

# Bifunctional Triterpene/Sesquiterpene Cyclase: Tetraprenyl- $\beta$ -curcumene Cyclase Is Also Squalene Cyclase in *Bacillus megaterium*

Tsutomu Sato,<sup>†</sup> Hiroko Hoshino,<sup>†</sup> Satoru Yoshida,<sup>†</sup> Mami Nakajima,<sup>‡</sup> and Tsutomu Hoshino<sup>†</sup>

<sup>†</sup>Department of Applied Biological Chemistry, Faculty of Agriculture, and Graduate School of Science and Technology, Niigata University, Ikarashi 2-8050, Nishi-ku, Niigata 950-2181, Japan

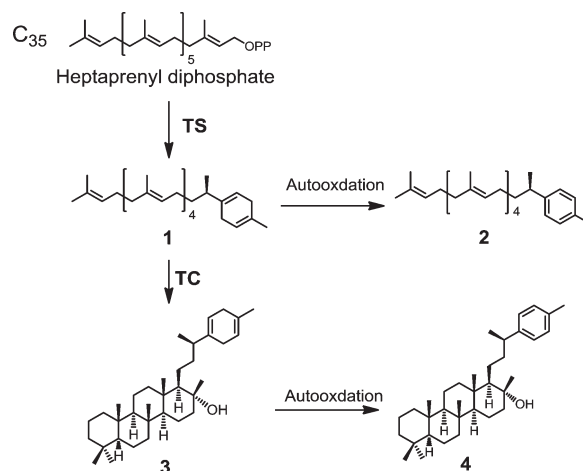
<sup>‡</sup>Center for Instrumental Analysis, Niigata University, Ikarashi 2-8050, Nishi-ku, Niigata 950-2181, Japan

**S** Supporting Information

**ABSTRACT:** This study demonstrates that a tetraprenyl- $\beta$ -curcumene cyclase, which was originally identified as a sesquiterpene cyclase that converts a head-to-tail type of monocycle to a pentacycle, also cyclizes a tail-to-tail type of linear squalene into a bicyclic triterpenol, 8 $\alpha$ -hydroxypolypoda-13,17,21-triene. The 8 $\alpha$ -hydroxypolypoda-13,17,21-triene was found to be a natural triterpene from *B. megaterium*. It was also demonstrated that cyclizations of both tetraprenyl- $\beta$ -curcumene and squalene occurred with a purified *B. megaterium* TC homologue in the same reaction mixture. These results suggest that the tetraprenyl- $\beta$ -curcumene cyclase is bifunctional, cyclizing both tetraprenyl- $\beta$ -curcumene and squalene in vivo. This is the first report describing a bifunctional terpene cyclase, which biosynthesizes two classes of cyclic terpenes with different numbers of carbons as natural products in the organism.

A recent biosynthetic study on the C<sub>35</sub> terpenes from *Bacillus subtilis* identified a novel terpene cyclase, tetraprenyl- $\beta$ -curcumene synthase (TS), which converted heptaprenyl diphosphate to tetraprenyl- $\beta$ -curcumene (**1**) (Scheme 1) and had no sequence homology with any of the known terpene cyclases.<sup>1</sup> Since this result was the first to demonstrate on both the gene and enzyme levels that C<sub>35</sub> terpenes were biosynthesized via the cyclization of the linear C<sub>35</sub> isoprenoid, we proposed a novel family name for these C<sub>35</sub> terpenes: sesquiterpenes.<sup>1</sup> An enzymatic reaction involving tetraprenyl- $\beta$ -curcumene cyclase (TC), which cyclizes **1** into C<sub>35</sub> terpenol (**3**), has also been demonstrated in vitro (Scheme 1).<sup>1</sup> The primary structure of the *B. subtilis* TC is similar to that of the squalene-hopene cyclase (SHC) and has two characteristic motifs conserved in the SHCs: the DXDDTA and QW motifs.<sup>2</sup> The SHC catalyzes the conversion of squalene (C<sub>30</sub>; **8**) to pentacyclic triterpenes, hopene (**9**) and hopanol (**10**) (Scheme 2), and has been investigated in depth to elucidate the common catalytic mechanism among the triterpene cyclase family members.<sup>2</sup> This was achieved by determining crystal structures, using site-directed mutagenesis, and analyzing products biosynthesized from substrate analogues.<sup>2</sup> Despite the similarity of the TC to the SHC, Bosak et al. reported that cell lysates of *E. coli* overexpressing *B. subtilis* TC could not react with **8**.<sup>3</sup> However, it

