early chemical incorporation studies using isotopically labeled precursors

suggested that 3ABA is derived from glucose via the shikimate pathway, possibly diverging at dehydroquininate or dehydroshikimate (Rinehart et al. 1980). This was later confirmed by Kudo and co-workers, where 3ABA was formed from dehydroshikimate by the action of a single protein, PctV (PtmT), a unique PLP-dependent aminotransferase-aromatase enzyme (Figure 3) (Hirayama et al. 2013, Hirayama et al. 2015). Inactivation of *ptmT* in *S. pactum* resulted in a strain that was no longer able to produce pactamycin, and chemical complementation with 3ABA and [1¹³C]-3ABA was able to rescue the production of pactamycin (Almabruk et al. 2013). In addition, supplementing the *ptmT* mutant strain with 3-amino-5-fluorobenzoate resulted in the production of 5''-fluoropactamycin and 5''-fluoropactamycate which further confirmed a direct involvement of 3ABA in pactamycin biosynthesis (Almabruk et al. 2013).

Proposed biosynthetic pathways to 3-aminoacetophenone

Based on bioinformatic data and the putative functions of genes within the pactamycin cluster, two reasonable biosynthetic routes have been proposed for the conversion of 3ABA to 3AAP. The first pathway involves activation of 3ABA to the adenylate form by the action of the putative AMP-forming acyl-CoA synthetase PctU (PtmS). This is followed by coupling of 3ABA-AMP and acetyl-CoA to give a β -ketoacyl-CoA thioester (Kudo et al. 2007). The product is then hydrolyzed by the putative hydrolase PtmO and the resulting β -ketoacid undergoes spontaneous decarboxylation to give 3-aminoacetophenone (3AAP) (Figure 3). It has also been shown that 3AAP is glycosylated by the glycosyltransferase enzyme PctL to give *N*-acetylglucosaminyl-3AAP (Kudo et al. 2007).

The second pathway involves a complex of discrete polyketide synthase proteins, PtmS, PtmI, and PtmK, where 3ABA is activated by PtmS to 3ABA-AMP and subsequently loaded to the acyl carrier protein PtmI (Ito et al. 2009). Subsequently, decarboxylative Claisen condensation between 3ABA-ACP and malonyl-ACP catalyzed by the putative β -ketoacyl-ACP synthase PtmK would give a β -ketoacyl intermediate, which is then released from the ACP (PtmI) by PtmO and undergoes spontaneous decarboxylation to produce 3AAP. Gene inactivation experiments revealed that *ptmS*, *ptmI*, and *ptmK* are necessary for the formation of 3AAP, and the gene products may function in a coordinated fashion (Abugrain et al. 2017). Interestingly, chemical complementation of *ptmT* mutant with 3AAP or GlcNAc-3AAP was not able to rescue the production of pactamycin, indicating that free 3AAP and its glycosylated product are not involved in the pathway. This leads to the notion that glycosylation of β -ketoacyl intermediate may occur while it is still tethered to the acyl carrier protein PtmI.

The formation of the aminocyclopentitol moiety of pactamycin

The mechanism underlying the formation of the five-membered ring structure of pactamycin has so far been a matter of speculation. An incorporation study using isotopically labeled precursors showed that it is derived from glucose – presumably via *N*-acetylglucosamine. This unusual conversion of glucose to a five-membered ring cyclopentitol has been proposed to be catalyzed by a radical SAM-dependent enzyme (PtmC). Indeed, inactivation of *ptmC*

in *S. pactum* completely abolishes the production of pactamycin (Ito et al. 2009). However, no biochemical evidence is yet available to show this remarkable bioconversion.

Recently, biochemical characterization of PctP (an NAD⁺-dependent dehydrogenase) and PctC (a PLP-dependent aminotransferase) revealed their ability to convert GlcNAc-3AAP to its corresponding 3'-amino derivative (Hirayama et al. 2018). PctP can also oxidize GlcNAc-3ABA and GlcNAc-3-aminophenyl- β -oxopropanoic acid ethyl ester, but it does not accept UDP-GlcNAc, glucosaminyl-3AAP, and 4'-C-methyl-N-acetyl-D-galactosaminyl-3-aminoacetophenone as substrates (Hirayama et al. 2018). The fact that UDP-GlcNAc is not a substrate of PctP suggests that the oxidation and transamination reactions do not take place before glycosylation. On the other hand, while PctP and PctC can convert GlcNAc-3AAP to its 3'-amino derivative, the substrate is not an intermediate in the pactamycin pathway. Therefore, it may be speculated that in pactamycin biosynthesis PctP (PtmN) and PctC (PtmA) act on a glycosylated product that is still attached to an acyl carrier protein or a coenzyme A (Figure 4). Detailed transformation from the glycosylated product to the known aminocyclopentitol TM-101 remains unclear, but may involve urea formation, two C-methylations, ring contraction, deacetylation, hydrolysis and decarboxylation.

