HOKKAIDO UNIVERSITY

Title	Stepwise cyclopropanation on the polycyclopropanated polyketide formation in jawsamycin biosynthesis
Author(s)	Hiratsuka, Tomoshige; Suzuki, Hideaki; Minami, Atsushi et al.
Citation	Organic & biomolecular chemistry, 15(5), 1076-1079 https://doi.org/10.1039/c6ob02675c
Issue Date	2017-02-07
Doc URL	https://hdl.handle.net/2115/68285
Type	journal article
File Information	OBC_20161221_revised Txt.pdf

Hokkaido University Collection of Scholarly and Academic Papers : HUSCAP

Please do not adjust margins

Journal Name

COMMUNICATION

Stepwise Cyclopropanation on the Polycyclopropanated Polyketide Formation in Jawsamycin Biosynthesis

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Tomoshige Hiratsuka, Hideaki Suzuki, Atsushi Minami and Hideaki Oikawa*

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).[1] In contrast to most cyclopropane-containing natural products such as duocarmycin[2] and curacin A,[3] **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).[4] Total syntheses of **1** and U-106305 have established their relative and absolute stereochemistries,[5] suggesting that the cyclopropanations occur with a high enantiofacial selectivity to afford the same configurations.

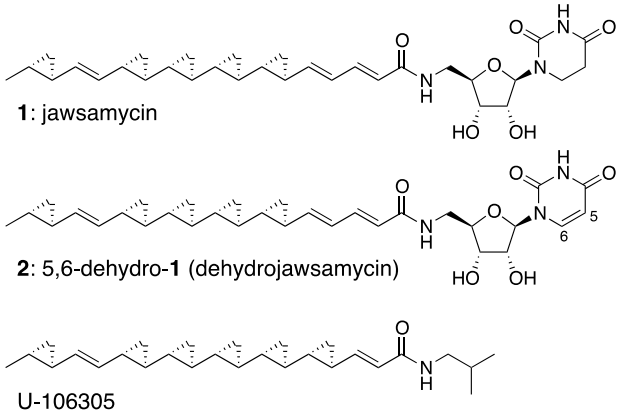
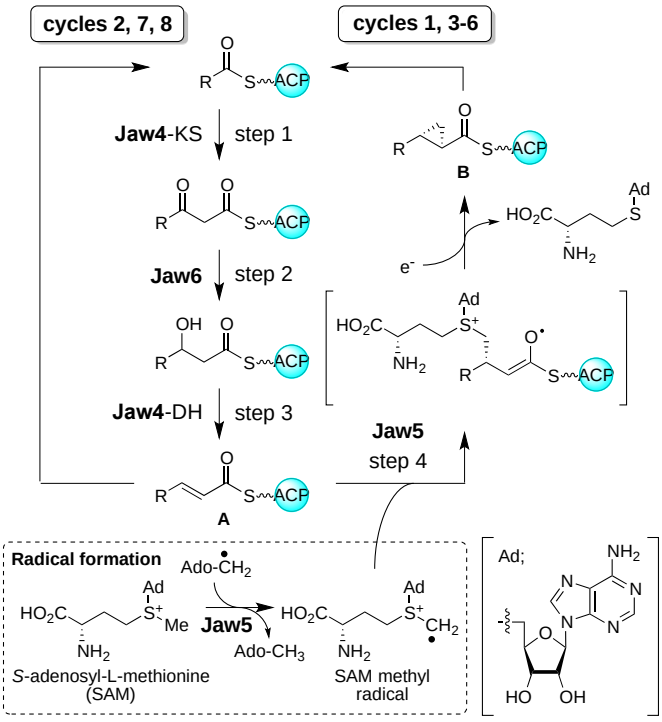


Figure 1 Structures of polycyclopropane-containing natural products.

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.[6] Reconstitution of the minimal polyketide synthases (PKSs) in *S. lividans* demonstrated that three enzymes, including the iterative type-I PKS Jaw4 (KS-AT-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)



Scheme 1 Proposed chain elongation mechanism catalyzed by Jaw456.