**Scheme 1.** Proposed Pathway for the Biosynthesis of the Sesquiterpenes in *B. subtilis*<sup>a</sup>



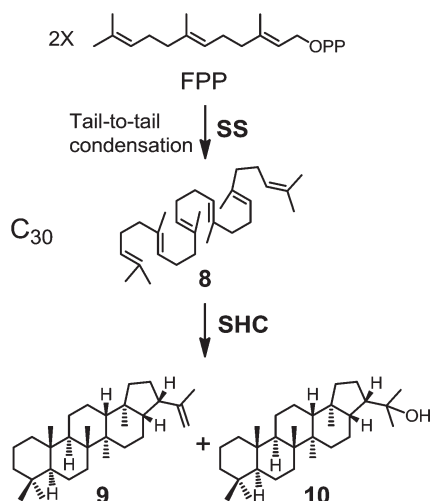
<sup>a</sup>The nonenzymatic synthetic pathways of **1**  $\rightarrow$  **2** and **3**  $\rightarrow$  **4** have previously been described by Takigawa et al.<sup>7</sup>

was possible that the overexpressed enzyme was not functional, because no enzymatic activity was confirmed using any substrate. Thus, we retested the cyclization of **8** with the enzymatic reaction system using recombinant TC from *B. subtilis*, which was shown to convert **1** to **3**. The reaction of recombinant SHC from *Alicyclobacillus acidocaldarius* with **1** was also analyzed and compared with the reaction of the TC with **1**. As a result, we found an additional function for TC, which also produces bicyclic triterpene from **8** in *B. megaterium* cells. To date, no bifunctional terpene cyclase, which biosynthesizes two classes of cyclic terpenes with different numbers of carbons as natural products in the organism, has yet been reported. It is also noteworthy that the bifunctional triterpene/sesquiterpene cyclase constructs two different cyclic scaffolds—the 6/6/6/6-fused tetracyclic and 6/6-fused bicyclic ring systems—from **1** and **8**, respectively.

The substrates **1** and **8** were separately incubated with the TC, which was purified by the methods described in our previous report,<sup>1</sup> and the reaction products were analyzed by GC-MS (Figure 1). It was confirmed that the prepared TC could cyclize