Tailoring processes in pactamycin biosynthesis

One of the most intriguing features of pactamycin biosynthesis is the wealth of its tailoring processes. These include a tandem C-methylation, N-methylations, hydroxylation, and the attachment of 6MSA. Earlier studies using gene inactivation and metabolic analysis confirmed the function of some of the genes involved in the tailoring processes. Those include *ptmQ* (an iterative type I polyketide synthase gene), *ptmD* (a SAM dependent N-methyltransferase gene), and *ptmH* (a radical SAM-dependent C-methyltransferase gene). Inactivation of *ptmQ* in *S. pactum* resulted in a mutant that produces de-6MSA-pactamycin, indicating that PtmQ is responsible for the formation of 6MSA (Ito et al. 2009). Inactivation of *ptmD* resulted in a mutant that produces N,N-didemethylpactamycin and N,N-didemethyl-7-deoxypactamycin (Lu et al. 2011), whereas inactivation of *ptmH* led to the production of de-6MSA-7-demethyl-7-deoxypactamycin (TM-025) and 7-demethyl-7-deoxypactamycin (TM-026) (Lu et al. 2011, Almabruk et al. 2013). However, while the products are generally consistent with the putative functions of the genes, they appear to have been modified further by the succeeding tailoring enzymes downstream in the pathway. The unspecific modifications by these enzymes have made it difficult to determine the actual sequence of the tailoring steps.

However, further studies using double and triple knockout mutants of *S. pactum* were able to interrogate the step-by-step sequence of the tailoring processes in pactamycin biosynthesis (Abugrain et al. 2016). For example, inactivation of both *ptmD* and *ptmH* resulted in a mutant strain that produced TM101 (Figure 5), suggesting that C-methylation at C-1 and C-5 as well as urea formation take place early in the pathway. On the other hand, inactivation of both *ptmD* and *ptmQ* resulted in a mutant strain that produced TM102, indicating that TM-101 is converted to TM-102 by the radical SAM-dependent C-methyltransferase PtmH, and TM-102 is the natural substrate for PtmD. The detection of de-6MSA-7-deoxypactamycin (jogyamycin) in the wild-type *S. pactum* (Iwatsuki et al.

2012) also confirms that TM-102 is the actual substrate for PtmD. Thus, PtmD catalyzes the *N*-methylation of TM-102 to give de-6MSA-7-deoxypactamycin, which is then converted to 7-deoxypactamycin by 6MSA attachment. This esterification reaction is catalyzed by a KAS III-like protein, PtmR, a highly promiscuous enzyme that can transfer 6MSA directly from the iterative type I PKS PtmQ to the primary hydroxy group of the aminocyclopentitol core (Abugrain et al. 2017). Interestingly, the *ptmQ* mutant did not only produce jogyamycin but also de-6MSA-pactamycin (Ito et al. 2009), indicating that the latter compound is a shunt product resulting from a C-7 hydroxylation reaction (Figure 5) (Abugrain et al. 2016). The cytochrome P450 monooxygenase PtmY may play a role in this reaction, as it has been proposed to catalyze the conversion of 7-deoxypactamycin to the final product pactamycin. However, inactivation of *ptmY* in *S. pactum* did not completely block the hydroxylation reaction, indicating a cross complementation by other enzyme(s) in the cells.

