UC San Diego

UC San Diego Previously Published Works

Title

Biosynthetic pathway of aliphatic formates via a Baeyer–Villiger oxidation in mechanism present in astigmatid mites

Permalink

https://escholarship.org/uc/item/9qm3s61s

Journal

Proceedings of the National Academy of Sciences of the United States of America, 114(10)

ISSN

0027-8424

Authors

Shimizu, Nobuhiro
Sakata, Daisuke
Schmelz, Eric A
et al.

Publication Date

2017-03-07

DOI

10.1073/pnas.1612611114

Peer reviewed

eScholarship.org Powered by the California Digital Library
University of California

Biosynthetic pathway of aliphatic formates via a Baeyer–Villiger oxidation in mechanism present in astigmatid mites

Nobuhiro Shimizu[a,1], Daisuke Sakata[a], Eric A. Schmelz[b], Naoki Mori[c], and Yasumasa Kuwahara[d,e]

[a]Faculty of Bioenvironmental Science, Kyoto Gakuen University, Kameoka 621-8555, Japan; [b]Section of Cell and Developmental Biology, University of California, San Diego, La Jolla, CA 92093-080; [c]Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Sakyo-ku, Kyoto 606-8502, Japan; [d]Biotechnology Research Center and Department of Biotechnology, Toyama Prefectural University, Imizu, Toyama 939-0398, Japan; and [e]Asano Active Enzyme Molecule Project, Exploratory Research for Advanced Technology, Japan Science and Technology Agency, Imizu, Toyama 939-0398, Japan

Edited by Jerrold Meinwald, Cornell University, Ithaca, NY, and approved January 30, 2017 (received for review July 30, 2016)

Astigmatid mites depend on bioactive glandular secretions, pheromones, and defensive agents to mediate intra- and interspecies interactions. Aliphatic formates, such as (*Z*,*Z*)-8,11-heptadecadienyl formate (8,11-F17) and (*Z*)-8-heptadecenyl formate (8-F17), are rarely encountered natural products that are abundant in *Sancassania* sp. Sasagawa (Acari: Acaridae) mite secretions. Linoleic acid and oleic acid are predicted as key intermediates in the synthesis of the closely related aliphatic formates. To gain insight in this biosynthetic pathway, acarid mite feeding experiments were conducted using ^{13}C-labeled precursors to precisely track incorporation. Analyses using ^{13}C NMR spectroscopy demonstrated that the ^{13}C-labeling pattern of the precursors was detectable on formates in exocrine secretions and likewise on fatty acids in total lipid pools. Curiously, the results demonstrated that the formates were biosynthesized without the dehomologation of corresponding fatty acids. Careful examination of the mass spectra from labeling experiments revealed that the carbonyl carbon of the formates is originally derived from the C-1 position of the fatty acids. Consistent with a Baeyer–Villiger oxidation reaction, labeling studies support the insertion of an oxygen atom between the carbonyl group and carbon chain. Empirical data support the existence of a Baeyer–Villiger monooxygenase responsible for the catalyzation of the Baeyer–Villiger oxidation. The predicted existence of a Baeyer–Villiger monooxygenase capable of converting aliphatic aldehydes to formates represents an exciting opportunity to expand the enzymatic toolbox available for controlled biochemical synthesis.

astigmatid mite | aliphatic formate | hydrocarbon | biosynthetic pathway | Baeyer–Villiger oxidation

Long-chain hydrocarbons in insects are key precursors to the construction of waterproof cuticles and further function in chemical communication, both within and between species (1). An array of hydrocarbons in astigmatid mites have also been characterized yet, unlike insects, comparatively short carbon chains ($<C_{20}$) predominate. One of the most widely distributed saturated hydrocarbons in the Astigmata is tridecane. With respect to unsaturated hydrocarbons, (*Z*)-8-heptadecene (8-C17) is widely distributed in Acaridae and Histiostomatidae, and (*Z*, *Z*)-6,9-heptadecadiene (6,9-C17) in Acaridae (2). As rarely detected chemicals in nature, 8-C17 serves as a sex pheromone in European house dust mites (*Dermatophagoides pteronyssinus*) (3), and similarly 6,9-C17 functions as an alarm pheromone in *Tortonia* sp., which attacks the nests of megachilid bees (*Osmia cornifons*) (4).

Linoleic acid [LA; (*Z*,*Z*)-9,12-octadecadienoic acid] has been experimentally proven to function as a precursor of 6,9-C17 in previous isotopic labeling studies with the mite (*Carpoglyphus lactis*) (5). *Carpoglyphus lactis* is able to synthesize LA de novo from a simple precursor (5, 6) and uses neral [(*Z*)-3,7-dimethyl-2,6-octadienal] as the alarm pheromone. Neral also reportedly functions as an alarm pheromone in several mite species and has been demonstrated to be a biosynthetic product of the mevalonate pathway in *C. lactis* (7). Mechanisms for lipid metabolism associated with mite secretory components have been gradually clarified (5–7). After being reported as an alarm pheromone of the mold mite (*Tyrophagus putrescentiae*) in 1975 (8), neryl formate [(*Z*)-3,7-dimethyl-2,6-octadienyl formate] has been subsequently identified as an alarm pheromone in several species of acaridae (2). Lardolure [(1*R*,3*R*,5*R*,7*R*)-1,3,5,7-tetramethyldecyl formate] has been identified as an aggregation pheromone in *Lardoglyphus konoi* (9, 10), and also shows induced activation in *C. lactis*, *Aleuroglyphus ovatus*, and *T. putrescentiae* (11). One of the lipidic components in mites, α-acaridial [2(*E*)-(4-methyl-3-pentenyl)butenedial] has been demonstrated to function as a haptenic allergen that induced allergic contact dermatitis (12). The well-known house dust mites, *D. pteronyssinus* and *Dermatophagoides farinare*, are both major allergens, causing atopic diseases, such as asthma, rhinitis, and atopic dermatitis (13). Recent studies have shown that lipidic components, such as formate and hydrocarbon, function to attract the house dust mites and have the potential to be used for mite control (3, 13). Unlike more ubiquitous lipids, the biosynthetic pathways of chain-like formates present in mites have not been previously examined.

