### Instructions for use

#### Title
Stepwise cyclopropanation on the polycyclopropanated polyketide formation in jawsamycin biosynthesis

#### Author(s)
Hiratsuka, Tomoshige; Suzuki, Hideaki; Minami, Atsushi; Oikawa, Hideaki

#### Citation
Organic & biomolecular chemistry, 15(5): 1076-1079

#### Issue Date
2017-02-07

#### Doc URL
http://hdl.handle.net/2115/68285

#### Type
article (author version)

#### File Information
OBC_20161221_revised Txt.pdf
Jawsamycin is a polyketide-nucleoside hybrid with a unique polycyclopropane moiety on a single polyketide chain. The unexpected isolation of cyclopropane-deficient jawsamycin analogs allowed us to propose a stepwise cyclopropanation mechanism for the enzymatic synthesis of this polyketide. The concise timing of the cyclopropanation could be regulated by a delicate balance between reaction rates of the condensation and cyclopropanation reactions.

Jawsamycin (FR-900848; 1), which was isolated from Streptoverticillium fervens HP-891, is an antifungal agent with potent activities against various phytopathogenic fungi (Figure 1). In contrast to most cyclopropane-containing natural products such as duocarmycin and curacin A, 1 has a unique polycyclopropane skeleton with the same stereochemistry for all of the cyclopropane rings on its single polyketide chain. Notably, U-106305, which was isolated from Streptomyces sp. UC11136, is the only other polyketide with a similar polycyclopropane skeleton (Figure 1). Total syntheses of 1 and U-106305 have established their relative and absolute stereochemistries, suggesting that the cyclopropanations occur with a high enantiofacial selectivity to afford the same configurations.

Recently, we identified the biosynthetic gene cluster of 1, which consists of nine open reading frames (jaw1–jaw9) and revealed the biosynthetic machinery by in vivo and in vitro analyses. Reconstitution of the minimal polyketide synthases (PKSs) in S. lividans demonstrated that three enzymes, including the iterative type-I PKS Jaw4 (KS-DH-ACP); the trans-acting ketoreductase (KR) Jaw6; and the radical SAM enzyme Jaw5, participate in the construction of the polyketide chains. Previous results showed that a SNAC analog of the cyclopropanated diketide intermediate (B; R = Me, Scheme 1)

Figure 1 Structures of polycyclopropane-containing natural products.

Scheme 1 Proposed chain elongation mechanism catalyzed by Jaw456.
was enantioselectively incorporated into 1, and that no jawsamycin analogs bearing a polunsaturated polyketide were produced in a jaw5 deletion mutant. Based on these results, we proposed the following stepwise cyclopropanation mechanism (Scheme 1): 1) Jaw4 and Jaw6 would mediate the construction of the α,β-unsaturated thioester A according to the functions of their individual domains (steps 1–3); and 2) Jaw5 would catalyze the cyclopropanation of A using S-adenosyl-L-methionine to give B (step 4). Of particular interest is that the cyclopropanation occurs in an iterative manner (cycles 1–6 except cycle 2, Scheme 1). However, direct evidences to support a biosynthetic hypothesis or a detailed regulatory mechanism involving Jaw456 are scarce. Here, we describe the isolation and characterization of cyclopropane deficient analogs and provide a biosynthetic rationale to account for the enzymatic polycyclopropanation.