Biosynthetic engineering of pactamycin

In conjunction with the advance of molecular genetic tools in recent years, biosynthetic engineering has played a more significant role in generating complex natural products. Taking advantage of the genetic information for pactamycin biosynthesis and functional understanding of the corresponding enzymes, new analogues of pactamycin with improved biological properties have been successfully generated. Pactamycin analogues such as de-6MSA-pactamycin, de-6MSA-7-demethyl-7-deoxypactamycin (TM-025), 7-demethyl-7-deoxypactamycin (TM-026), de-6MSA-*N,N*-didemethyl-7-demethyl-7-deoxypactamycin (TM-101), and de-6MSA-*N,N*-didemethyl-7-deoxypactamycin (TM-102) have been obtained through this approach (Ito et al. 2009, Lu et al. 2011, Abugrain et al. 2016). Some of these compounds, while still maintaining their anti-plasmodial activity, have no substantial antibacterial activity and are less toxic than pactamycin against mammalian cells. For example, TM-025 and TM-026 showed potent antimalarial activity with IC₅₀ values of ~ 25 nM against both *P. falciparum* D6 (chloroquine sensitive) and Dd2 (chloroquine resistant) strains, but they appear to be 10 – 30 times less toxic than pactamycin against HCT116 cells line (Lu et al., 2011). This improved selectivity index suggests distinct ribosomal binding selectivity or mechanisms of action of the compounds in *Plasmodium* and bacterial or mammalian cells. TM-101 and TM-102 were also active against chloroquine-sensitive (D6) and chloroquine-resistant (Dd2 and 7G8) strains of *P. falciparum* with TM-102 (IC₅₀ 37–67 nM) being 3.5–6.5 times more active than TM-101 (IC₅₀ 218–271 nM). However, TM-102 was about 10 times less active than pactamycin against *P. falciparum* strains and also much less toxic than pactamycin against HCT116 and HEPG2 (hepatocellular carcinoma) cancer cell lines (Abugrain et al., 2016).

Genetically engineered strains of *S. pactum* have also been used for mutasynthesis of unnatural pactamycin analogues. Chemical complementation of the *ptmT/ptmH* mutant strain with 2-fluoro-5-aminobenzoic acid resulted in fluorinated TM-025 and TM-026. These fluorinated compounds also showed potent antimalarial activity against chloroquine-sensitive (D6) and chloroquine-resistant (Dd2 and 7G8) strains of *P. falciparum* with IC₅₀ values in low nM concentrations (<40 nM), comparable to those of TM-025 and TM-026 (Almabruk et al., 2013).

As noted above, a β -ketoacyl-ACP synthase (KAS) III-like protein (PtmR) which functions as an acyltransferase that catalyzes the transfer of 6MSA to the aminocyclopentitol core has been identified (Abugrain et al. 2017). This enzyme is highly promiscuous, which is able to utilize a number of de-6MSA-pactamycin analogues and a variety of *N*-acetylcysteamine thioesters as substrates to produce new derivatives of pactamycin (Figure 6). This finding has led to the development of a chemoenzymatic approach that enables quick production of pactamycin derivatives for structure-activity relationship studies.

Regulatory system in pactamycin biosynthesis

As observed in many other biosynthetic pathways in *Streptomyces*, pactamycin biosynthesis may be regulated by a complex regulatory system involving pathway-specific regulatory proteins, which control the transcription of the structural genes, and global regulatory systems. Analysis of the pactamycin biosynthetic gene cluster revealed two putative pathway-specific regulatory genes *ptmF* and *ptmE* in the cluster (Lu et al. 2018). PtmF contains a DNA-binding winged HTH domain and a putative response regulator that belongs to the Trans_reg_C family (Chinnadurai 2007). Phylogenetically, PtmF was found to be closely related to the DeoR transcriptional regulator and shares high similarity with the OmpR-like DNA-binding motif of two component regulators, which is the characteristic of the SARP family. On the other hand, the deduced product of *ptmE* contains a nucleotide-binding motif (p-loop) at the *N*-terminal and is proposed to have kinase activity. Inactivation of *ptmF* and *ptmE* genes in *S. pactum* completely abolished pactamycin production, indicating that PtmE and PtmF are positive regulators which control the expression of the whole pactamycin biosynthetic gene cluster. However, introduction of a second copy of *ptmF* and *ptmE* into *S. pactum* did not affect the pactamycin titer, suggesting the involvement of other systems in controlling the pathway (Lu et al. 2018). In addition, the production of pactamycin is affected by inorganic phosphate, presumably through the PhoR-PhoP system. Cultivation of *S. pactum* in a medium containing phosphate more than 2 mM resulted in diminished transcription of *ptmF* and *ptmE* as well as lower production of pactamycin. However, inactivation of the *phoP* gene in *S. pactum* annihilated or diminished the effect of high phosphate concentration on pactamycin production.