Significance

Aliphatic formates are commonly detected in astigmatid mite secretions and have been considered for roles in pheromone communication. Despite the natural occurrence of aliphatic formates, details surrounding the biosynthetic pathways remain unclear. To probe the biosynthetic mechanism, we performed ^{13}C-incorporation experiments targeting two different chain-like aliphatic formates by feeding with suitable labeled precursors. Results support a mechanism whereby formates are generated via the Baeyer–Villiger oxidation of aldehydes as direct precursors. The enzyme that catalyzes this reaction has yet to be identified in animals, microbes, and plants. Results from the labeling studies inform approaches to identify the specific gene and encoded enzyme responsible for aliphatic formate biosynthesis, which will enable a facile dehomologation method in the field of organic synthesis.

Author contributions: N.S. designed research; N.S. and D.S. performed research; N.S., D.S., N.M., and Y.K. analyzed data; and N.S., E.A.S., N.M., and Y.K. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Freely available online through the PNAS open access option.

[1]To whom correspondence should be addressed. Email: shimizu@kyotogakuen.ac.jp.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1612611114/-/DCSupplemental.

In our previous efforts, two formates, specifically (*Z*,*Z*)-8,11-heptadecadienyl formate (8,11-F17) and (*Z*)-8-heptadecenyl formate (8-F17), were identified as the main secretion component of unidentified acarid mite (*Sancassania* sp. Sasagawa) (14). Based on the double-bond position, LA was hypothesized to be the biosynthetic precursor of 8,11-F17 and, similarly, oleic acid (OA) for 8-F17. If LA and OA are the biosynthetic precursors of the unsaturated hydrocarbons 6,9-C17 and 8-C17 in *Sancassania* sp. Sasagawa, new mechanistic insights involved in chain shortening and formate production are needed to understand the predictably unique steps involved. To provide a chemical framework for understanding the biosynthetic pathway of aliphatic formates, we performed experiments in which ^{13}C-labeled precursors were administered to mites for the determination of ^{13}C-labeling patterns of formate moiety using ^{13}C NMR spectroscopy and gas chromatography-mass spectrometry (GC-MS). Results support an unusual biochemical mechanism in the generation of aliphatic formates, of which there are no previously reported cases. To the best of our knowledge, the enzyme that catalyzes this reaction is yet to be identified in animals, microbes, and plants.

Results

GC-MS Analysis Demonstrate Incorporation of ^{13}C-Labeled Acetate into 8,11-F17 and 8-F17. Analyses of *Sancassania* sp. Sasagawa mite glandular secretions by GC-MS revealed two types of predominant hydrocarbons, specifically 6,9-C17 [retention time (RT) = 15.25 min] and 8-C17 (RT = 15.32 min) and two types of formates, specifically 8,11-F17 (RT = 18.91 min), 8-F17 (RT = 18.95 min) as the main components (Fig. 1). Examination of the mass spectrum of 8,11-F17 revealed *m/z* 280 as a molecular ion (Fig. S1*A*). In contrast, the predicted molecular ion of 8-F17 was not clearly observable and instead the M^+–HCOOH *m/z* 236 ion predominated (Fig. S1*C*). To gain insights into the origin of these molecules, *Sancassania* sp. Sasagawa mites were reared in an agar medium with dry yeast as the main ingredient in the presence of 10% [1-^{13}C] acetate. To examine the pattern of ^{13}C incorporation into formates 8,11-F17 and 8-F17, the mites secretions analyzed by GC-MS 7 d after the initiation cultivation with [1-^{13}C] acetate. In the mass spectrum of 8,11-F17 derived from mites cultivated in unlabeled (control) media, the M^++3 ion (*m/z* 283) was identified to be the highest molecular-weight isotopic parent ion detectable. However, when [1-^{13}C] acetate was fed to the mites, the M^++10 ion (*m/z* 290) was identified to be the largest isotopic ion (Fig. S1*B*). Similarly, in the mass spectrum of 8-F17, the M^+–HCOOH+2 ion (*m/z* 238) was identified to be the largest isotope ion in the control. However, when [1-^{13}C] acetate was fed to the mites, the M^+–HCOOH+8 ion (*m/z* 244) was identified to be the largest isotope ion (Fig. S1*D*). These results confirm that 1-^{13}C acetate is incorporated into aliphatic formates, such as 8,11-F17 and 8-F17, 7 d after the administration experiment.

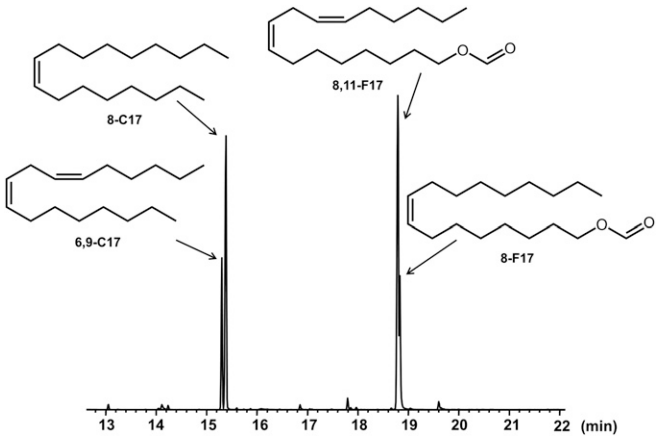

Fig. 1. Mites (*Sancassania* sp. Sasagawa) contain abundant C17 unsaturated hydrocarbons and C17 aliphatic formates. Typical GC of extracts from *Sancassania* sp. Sasagawa. In order of GC retention time: (*Z*,*Z*)-6,9-heptadecadiene (6,9-C17), (*Z*)-8-heptadecene (8-C17), (Z,Z)-8,11-heptadecadienyl formate (8,11-F17), and (Z)-8-heptadecenyl formate (8-F17).

^{13}C NMR Analysis of ^{13}C-labeled LA and OA Yield Predictable Conserved Patterns of Chain Elongation. Mites cultivated in media including 10% [1-^{13}C] acetate were separated from the culture media by suspension in saturated saline. The obtained mites of all developmental stages were immersed in hexane, and their total surface secretions were extracted. After mite bodies were isolated from hexane, total mite lipids were extracted using chloroform:methanol (2:1). After hydrolysis of the extracts by sodium methoxide, the products were treated with trimethylsilyldiazomethane to yield the corresponding fatty acid methyl esters. After isolation of LA methyl ester (LAME) and OA methyl ester (OAME) by a 10% (wt/wt) $AgNO_3$–SiO_2 column, each ester was analyzed by ^{13}C NMR.