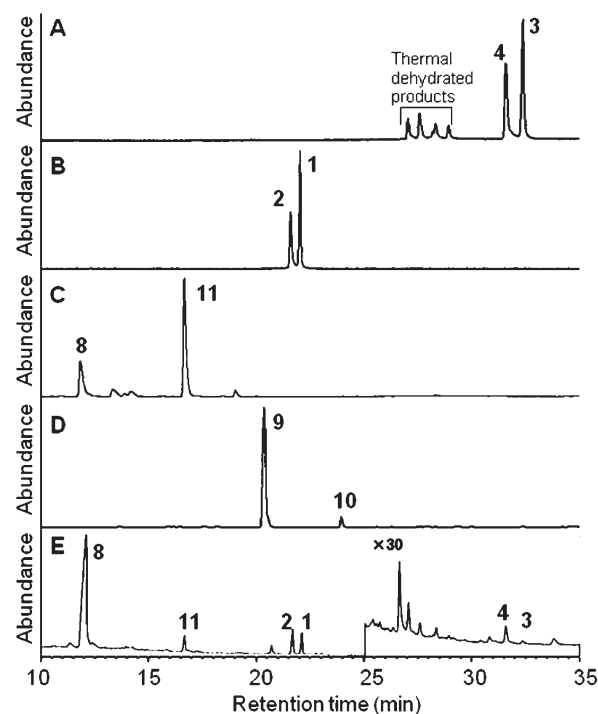
**Received:** June 29, 2011

**Published:** October 07, 2011

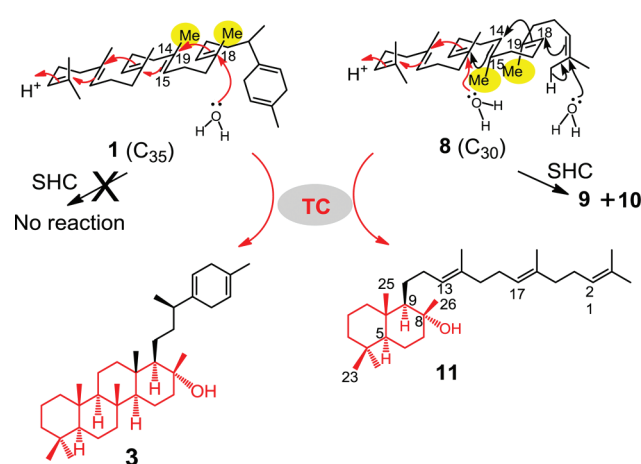
**Scheme 2. Pathway for the Biosynthesis of the Hopene (9) and the Hopanol (10)**


its natural substrate **1** into its usual product **3** (Figure 1A), suggesting that the purified enzymes were functional. The compound **4** and its thermally dehydrated products, which were detected in the chromatogram of Figure 1A, are synthesized by the nonenzymatic reaction of **3** (Scheme 1).<sup>1,4</sup> We revealed that the TC produced a significant GC-MS peak of **11** from **8** (Figure 1C). In order to determine the structure of **11**, 20 mg of **8** was incubated with cell-free extracts prepared from *E. coli* BL21 (DE3) cells overexpressing recombinant TC as described before.<sup>1</sup> *n*-Hexane extracts from the reaction mixture were purified by silica gel column chromatography and SiO<sub>2</sub> HPLC to yield 3.7 mg of pure **11**. Using NMR (<sup>1</sup>H, <sup>13</sup>C, DEPT, COSY, HOHAHA, NOESY, HMQC, and HMBC) and MS (ESI and EI), the compound **11** was shown to be 8 $\alpha$ -hydroxypolypoda-13,17,21-triene (Scheme 3),<sup>5</sup> which is found in the fern *Polypodium niponicum*. The molecular formula of **11** was determined to be C<sub>30</sub>H<sub>52</sub>O on the basis of the HR-ESI-MS results. In the <sup>1</sup>H NMR spectrum, three olefinic protons ( $\delta_{\text{H}}$  5.56, 5.45, 5.37, each 1H, t, *J* = 6.9 or 7.0) and four allylic methyl protons ( $\delta_{\text{H}}$  1.84, 1.81, 1.75, 1.69, each 3H, s) were found, indicating that three double bonds remained unchanged. The remaining four methyl protons appeared at higher field ( $\delta_{\text{H}}$  1.18, 0.97, 0.88, 0.82, each 3H, s), suggesting that four tertiary methyl groups were in the cyclized bicyclic ring. The relative stereochemistry of **11** was determined by observing the following NOE: H-5/H-9, Me-23/Me-25, and Me-25/Me-26. The specific optical rotation ( $[\alpha]_{\text{D}} = -1.8^\circ$ ) was nearly identical with the published value ( $[\alpha]_{\text{D}} = -0.9^\circ$ ),<sup>5</sup> suggesting the absolute configuration of **11** shown in Scheme 3. Thus, it was clear that the TC produces bicyclic **11** by the cyclization of **8**. The recombinant TC prepared by Bosak et al. may have no enzymatic activity toward substrates **1** and **8**.<sup>3</sup>