We previously achieved the heterologous expression of the jaw genes in Streptomyces lividans TK23 (except for a reductase jaw1), which allowed us to isolate dehydrojawsamycin (2) (Figure 1). Considerable amounts of the analogs of 2 were also identified in this transformant. LC-MS analysis revealed regularly shifted molecular ion peaks, which were most likely attributed to analogs lacking CHO (14 m.u.) and C3H2 (26 m.u.) moieties (Figure 2). For structural determination of those analogs, large-scale fermentation was then conducted. Repeated chromatography of the crude metabolites led to isolation of five analogs, including compounds 3, 4, and 2a–4a. HR-MS analysis showed the molecular formula of each analog: 3 (C30H33N3O6), 4 (C29H32N3O6), 2a (C31H33N3O6), 3a (C29H32N3O6), and 4a (C28H31N3O6). The molecular formula of 2a proved that its structure differs from that of 2 (C32H34N3O6) in terms of the number of cyclopropane moieties. The 1H-NMR and COSY spectra of 2a showed that there were eight olefinic protons attached to a methyl-terminal- and a carboxy-terminal conjugated diene. The terminal allylic methyl protons were shifted downfield (1.66 ppm) compared with those of 2 (1.00 ppm). Characteristic signals of 5'-amino-5'-deoxyuridine (AdU) were also observed. Further extensive NMR analyses revealed that 2a contained a C16-polyketide backbone harboring four contiguous cyclopropanes flanked by two conjugated diene moieties. The polyketide structure of 2a was described as C16-CP4 (1278) to indicate the chain length (Cx), the number of cyclopropane moieties (CPy), and the positions of double bonds (z in a parenthesis), respectively. In contrast to 2a, MS analysis indicated that analogs 3, 4, 3a, and 4a have a C16-polyketide backbone with a variable number of cyclopropane moieties (three to five). The 1H-NMR spectrum of 3 (cyclopropane number: 5) showed two isolated olefin moieties.

![Scheme 2](image-url)

Scheme 2. Proposed biosynthetic pathway for 2–4 and 2a–4a. Compounds 2a–4a as well as 3 and 4 were isolated in this study. The numbering of the double bonds is shown in the upper part of the polyketide structure.

Figure 2. MS spectrum of the crude metabolites from the transformant producing 2.
Furthermore, the similarity in the chemical shifts of the carboxy-terminal signals of 3 and U-106305 suggested that one of the cyclopropane moieties was located next to an α,β-unsaturated amide. Detailed NMR analyses revealed that C16-CP5-(27)-AdU 3 contained one isolated and four contiguous cyclopropanes on a C16 polyketide chain. The structures of C16-CP3-(267)-AdU 4, C16-CP2-(127)-AdU 3a, and C16-CP3-(1267)-AdU 4a were determined by NMR analysis (Scheme 2). Compounds 2a, 3a, and 4a featured the same polycyclopropanation pattern as 2, 3, and 4, respectively, but all lacked an isolated cyclopropane at their methyl-terminal.

Other than the isolated dehydrojawsamycin analogs described above, LC-MS analysis of the crude extracts revealed the production of several minor dehydrojawsamycin analogs with a shorter polyketide chain. The compounds 2 and analogs were then analyzed by a liquid-chromatography high resolution tandem mass spectrometry (LC-HR-MS/MS). Several key elimination fragments (i.e., Fa, C6H2O2N2; Fb, C6H2O2N2; Fc, C6H2O2N2; Fd, C6H2O2N2; Fe, C6H2O2N2) were observed (Figures 3 and S2), which indicated that each analog has a 5′-amino-5′-deoxyuridine moiety. These fragments could also be used to speculate the length and the number of cyclopropanes in the polyketide chain. Feeding experiments with L-[Me-13]C1]methionine led to the efficient incorporation of a 13C-labeled methylene group into the cyclopropane moieties of the polyketide chain, resulting in expected mass shifts for each analog (Figures S3–S8). Consequently, 25 polyketide analogs with variable chain lengths were identified.

A similar set of 1 analogs (23 analogs) was identified in 1 producing S. lividans transformant containing all of the jaw genes (Figures S1 and S9–S13). Among them, C18-CP3-AdU and C16-CP2-(27)-AdU were also detected in the crude metabolites of S. fervens HP-891, albeit in low yields (Figure S14). These minor analogs were classified according to the length and number of cyclopropane units in their polyketide chains (Tables 1 and S1), showing that a limited number (one to three) of isomers was produced by the 1 and 2 producing transformants. The putative structures of these isomers will be discussed in the following paragraph.