The ^{13}C-isotopic abundances of each carbon atom of ^{13}C-labeled LAME are summarized in Fig. S2 and Table S1. Chemical shifts for each carbon atom were assigned with reference to LAME ^{13}C NMR data (5). Regarding the relative ^{13}C-isotopic abundance values, the C-2 carbon atom was set as the standard signal and ^{13}C-isotopic abundance was calculated for each carbon atom. In the mite lipid-derived ^{13}C-labeled LAME, the odd-numbered carbon atoms 1, 3, 5, 7, 9, 11, 13, 15, and 17 showed strong signals from ^{13}C. The individual levels of ^{13}C-isotopic abundance of these nine carbon atoms were 750–1053%. Conversely, the ^{13}C-isotopic abundances of even-numbered carbon atoms—that is, 2 (standard signal), 4, 6, 8, 10, 12, 14, 16, and 18—were low at −22 to 15%; these were therefore judged not to be enriched by ^{13}C atoms.

The ^{13}C-isotope abundances of each carbon atom of ^{13}C-labeled OAME are summarized in Fig. S3 and Table S2. Chemical shifts of each carbon atom were assigned with reference to the previously described LAME ^{13}C NMR data. Regarding calculations of the relative strength of the carbon signal, the C-2 carbon atom was set as the standard signal in the same manner as for LAME. In mite lipid-derived ^{13}C-labeled OAME, odd-numbered carbon atoms 1, 3, 5, 7, 9, 11, 13, 15, and 17 were strongly labeled by ^{13}C. The ^{13}C-isotopic abundances of these nine carbon atoms were 689–919%. Conversely, ^{13}C-isotopic abundances of even-numbered carbon atoms—that is, 2 (standard signal), 4, 6, 8, 10, 12, 14, 16, and 18—were low at −15 to 37%, and these were judged to not be enriched by ^{13}C.

Contrasting Fatty Acids, ^{13}C NMR Analysis of 8,11-F17 and 8-F17 Demonstrate Carbonyl and Even ^{13}C-labeling of Carbons. After mite hexane extract was prepared as previously described, 8,11-F17 and 8-F17 were isolated via a 10% (wt/wt) $AgNO_3$-SiO_2 column, each ^{13}C NMR spectrum was measured. ^{13}C NMR spectra of chemically synthesized 8,11-F17 and 8-F17 were also measured as a control.

The ^{13}C NMR spectra and data of ^{13}C-labeled and ^{13}C-unlabeled 8,11-F17 are summarized in Fig. 2 and Table S3. All chemical shifts of the carbon atoms were assigned with reference to ^{13}C NMR data of the analogous molecules 6,9-C17 (5). The carbonyl carbon and the even-numbered carbons at 2, 4, 6, 8, 10, 12, 14, and 16 detected in mite-derived ^{13}C-labeled 8,11-F17 were consistent with strong ^{13}C labeling. The even-numbered carbons correspond to the C-1 carbon of the ^{13}C-labeled acetyl units before the carbon chain is elongated. From the ^{13}C NMR data, we conclude that [1-^{13}C] acetate was incorporated into 8,11-F17. Conversely, odd-numbered carbons at 1, 3, 5, 7, 9, 11, 13,

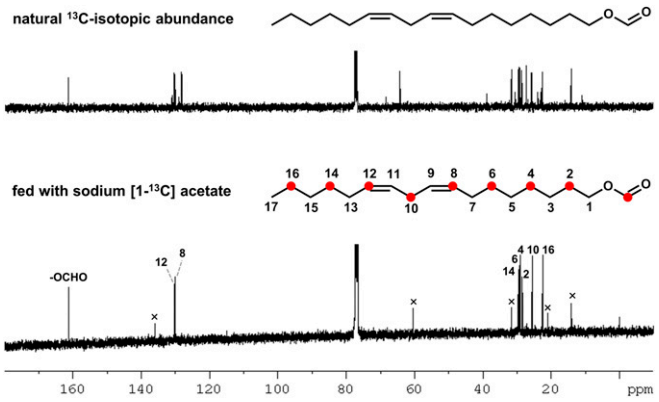

Fig. 2. Mite incorporation of [1-^{13}C] acetate into the aliphatic formate 8,11-F17 reveals even carbon chain ^{13}C-labeling. ^{13}C NMR spectra of ^{12}C-unlabeled and ^{13}C-labeled 8,11-F17. Filled circles indicate significantly ^{13}C-enriched atoms. ×: contaminant signal.

15, and 17 were not enriched with ^{13}C and no incorporation signal was detected.

^{13}C NMR spectra and data of ^{13}C-labeled and ^{13}C-unlabeled 8-F17 are summarized in Fig. 3 and Table S4. All chemical shifts of the carbon atoms are assigned with reference to 8,11-F17 (Table S3). Similar to ^{13}C-labeled 8,11-F17, the signals from the carbonyl carbon and the even-numbered carbons at 2, 4, 6, 8, 10, 12, 14, and 16 in mite-derived ^{13}C-labeled 8-F17 were consistent with ^{13}C labeling. The ^{13}C NMR data also confirmed the incorporation of [1-^{13}C] acetate into 8-F17. Conversely, odd-numbered carbons at 1, 3, 5, 7, 9, 11, 13, 15, and 17 were not enriched with ^{13}C and no incorporation signal was detected.

Incorporation of 1-^{13}C–Labeled Stearic Acid Demonstrates Origins of 8,11-F17 and 8-F17 Carbonyl Carbons. To examine the incorporation and origin of ^{13}C atoms into the formyl moiety present in 8,11-F17 and 8-F17, mites were fed on a mixture of dry yeast and [1-^{13}C] stearic acid [1:4 (wt/wt)] every 4 d, and the secretions of mites were analyzed every 7 d. When raised with food containing no additional ^{13}C-label, the abundance of the isotopic ion of 8,11-F17 (M^++1 ion, *m/z* 281) was 0.20 for M^+ ion (*m/z* 280). A week after feeding the [1-^{13}C] stearic acid, the values doubled. After 3 wk, they were 6-fold, and after an additional 3 wk they were 30-fold (Fig. 4). According to these data, it is possible that the ^{13}C atoms were incorporated into the formyl moiety of 8,11-F17 molecules.