Division of chemistry, Graduate School of Science, Hokkaido University, Sapporo 060-0810, Japan
E-mail: hoik@sci.hokudai.ac.jp
Fax: +81-11-706-3448; Tel: +81-11-706-2622
Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

Please do not adjust margins

Please do not adjust margins

was enantioselectively incorporated into **1**,[7] and that no jawsamycin analogs bearing a polyunsaturated polyketide were produced in a *jaw5* deletion mutant.[6] 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[8] (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).[6] 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 CH_2 (14 m.u.) and C_2H_2 (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** ($C_{30}H_{39}N_3O_6$), **4** ($C_{29}H_{37}N_3O_6$), **2a** ($C_{31}H_{39}N_3O_6$), **3a** ($C_{29}H_{37}N_3O_6$), and **4a** ($C_{28}H_{35}N_3O_6$). The molecular formula of **2a** proved that its

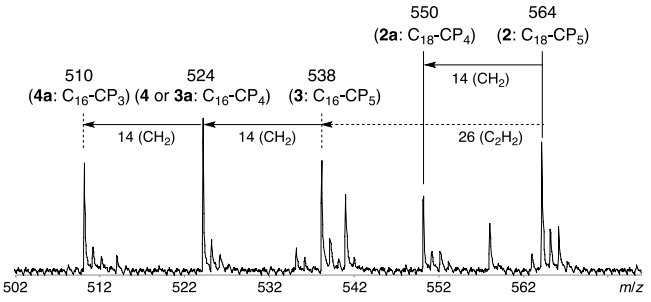
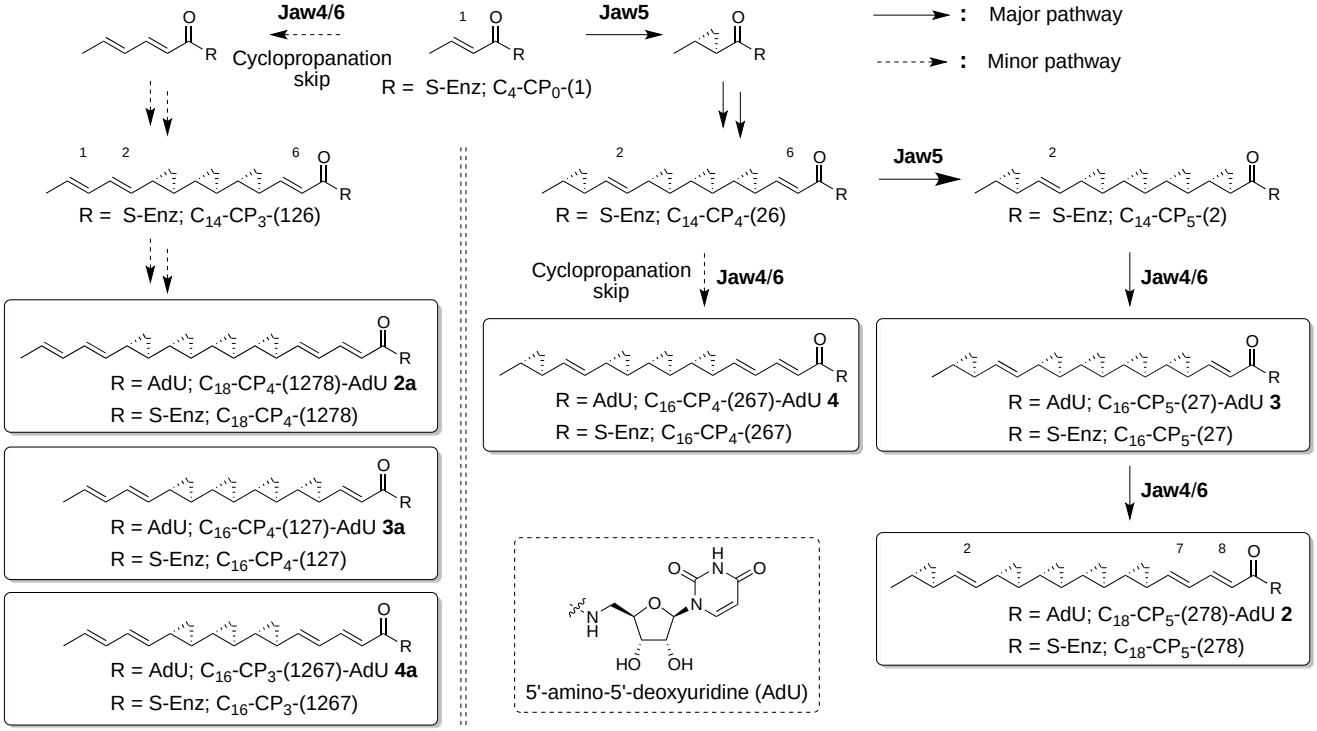