Based on these results, we have proposed detailed mechanisms for the elongation of the chains in 1 and 2, which are shown in Schemes 2 and S1. For dehydrojawsamycin analogs harboring a methyl-terminal cyclopropane (2–4), a putative C14-CP4-(26) was regarded as a common intermediate. A cyclopropanation followed by a chain elongation of C14-CP4-(26) would afford C16-CP5-(27) and C18-CP6-(278) via C14-CP5-(2). Alternatively, skipping the cyclopropanation of C14-CP4(26) would yield C16-CP4-(26). All of the resulting PKS-tethered polyketides would then be cleaved by the action of promiscuous acyltransferase Jaw2 to give 2–4. Polyketides with a methyl-terminal conjugated diene such as C18-CP2-(1278), C16-CP4-(127), and C14-CP5-(1267) could also be biosynthesized in a similar manner from C14-CP5-(1267), the terminal diene moiety of which could be constructed by skipping the cyclopropanation of C2-CP2-(1) (Scheme S1). Taken together, these mechanistic considerations suggested that the α,β-unsaturated polyketide A was a branch point in the enzymatic polyketide synthesis and that the timing of the cyclopropanation could be regulated by a delicate balance between the reaction rates of the condensation (step 1) and cyclopropanation (step 4) steps, which would be catalyzed by the KS domain of Jaw4 and Jaw5, respectively. This hypothetical regulatory mechanism could be related to the natural deconstruction system, with the catalytic domains responsible for the construction of the polyketides being separated into three different enzymes (Jaws 4, 5 and 6).

In the case of PksA, which is a non-reducing fungal iterative PKS involved in aflatoxin biosynthesis, the application of an artificial deconstruction approach led to the functional characterization of each domain that balance of the kinetics and cooperative controls facilitated the correct polyketide elongation cycle. The similarities in these mechanisms could therefore support our hypothesis. However, several other possibilities including that Jaw5 has preferred or strict substrate specificities to control the timing of the cyclopropanation cannot be excluded.

Although most of the cyclopropanation skipping steps occurred in cycles 1 and 6, the production of minor polyketides with C16-CP3, C14-CP5, C14-CP2, and C12-CP1 indicated that the cyclopropanation step could also be skipped at cycles 3, 4 and 5. Putative structures for these isomers are shown in Table S2. A simple extension of this model allowed us to propose a mechanism for the biosynthesis of the polyketide in U-106305. An initial cyclopropanation followed by the chain elongation of C16-CP5-(27) would afford C18-CP6-(28) with five contiguous

---

**Table 1.** Number of polyketide isomers of 2 analogs. Compounds, that were not observed by LC-HR-MS/MS analysis, are indicated as horizontal bars (-). C10-CP5, C12-CP5, and C12-CP4 were not biosynthetically available and the corresponding columns are filled by grey colour.

<table>
<thead>
<tr>
<th>C18</th>
<th>C16</th>
<th>C14</th>
<th>C12</th>
<th>C10</th>
<th>C8</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>-</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>
cyclopropanes (Scheme S2). To our knowledge, imprecise programming of the polyketide biosynthetic machinery for iterative PKS-catalyzed processes has been reported in TENS,\textsuperscript{12} a fungal highly reducing iterative PKS-nonribosomal synthetase hybrid, and in Bref-PKS,\textsuperscript{13} a fungal highly reducing PKS.

In summary, we have identified cyclopropane and/or acetate unit-deficient dehydrojawsamycin analogs from a previously constructed \textit{S. lividans} transformant harboring \textit{jaw} genes. The polyketide structures of these analogs allowed us to propose the biosynthetic logic on the mechanisms responsible for their enzymatic polycyclopropanation. Significantly, the balance between the reaction rates of the condensation and cyclopropanation reactions toward the \(\alpha,\beta\)-unsaturated polyketide appeared to be critical to the synthesis of the unique polyketide skeletons found in these systems.

This work was financially supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan (JSPS KAKENHI Grant Number JP15H01835 (H. O.) and JP16H06446 (A. M.)). We thank Ms. Seiko Oka (Hokkaido University) for her kind cooperation on the LC-HR-MS/MS analysis.

Notes and references