In the case of 8-F17, we did not determine whether ^{13}C had been incorporated into the formyl moiety because there was a complete dissociation of H^{13}COOH from the M^+ ion, thus making the M^+ ion unobservable in electron ionization (EI) mode. However, the fact that no increases were observed in the amount of M^+−HCOOH+1 ions (*m/z* 237) in 8-F17 is consistent with the lack of ^{13}C incorporation anywhere else except the formyl moiety in the molecule (Fig. S4). To detect the molecular ion of 8-F17, we analyzed ^{13}C-labeled and ^{13}C-unlabeled 8-F17 by chemical ionization (CI). When raised with food not containing ^{13}C-labeled stearic acid, the quasi-molecular ion of 8-F17 (M^++H ion, *m/z* 283) was detected (Fig. 5). Five weeks after feeding the [1-^{13}C] stearic acid, M^++H+1 ion (*m/z* 284) was detected together with the M^++H+1−H^{13}COOH ion (*m/z* 237). These results are highly consistent with a single ^{13}C atom incorporation specifically into the formyl moiety of the 8-F17.

In the case of 6,9-C17 and 8-C17 hydrocarbon biosynthesis, $^{13}CO_2$ would be dissociated from linoleyl aldehyde and oleyl aldehyde, respectively, during the decarbonylase reaction leading to cuticular hydrocarbon biosynthesis (15). By feeding with [1-^{13}C] stearic acid, the corresponding hydrocarbons were not labeled with ^{13}C atom caused by loss of $^{13}CO_2$ (Fig. S5). Collectively, the current data are consistent with the ^{13}C atom derived from [1-^{13}C] stearic acid as selectively present in the formyl moiety and not significantly incorporated into other carbon positions.

Discussion

To elucidate the biosynthesis of aliphatic formates, specifically 8,11-F17 and 8-F17, in an arthropod model, ^{13}C-labeled acetate was administered to mites (*Sancassania* sp. Sasagawa) following by targeted GC-MS and ^{13}C NMR analyses. ^{13}C was accurately incorporated into the formates 8,11-F17 and 8-F17, and the specific carbon location of its biosynthesis precursor LA and OA was determined. In the biosynthesis of fatty acids, the carbon chain is elongated by malonyl-CoA (C_2); this process is initiated with an acetyl-CoA (C_2). A result of incorporating [1-^{13}C] acetate into mite lipids, odd-numbered ^{13}C labeled LA and OA are generated through elongation and desaturation (Fig. 6). These data agree well with the results of a previous study, where ^{13}C was inserted into an LA carbon chain in a regular manner by administering ^{13}C-labeled glucose to *C. lactis* (5).

In contrast to established patterns for fatty acids, when the labeling pattern of formates 8,11-F17 and 8-F17 are considered, even-numbered carbon atoms are labeled with ^{13}C in a regular manner. The most parsimonious hypothesis is that the carboxyl group of LA and OA has been decreased by one carbon. However, when we consider that a carbonyl carbon of formate also remains labeled with ^{13}C, then it is further possible that formate is generated by an oxygen atom inserted between the fatty acid carboxyl carbon and the α carbon (Fig. 6). An alternative and more conservative hypothesis is that C18 fatty acids are converted to C17 molecules with primary alcohols through a process of α-oxidation and reduction, followed by the reconstitution of a C18 molecule by the conjugation to formate. Although biologically plausible, such a mechanism is highly disfavored considering empirical data that the carbonyl carbon of formate present in 8-F17 is concentrated with ^{13}C (Fig. 5). Consequently, the currently proposed mechanism of aliphatic formate biosynthesis would proceed via Baeyer–Villiger oxidation following reduction of fatty acids (aliphatic acyl CoA) to aldehydes (Fig. 7). To verify that aldehydes are direct precursors, an isotopically labeled aldehyde was prepared for an administration experiment on mites. However, exogenous aldehyde feeding experiments resulted in significant miticide activity, thus forcing consideration of alternative experiments. Aldehydes

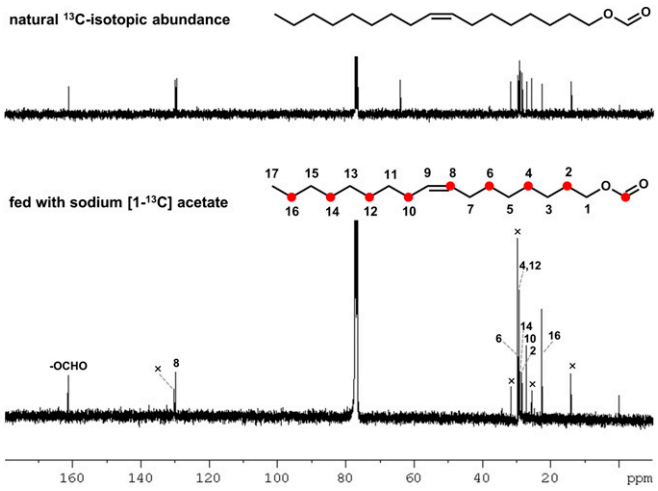

Fig. 3. Mite incorporation of [1-^{13}C] acetate into the aliphatic formate 8-F17 also reveals even carbon chain ^{13}C-labeling. ^{13}C NMR spectra of ^{12}C-unlabeled and ^{13}C-labeled 8-F17. Filled circles indicate significantly ^{13}C-enriched atoms. ×: contaminant signal.

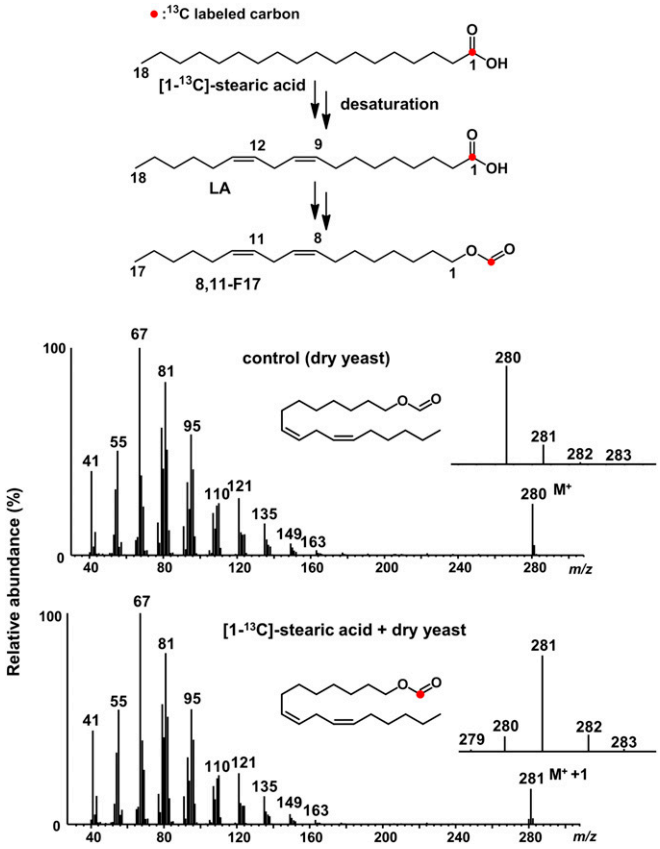

Fig. 4. Mites fed [1-^{13}C] stearic acid as a precursor specifically retain ^{13}C-labeled formate present in 8,11-F17. Mass spectra of ^{12}C-unlabeled and ^{13}C-labeled 8,11-F17 in EI mode. Filled circles indicate significantly ^{13}C-enriched atoms.