Figure 2. MS spectrum of the crude metabolites from the transformant producing **2**.

structure differs from that of **2** ($C_{32}H_{41}N_3O_6$) in terms of the number of cyclopropane moieties. The ^{1}H-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 C_{18}-polyketide backbone harboring four contiguous cyclopropanes flanked by two conjugated diene moieties. The polyketide structure of **2a** was described as C_{18}-CP_4-(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 C_{16}-polyketide backbone with a variable number of cyclopropane moieties (three to five). The ^{1}H-NMR spectrum of **3** (cyclopropane number; 5) showed two isolated olefin moieties.



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.

This journal is © The Royal Society of Chemistry 20xx

Please do not adjust margins

Please do not adjust margins

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 C_{16}-CP_5-(27)-AdU **3** contained one isolated and four contiguous cyclopropanes on a C_{16} polyketide chain. The structures of C_{16}-CP_4-(267)-AdU **4**, C_{16}-CP_4-(127)-AdU **3a**, and C_{16}-CP_3-(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).[9] Several key elimination fragments (i.e., Fa, $C_4H_4O_2N_2$; Fb, $C_6H_8O_4N_2$; Fc, $C_8H_{10}O_5N_2$; Fd, $C_9H_{13}O_5N_3$; Fe, $C_{10}H_{13}O_6N_3$) 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-$^{13}C_1$]methionine led to the efficient incorporation of a ^{13}C-labelled methylene group into the cyclopropane moieties of the polyketide chain,[10] resulting in expected mass shifts for each analog (Figures S3–S8). Consequently, 25 polyketide analogs with variable chain lengths were identified.

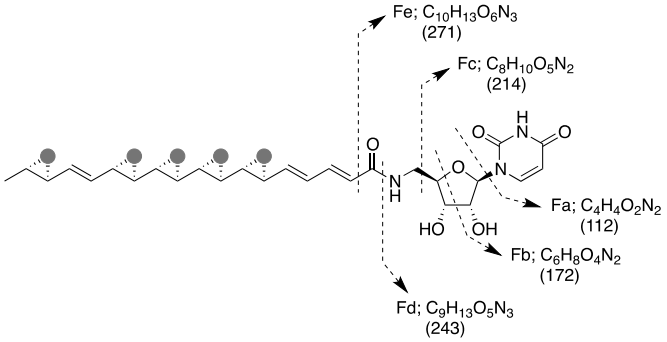


Figure 3. Characteristic MS fragments of dehydrojawsamycin **2**. Carbon-13 labels are shown in the grey circles. The molecular weight of each elimination fragment is shown in the parentheses.

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 (-). C_{10}-CP_5, C_8-CP_5, and C_8-CP_4 were not biosynthetically available and the corresponding columns are filled by grey colour.

	CP_5	CP_4	CP_3	CP_2	CP_1	CP_0
C_{18}	1	2	1	-	-	-
C_{16}	1	2	3	1	-	-
C_{14}	1	1	2	2	1	-
C_{12}	-	1	1	1	2	-
C_{10}		-	-	-	1	-
C_8			-	-	1	1

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, C_{18}-CP_4-AdU and C_{16}-CP_5-(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 C_{14}-CP_4-(26) was regarded as a common intermediate. A cyclopropanation followed by a chain elongation of C_{14}-CP_4-(26) would afford C_{16}-CP_5-(27) and C_{18}-CP_5-(278) via C_{14}-CP_5-(2). Alternatively, skipping the cylopropanation of C_{14}-CP_4-(26) would yield C_{16}-CP_4-(267). All of the resulting PKS-tethered polyketides would then be cleaved by the action of promiscuous acyltransferase Jaw2[6] to give **2**–**4**. Polyketides with a methyl-terminal conjugated diene such as C_{18}-CP_4-(1278), C_{16}-CP_4-(127), and C_{16}-CP_3-(1267) could also be biosynthesized in a similar manner from C_{14}-CP_3-(126), the terminal diene moiety of which could be constructed by skipping the cyclopropanation of C_4-CP_0-(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.[11] 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 C_{16}-CP_3, C_{14}-CP_3, C_{14}-CP_2, and C_{12}-CP_1 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 C_{16}-CP_5-(27) would afford C_{18}-CP_6-(28) with five contiguous