Conclusions

While pactamycin possesses remarkable biological activity, due to the significant and broad-range cytotoxicity its development as a therapeutic agent has stalled. However, there has been new hope for the future of this highly active class of natural products, attributed mostly to the recent successes in its chemical synthesis and biosynthetic engineering. While our understanding of the biosynthesis of pactamycin is fragmentary, on-going efforts on this front are expected to provide more insights into its mode of formation in nature. Nonetheless, based on the current state of knowledge, a number of elements have been noted that underline the uniqueness of its machinery: 1) the occurrence of the structural components of pactamycin, e.g., the 3-aminoacetophenone unit, its precursor 3-aminobenzoic acid, and the aminocyclopentitol core, are extremely rare in nature; 2) the mode of formation of pactamycin encompasses several major pathways: the shikimate pathway, the amino sugar pathway, and the acetate pathway (the iterative type I and type II

PKSs); and 3) many enzymes involved in pactamycin biosynthesis are somewhat unusual and promiscuous in nature. In addition, the entire pactamycin pathway in *S. pactum* appears to be regulated by two pathway specific regulators, PtmF and PtmE.

Another interesting but perhaps less desired feature of pactamycin is its ability to inhibit protein synthesis in both prokaryotes and eukaryotes. This broad-spectrum inhibitory activity is responsible for the wide-range toxicity of the antibiotic, which may be modulated by modifying its chemical structure. In fact, alteration of the pactamycin structure through biosynthetic engineering has resulted in a number of new pactamycin analogues, some of which exhibit improved target selectivity. This accomplishment highlights the suitability of the biosynthetic method as a feasible approach to generate new analogues of complex natural products. In addition, the promiscuity of the structural enzymes may be exploited for chemoenzymatic production of structurally diverse derivatives, underscoring the importance of a deep understanding of the enzymes involved in natural product biosynthesis.

Acknowledgments

The authors thank Philip Proteau for a critical reading of this manuscript.

Funding

This work was supported by grant AI129957 from the National Institute of Allergy and Infectious Diseases. The content is solely the responsibility of the authors and does not represent the official views of the National Institute of Allergy and Infectious Diseases, or the National Institutes of Health (NIH).

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pactamycin: $R^1=OH$, $R^2=H$, $R^3=H$

8''-hydroxypactamycin: $R^1=OH$, $R^2=OH$, $R^3=H$

7-deoxypactamycin: $R^1=H$, $R^2=H$, $R^3=H$

8''-hydroxy-7-deoxypactamycin: $R^1=H$, $R^2=OH$, $R^3=H$

5''-fluoropactamycin: $R^1=OH$, $R^2=H$, $R^3=F$

pactamycate: $R^1=H$, $R^2=H$

8''-hydroxypactamycate: $R^1=OH$, $R^2=H$

5''-fluoropactamycate: $R^1=H$, $R^2=F$

pactalactam: $R^1=OH$, $R^2=H$

7-deoxypactalactam: $R^1=H$, $R^2=H$

8''-hydroxy-7-deoxypactalactam: $R^1=H$, $R^2=OH$

de-6MSA-7-deoxypactamycin
(jogamycin)

Figure 1.

Chemical structures of pactamycin and its congeners

Figure 2.

Biosynthetic origin of pactamycin. The aminocyclopentitol core is derived from glucose, presumably via *N*-acetylglucosamine; the 3-aminoacetophenone moiety is derived from the shikimate pathway; and the 6-methylsalicylyl moiety is derived from four molecules of acetate by an iterative type I polyketide synthase enzyme. The methyl groups and C-7 are derived from methionine.

Figure 3. Biosynthesis of 3-aminobenzoic acid and proposed pathways to 3-aminoacetophenone. **A.** Formation of a β -ketoacyl intermediate by Claisen condensation involving acetyl-CoA. **B.** Formation of a β -ketoacyl intermediate by Claisen condensation involving discrete PKS proteins.

Figure 4.

Proposed mode of formation of the 3-aminocyclopentitol ring.

Figure 5. Tailoring steps in pactamycin biosynthesis. Many tailoring enzymes in pactamycin biosynthesis have relaxed substrate specificity.

Figure 6.

The biosynthesis and transfer of the 6-methylsalicylyl moiety. **A.** 6-methylsalicylyl moiety is produced by the iterative type I PKS PtmQ and transferred to de-6MSA-pactamycin by a KAS III-like enzyme PtmR. **B.** PtmR recognizes various *N*-acetylcysteamine thioesters as substrates to produce a suite of pactamycin analogues.