are known intermediates of hydrocarbons in insects (15–18), and our own studies make it increasingly clear that aldehydes are also intermediates of hydrocarbons in acaridae. Because mites (*Sancassania* sp. Sasagawa) secrete hydrocarbons and formates at the same time, it is hypothesized that two different types of enzymes act on aldehydes as a common substrate in the secretory glands and generate hydrocarbons and formates separately (Fig. 7).

Feeding experiments with [1-^{13}C] stearic acid were carried out to determine if the formate 8,11-F17 is generated without the process of LA dehomologation. According to the previously described [1-^{13}C] acetate administration experiment, because LA has been verified to be de novo biosynthesized, when [1-^{13}C] stearic acid is taken into the body of mites, it should be unsaturated and generate [1-^{13}C] LA. Given our hypothesis that 8,11-F17 is synthesized from LA, we predicted that [formyl-^{13}C] 8,11-F17 (*m/z* 281) should be obtained. When mite secretions were analyzed by GC-MS, the strength of the M^++1 ion (*m/z* 281) of 8,11-F17 increased over time during the administration experiment, and at 6 wk the ion strength was 30 times stronger compared with the control (Fig. 4). Given the rather surprising and strong 1-^{13}C enrichment measured, we present these findings as evidence that 8,11-F17 is biosynthesized without going through the process of dehomologation, which—if predominant—would oppositely remove the 1-^{13}C label present. We propose that this process similarly occurs for 8-F17, although we do present any parallel GC/EI-MS evidence for this example because of suboptimal ionization parameters. However, using an alternative soft CI approach, we analyzed 8-F17 after feeding mites with [1-^{13}C] stearic acid and successfully obtained M^++H+1 ion (*m/z* 284) derived from [formyl-^{13}C] 8-F17 in mass spectrum (Fig. 5). This provides additional supporting evidence that 8-F17 is also biosynthesized without going through the process of dehomologation. In contrast, the findings that M^++1 parent ions of 6,9-C17 and 8-C17 do not increase are consistent with the generation of hydrocarbons (6,9-C17 and 8-C17) by decarbonylation of 1-^{13}C–labeled aldehyde (Fig. S5). Considering all current results, we propose the involvement of a Baeyer–Villiger oxidation mechanism in the generation of aliphatic formates. The reaction mechanism involves insertion of an oxygen atom between the carbonyl group of aldehyde, the reduced product of LA (linoleoyl-CoA) and OA (oleoyl-CoA), and the carbon chain.

In 1948, indication of a biological Baeyer–Villiger oxidation was demonstrated for the first time during a series of microbiological studies on steroids (19). Since then, Baeyer–Villiger monooxygenase, which changes cyclic ketones to lactones and aliphatic and aromatic ketones to corresponding esters, has been predominantly found in microbes (20, 21). In plants, brassinolide has the highest physiological activity among brassinosteroid hormones and is essential in the promotion of extension growth; it is also known to be generated by the Baeyer–Villiger oxidation of castasterone, a biosynthetic intermediate (22, 23). To the best of our knowledge, we are unaware of enzymes capable of Baeyer–Villiger oxidations in animals. Baeyer–Villiger monooxygenases are useful

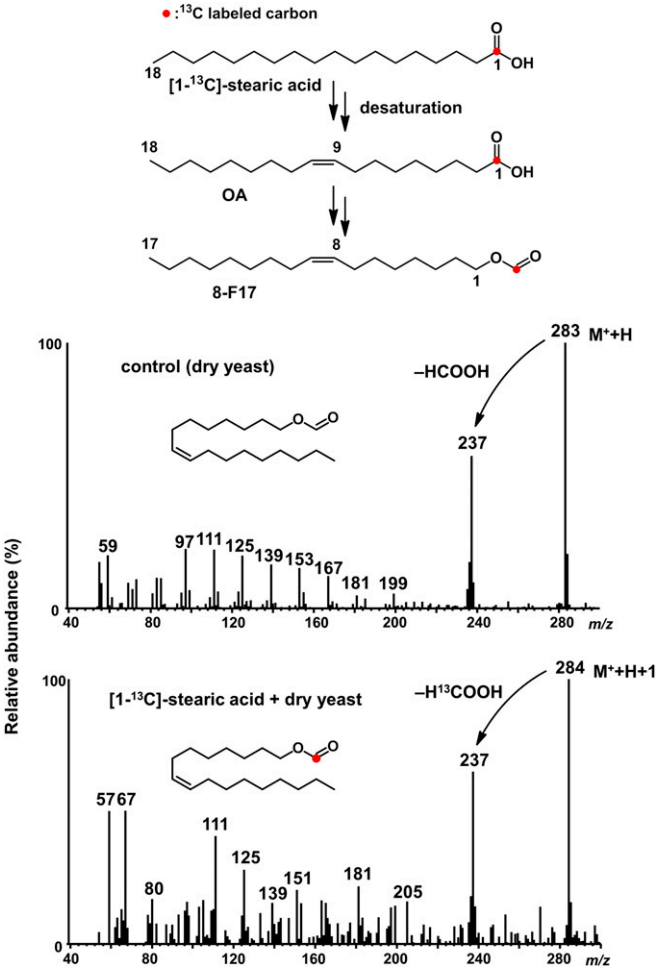

Fig. 5. Mites fed [1-^{13}C] stearic acid as a precursor specifically retain ^{13}C-labeled formate present in 8-F17. Mass spectra of ^{12}C-unlabeled and ^{13}C-labeled 8-F17 in CI mode. Filled circles indicate significantly ^{13}C-enriched atoms.