This journal is © The Royal Society of Chemistry 20xx

Please do not adjust margins

Please do not adjust margins

cyclopropanes (Scheme S2). To our knowledge, imprecise programming of the polyketide biosynthetic machinery for iterative PKS-catalyzed processes has been reported in TENS,[12] a fungal highly reducing iterative PKS-nonribosomal synthetase hybrid, and in Bref-PKS,[13] a fungal highly reducing PKS.

In summary, we have identified cyclopropane and/or acetate unit-deficient dehydrojawsamycin analogs from a previously constructed *S. lividans* transformant harboring *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 towards the α,β-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

1 M. Yoshida, M. Ezaki, M. Hashimoto, M. Yamashita, N. Shigematsu, M. Okuhara, M. Kohsaka and K. Horikoshi, *J. Antibiot*., 1990, **43**, 748.
2 M. Ichimura, T. Ogawa, S, Katsumata, K. Takahashi, I. Takahashi and H. Nakano, *J. Antibiot*., 1991, **44**, 1045.
3 W. H. Gerwick, P. J. Proteau, D. G. Nagle, E. Hamel, A. Blokhin and D. L. Slate, *J. Org. Chem.*, 1994, **59**, 1243.
4 M. S. Kuo, R. J. Zielinski, J. I. Cialdella, C. K. Marschke, M. J. Dupuis, G. P. Li, D. A. Kloosterman, C. H. Spilman and V. P. Marshall, *J. Am. Chem. Soc.*, 1995, **117**, 10629.
5 A. G. M. Barrett, W. W. Doubleday, K. Kasdorf and G. J. Tustin, *J. Org. Chem.*, 1996, **61**, 3280; J. R. Falck, B. Mekonnen, J. R. Yu and J. Y. Lai, *J. Am. Chem. Soc.*, 1996, **118**, 6096; A. G. M. Barrett, D. Hamprecht, A. J. P. White and D. J. Williams, *J. Am. Chem. Soc.*, 1997, **119**, 8608; J. Pietruszka, *Chem. Rev.*, 2003, **103**, 1051.
6 T. Hiratsuka, H. Suzuki, R. Kariya, T. Seo, A. Minami and H. Oikawa, *Angew. Chem. Int. Ed.*, 2014, **53**, 5423.
7 T. Tokiwano, H. Watanabe, T. Seo and H. Oikawa, *Chem. Commun.*, 2008, 6016.
8 C. Hertweck, *Angew. Chem. Int. Ed.*, 2009, **48**, 4688; D. H. Kwan and F. Schulz, *Molecules*, 2011, **16**, 6092.
9 D. Krug and R. Müller, *Nat. Prod. Rep.*, 2014, **31**, 768.
10 H. Watanabe, T. Tokiwano and H. Oikawa, *J. Antibiot.*, 2006, **59**, 607; H. Watanabe, T. Tokiwano and H. Oikawa, *Tetrahedron*, 2006, **47**, 1399.
11 J. M. Crawford, P. M. Thomas, J. R. Scheerer, A. L. Vagstad, N. L. Kelleher and C. A. Townsend, *Science*, 2008, **320**, 243; A. L. Vagstad, S. B. Bumpus, K. Belecki, N. L. Kelleher and C. A. Townsend, *J. Am. Chem. Soc.*, 2012, **134**, 6865.
12 A. A. Yakasai, J. Davison, Z. Wasil, L. M. Halo, C. P. Butts, C. M. Lazarus, A. M. Bailey, T. J. Simpson and R. J. Cox, *J. Am. Chem. Soc.*, 2011, **133**, 10990.
13 A. O. Zabala, Y. –H. Chooi, M. S. Choi, H. –C. Lin and Y. Tang, *ACS Chem. Biol.*, 2014, **9**, 1576.

 This journal is © The Royal Society of Chemistry 20xx

Please do not adjust margins