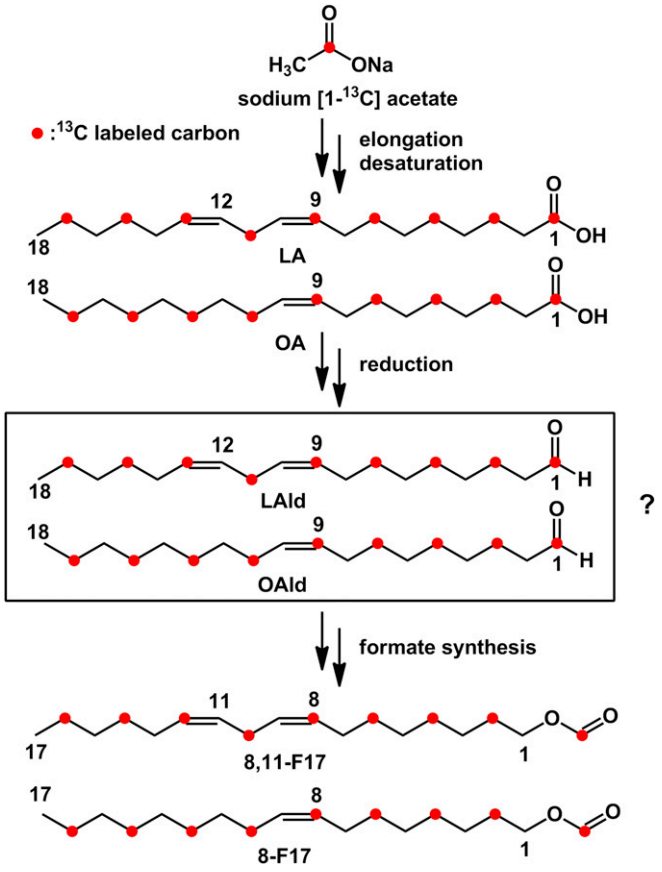

Fig. 6. Summary of measured ^{13}C-labeling patterns present in fatty acids and formates, specifically 8,11-F17 and 8-F17, after feeding with [1-^{13}C] acetate. Filled circles indicate significantly ^{13}C-enriched atoms.

as biocatalysts, especially in the selective synthesis of natural products and pharmaceuticals, given the ability to produce optically active lactones or esters via the enantioselective oxidation of racemic ketones (21).

As a classic Baeyer–Villiger reaction of aliphatic and aromatic aldehydes using peroxide, carboxylic acids are selectively generated (24). However, in 2010 generation of formate from aliphatic and aromatic aldehydes via a Baeyer–Villiger oxidation was demonstrated and enabled by a hypervalent difluoro-λ^3-bromane in water (25). Although there are cases of formate being generated from aromatic aldehyde in enzymatic Baeyer–Villiger oxidations that use cyclohexanone monooxygenase and 4-hydroxyacetophenone monooxygenase of microbial origin (26–28), an enzyme that generates formate from aliphatic aldehyde has yet to be discovered. In organic chemistry, an efficient transformation of a primary alcohol to a one-carbon–shorter carboxylic acid using *o*-iodoxybenzoic acid and iodine has recently been reported (29). In the present study, the predicted arthropod enzyme would catalyze a novel dehomologation and generate a one-carbon–shorter primary alcohol from an aldehyde via hydrolysis of formate. The substrate breath of substrate specificity of this enzyme still needs to be clarified; however, we expect useful applications requiring mild dehomologation in the synthesis of the labile bioactive molecules.

Methods

Chemical Analysis. Column chromatography was performed on Wakosil silica gel C-200 using the specified solvents. ^{1}H- and ^{13}C NMR spectra were recorded on a Bruker Biospin AC400M spectrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C) using tetramethylsilane as the internal standard. GC-MS was conducted using a Network GC System (6890N; Agilent Technologies) coupled with a mass-selective detector (5975 Inert XL; Agilent Technologies) operated at 70 eV using an HP-5MS capillary column (0.25 mm i.d. × 30 m with 0.25-μm film thickness; Agilent Technologies). Helium was used as the carrier gas at a flow rate of 1.00 mL/min, with a splitless mode at a temperature set to increase from 60 (2 min) to 290 °C at a rate of 10 °C/min. The temperature was maintained at 290 °C for 5 min. Positive CI mass spectra were acquired on the same GC system coupled with mass spectrometer (JMS-700 MStation, JOEL) operated at 200 eV using an DB-5MS capillary column (0.25 mm i.d. × 30 m with 0.25-μm film thickness; Agilent Technologies), with isobutane as reagent gas.

Mites. An unidentified *Sancassania* sp. Sasagawa strain (Acari: Acaridae) derived from hypopus attached to a Japanese rhinoceros beetle *Trypoxylus dichotomus* (L. 1771) originating from Kameoka city (Kyoto prefecture, Japan) was used in this study. Culture lines were established in our laboratory and maintained for several generations in a sterilized environment at 20 °C and 90% relative humidity in an agar medium, as reported previously (30). The internal transcribed spacer (ITS-II) region of this species consisted of 498, 496, or 492 bps (code name SS28, GenBank accession nos.: AB104962, AB104963, AB104964) as listed in Noge et al. (31).

Feeding Experiment with a ^{13}C-Labeled Compound-Enriched Diet. For the feeding experiment, mites were fed on an agar medium with dry yeast and sodium [1-^{13}C] acetate (>99% ^{13}C enrichment; Sigma-Aldrich) [9:1 (wt/wt)] for 7 d. No adverse effect was observed with the addition of sodium acetate to the medium. The rate at which ^{13}C atoms were incorporated into 8,11-F17 and 8-F17 was monitored by GC-MS on the second and seventh day. At each time interval, three mites were transferred to a conical glass insert (5 mm in diameter × 30 mm in height; Agilent Technologies) using a needle and soaked for 3 min in hexane (3 μL). The extract was directly subjected to GC-MS analysis. Another feeding experiment was conducted by using a mixture of dry yeast and [1-^{13}C] stearic acid (99% ^{13}C enrichment; Sigma-Aldrich) [1:4 (wt/wt)] for 6 wk. A set amount of feed (30–50 mg) was provided once every 4 d. The rate at which ^{13}C atoms were incorporated into 8,11-F17 and 8-F17 was monitored by GC-MS in EI or CI mode at given time intervals over 7 d. Secretion by the mites was extracted with hexane and analyzed by GC-MS as described above.

Isolation of 8,11-F17 and 8-F17 Incorporated with Sodium [1-^{13}C] Acetate. Mites of all developmental stages and both sexes were separated from the culture medium by suspension in a saturated saline. Medium-free mites (2.37 g) were then immersed in hexane (10 mL) for 10 min to obtain the secretions of the laterodorsal opisthonotal glands. After evaporation of the solvent, the extract was applied to a 10% (wt/wt) $AgNO_3$-SiO_2 column (2.8 g) and eluted with a mixture of ethyl acetate and hexane [1:10 (vol/vol)]. Purified 8,11-F17 (1 mg) and 8-F17 (<1 mg) were analyzed by ^{13}C NMR spectroscopy.

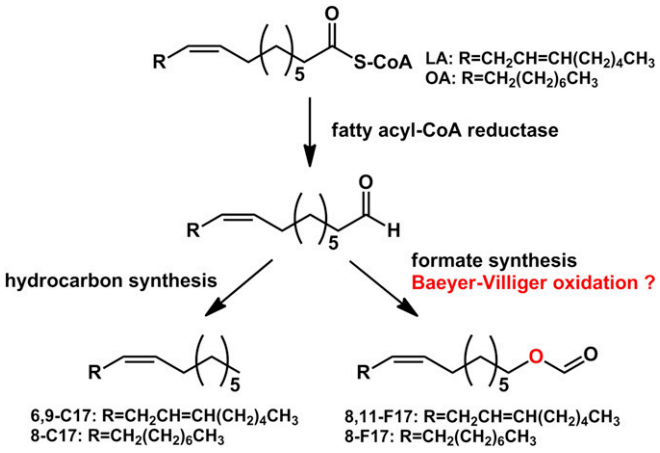

Fig. 7. Proposed role of an arthropod-associated Baeyer–Villiger type oxidation in the biosynthesis of aliphatic formates (8,11-F17 and 8-F17) and hydrocarbons (6,9-C17 and 8-C17) via aldehydes as common precursors.

Isolation of LA and OA Incorporated with Sodium [1-^{13}C] Acetate. After hexane extraction of the mites as described above, hexane-free mites were then extracted with chloroform:methanol [2:1 (vol/vol), 10 mL] for 3 d. After filtration, the filtrate was concentrated in vacuo and total lipids were hydrolyzed with sodium methoxide (100 mg) in methanol (5 mL) under reflux for 2 h. After acidifying the reaction mixture with 1N HCl at room temperature, ethyl acetate was added. The organic layer was successively washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The dry residue (17 mg) was dissolved with benzene:methanol [4:1 (vol/vol), 1 mL] and a trimethylsilyldiazomethane solution (0.6 M, 0.5 mL) was added to the solution at room temperature. After 30 min, the solvent was removed in vacuo, leaving fatty acid methyl esters (17 mg) in the form of oil. For isolation and purification of LAME and OAME, these esters were applied to a 10% (wt/wt) $AgNO_3$–SiO_2 column (1.7 g) and eluted with a mixture of ethyl acetate and hexane [1:10 (vol/vol)]. Purified LAME (3 mg) and OAME (3 mg) were analyzed, respectively, by ^{13}C NMR spectroscopy. Samples of authentic unlabeled LAME and OAME were obtained from a commercial source for use as a standard (Sigma-Aldrich).

Determination of ^{13}C-Labeling Patterns. ^{13}C NMR spectra of the compound labeled at the ^{13}C atoms and of the naturally occurring compound were recorded under the same experimental conditions. Chemical shifts were assigned for every ^{13}C NMR signal, and the signal integral for each respective carbon atom in the labeled compound was referenced to that of the compound with natural ^{13}C abundance, thereby affording the relative ^{13}C abundances for each position in the ^{13}C-labeled compounds.

Chemical Identification. Two formates 8,11-F17 and 8-F17 were synthesized to establish unbiased structures (14). Two hydrocarbons, 6,9-C17 and 8-C17, have been previously identified (32, 33). Two fatty acids, LA and OA, were identified by comparison of mass spectra and GC retention times with authentic samples. A commercial calibration standard containing bacterial fatty acid methyl esters (Catalog No. 47080-U, Sigma-Aldrich) was used as the authentic sample.

ACKNOWLEDGMENTS. This study was supported in part by Grant-in-Aid for Scientific Research Grant 24580167 (to N.S.) from the Japan Society for the Promotion of Science.

1. Blomquist GJ (2010) Structure and analysis of insect hydrocarbons. *Insect Hydrocarbons. Biology, Biochemistry, and Chemical Ecology*, eds Blomquist GJ, Bagnères A-G (Cambridge Univ Press, Cambridge, UK), pp 19–34.
2. Kuwahara Y (2004) Chemical ecology of astigmatid mites. *Advances in Insect Chemical Ecology*, eds Cardè RT, Millar JG (Cambridge Univ Press, Cambridge, UK), pp 76–109.
3. Steidle JLM, et al. (2014) Pheromonal communication in the European house dust mite, *Dermatophagoides pteronyssinus*, and its use for mite control. *Insects* 5(3): 639–650.
4. Kuwahara Y, et al. (1995) Chemical ecology of astigmatid mites XL. Identification of the alarm pheromone and new C_{17} hydrocarbons from *Tortonia* sp., a pest attacking the nest of *Osmia cornifrons*. *Appl Entomol Zool (Jpn)* 30:177–184.
5. Shimizu N, Naito M, Mori N, Kuwahara Y (2014) De novo biosynthesis of linoleic acid and its conversion to the hydrocarbon (*Z,Z*)-6,9-heptadecadiene in the astigmatid mite, *Carpoglyphus lactis*: Incorporation experiments with ^{13}C-labeled glucose. *Insect Biochem Mol Biol* 45:51–57.
6. Aboshi T, et al. (2013) Biosynthesis of linoleic acid in *Tyrophagus* mites (Acarina: Acaridae). *Insect Biochem Mol Biol* 43(11):991–996.
7. Morita A, et al. (2004) Neral (the alarm pheromone) biosynthesis *via* the mevalonate pathway, evidenced by D-glucose-1-^{13}C feeding in *Carpoglyphus lactis* and ^{13}C incorporation into other opisthonotal gland exudates. *J Pestic Sci* 29:27–32.
8. Kuwahara Y, Ishii S, Fukami H (1975) Neryl formate: Alarm pheromone of the cheese mite, *Tyrophagus putrescentiae* (Schrank) (Acarina, Acaridae). *Experientia* 31: 1115–1116.
9. Yen LTM, Wada Y, Matsumoto K, Kuwahara Y (1980) Pheromone study on acarid mites VI. Demonstration and isolation of an aggregation pheromone in *Lardoglyphus konoi* Sasa et Asanuma. *Jap J Sanit Zool* 31:249–254.
10. Yen LTM, Tominaga Y, Matsumoto K, Wada Y (1982) 1,3,5,7-Tetramethyldecyl formate, lardolure: Aggregation pheromone of the acarid mite, *Lardoglyphus konoi* (Sasa et Asanuma) (Acarina: Acaridae). *Agric Biol Chem* 46:2283–2291.
11. Kuwahara Y, Matsumoto K, Wada Y, Suzuki T (1991) Chemical ecology on astigmatid mites. XXIX. Aggregation pheromone and kairomone activity of synthetic lardolure (1*R*, 3*R*, 5*R*, 7*R*)-1,3,5,7-tetramethyldecyl formate and its optical isomers to *Lardoglyphus konoi* and *Carpoglyphus lactis* (Acari: Astigmata). *Appl Entomol Zool (Jpn)* 26: 85–89.
12. Sasai T, et al. (2008) Induction of allergic contact dermatitis by astigmatid mite-derived monoterpene, α-acaridial. *Biochem Biophys Res Commun* 375(3):336–340.
13. Skelton AC, Cameron MM, Pickett JA, Birkett MA (2010) Identification of neryl formate as the airborne aggregation pheromone for the American house dust mite and the European house dust mite (Acari: Epidermoptidae). *J Med Entomol* 47(5):798–804.
14. Shimizu N, Sakata D, Miyazaki H, Shimura Y, Kuwahara Y (2016) Identification and synthesis of (*Z,Z*)-8,11-heptadecadienyl formate and (*Z*)-8-heptadecenyl formate: Unsaturated aliphatic formates found in the unidentified astigmatid mite, *Sancassania* sp. Sasagawa (Acari: Acaridae). *Molecules* 21(5):619–627.
15. Qiu Y, et al. (2012) An insect-specific P450 oxidative decarbonylase for cuticular hydrocarbon biosynthesis. *Proc Natl Acad Sci USA* 109(37):14858–14863.
16. Reed JR, et al. (1994) Unusual mechanism of hydrocarbon formation in the housefly: cytochrome P450 converts aldehyde to the sex pheromone component (*Z*)-9-tricosene and CO_2. *Proc Natl Acad Sci USA* 91(21):10000–10004.
17. Reed JR, Quilici DR, Blomquist GJ, Reitz RC (1995) Proposed mechanism for the cytochrome P450-catalyzed conversion of aldehydes to hydrocarbons in the house fly, *Musca domestica*. *Biochemistry* 34(49):16221–16227.
18. Mpuru S, Reed JR, Reitz RC, Blomquist GJ (1996) Mechanism of hydrocarbon biosynthesis from aldehyde in selected insect species: Requirement for O_2 and NADPH and carbonyl group released as CO_2. *Insect Biochem Mol Biol* 26:203–208.
19. Turfitt GE (1948) The microbiological degradation of steroids. *Biochem J* 42(3): 376–383.
20. Pazmiño DT, Fraaije MW (2007) Discovery, redesign and applications of Baeyer-Villiger monooxygenases. *Future Directions in Biocatalysis*, ed Matsuda T (Elsevier, Amsterdam), pp 107–128.
21. Leisch H, Morley K, Lau PCK (2011) Baeyer-Villiger monooxygenases: More than just green chemistry. *Chem Rev* 111(7):4165–4222.
22. Yokota T, et al. (1990) Brassinolide is biosynthesized from castasterone in *Catharanthus roseus* crown gall cells. *Agric Biol Chem* 54:1107–1108.
23. Nomura T, et al. (2005) The last reaction producing brassinolide is catalyzed by cytochrome P-450s, CYP85A3 in tomato and CYP85A2 in *Arabidopsis*. *J Biol Chem* 280(18):17873–17879.
24. ten Brink GJ, Arends IWCE, Sheldon RA (2004) The Baeyer-Villiger reaction: New developments toward greener procedures. *Chem Rev* 104(9):4105–4124.
25. Ochiai M, Yoshimura A, Miyamoto K, Hayashi S, Nakanishi W (2010) Hypervalent λ^3-bromane strategy for Baeyer-Villiger oxidation: Selective transformation of primary aliphatic and aromatic aldehydes to formates, which is missing in the classical Baeyer-Villiger oxidation. *J Am Chem Soc* 132(27):9236–9239.
26. Branchaud BP, Walsh CT (1985) Functional group diversity in enzymatic oxygenation reactions catalyzed by bacterial flavin-containing cyclohexanone monooxygenase. *J Am Chem Soc* 107:2153–2161.
27. Kamerbeek NM, Olsthoorn AJJ, Fraaije MW, Janssen DB (2003) Substrate specificity and enantioselectivity of 4-hydroxyacetophenone monooxygenase. *Appl Environ Microbiol* 69(1):419–426.
28. Moonen MJH, Westphal AH, Rietjens IMCM, van Berkel WJH (2005) Enzymatic Baeyer-Villiger oxidation of benzaldehydes. *Adv Synth Catal* 347:1027–1034.
29. Xu S, Itto K, Satoh M, Arimoto H (2014) Unexpected dehomologation of primary alcohols to one-carbon shorter carboxylic acids using *o*-iodoxybenzoic acid (IBX). *Chem Commun (Camb)* 50(21):2758–2761.
30. Kuwahara Y (1999) Rearing of astigmatid mites by culture medium. *J Acarol Soc Jpn* 8: 58–59.
31. Noge K, et al. (2005) Identification of astigmatid mites using the second internal transcribed spacer (ITS2) region and its application for phylogenetic study. *Exp Appl Acarol* 35(1-2):29–46.
32. Kuwahara Y, et al. (1992) Chemical ecology on astigmatid mites XXXIII. Identification of (*Z,Z*)-6,9-heptadecadiene in the secretion of *Carpoglyphus lactis* (Acarina, Carpoglyphidae) and its distribution among astigmatid mites. *J Acarol Soc Jpn* 1:95–104.
33. Shimizu N, Mori N, Kuwahara Y (1999) Identification of the new hydrocarbon (*Z,Z*)-1,6,9-heptadecatriene as the secretory component of *Caloglyphus polyphyllae* (Astigmata: Acaridae). *Biosci Biotechnol Biochem* 63(8):1478–1480.