*B. subtilis* and several other *Bacillus* species, such as *B. amyloliquefaciens*, *B. atrophaeus*, *B. cellulosilyticus*, *B. pumilus*, and *B. megaterium*, contain homologues of the squalene synthase (SS) or phytoene synthase (PS) that catalyze the tail-to-tail condensation of two farnesyl diphosphates to **8** (Scheme 2) or two geranylgeranyl diphosphates to phytoene (C<sub>40</sub>) (Table 1).<sup>6</sup> However, the production of **8** and phytoene in the *Bacillus* species, except for *B. subtilis*,<sup>7</sup> has never been analyzed. It is possible that **8** is



**Figure 1.** GC-MS total ion chromatogram, used to analyze the enzymatic activity of purified TC and SHC with the substrates **1** and **8**, and the *n*-hexane extract from *B. megaterium* cells: (A) reaction mixture after the incubation of substrate **1** with the TC; (B) reaction mixture after the incubation of substrate **1** with the SHC; (C) reaction mixture after the incubation of substrate **8** with the TC; (D) reaction mixture after the incubation of substrate **8** with the SHC; (E) *n*-hexane extract from the *B. megaterium* cells.

**Scheme 3. Cyclization of 1 and 8 Catalyzed by the TC and the SHC<sup>a</sup>**


<sup>a</sup> This study revealed that the 8 $\alpha$ -hydroxypolypoda-13,17,21-triene (**11**) from **8** was formed by the TC and that no product from **1** was biosynthesized by the SHC.

formed by the SS or PS homologues and **11** is produced from **8** by the TC homologue in the *Bacillus* cells. In order to find the producer of both sesquiterpenes (**1**–**4**) and triterpenes (**8** and **11**), six species shown in Table 1 were cultured in sporulation medium

**Table 1.** Genes and Homologues of the Terpene Synthases and the Production of the Terpenes in *Bacillus* Species Cultured in Sporulation Medium<sup>a</sup>

species	genes and homologues			production of sesquiterpenes <sup>c</sup>		production of triterpenes	
	TS	TC	SS or PS	1 and 2	3 and 4	8	11
<i>B. subtilis</i>	BSU30500	BSU19320	BSU10810	+	+	— <sup>d</sup>	—
<i>B. atrophaeus</i>	BATR1942_13035	BATR1942_08330	BATR1942_03015	+	—	—	—
<i>B. amyloliquefaciens</i>	BAMF_2833	BAMF_2003	BAMF_1165	+	+	—	—
<i>B. cellulosilyticus</i>	Bcell_3320	Bcell_4159	Bcell_3989	+	—	—	—
<i>B. pumilus</i>	BPUM_2682	BPUM_1858	BPUM_1012	+	+	—	—
<i>B. megaterium</i>	BMD_4842	BMD_2134	BMD_0659, <sup>b</sup> BMD_3864 <sup>b</sup>	+	+	+	+

<sup>a</sup> Legend: +, detected; —, not detected. <sup>b</sup> *B. megaterium* has two SS/PS homologues. <sup>c</sup> The production of sesquiterpenes in *B. subtilis*, *B. amyloliquefaciens*, and *B. pumilus* was reported in ref 1. <sup>d</sup> Although the production of 8 in *B. subtilis* cultured in sporulation and vegetative medium could not be detected, its production in biofilm-inducing and the pigment-producing medium was confirmed.

and the lipid fractions analyzed by GC-MS. The triterpenes 8 and 11, which have the same retention times and mass spectrum profiles as the authentic samples, were successfully found in the lipids of *B. megaterium* cells in addition to the sesquiterpenes (1–4) (Figure 1E, Figure S4 (Supporting Information), and Table 1). The result is unique to our study, in that 11 has been found in bacteria. In contrast, the other five species produced only the sesquiterpenes (1 and 2 or 1–4) (Table 1). Since only one TC (or SHC) homologue exists in *B. megaterium* (Table 1), this TC would be bifunctional, producing 3 and 11 from 1 and 8 in vivo, respectively. It has recently been reported that the SS/PS homologue from *B. subtilis* (BSU10810 or YisP) converted FPP to 8 in vitro and was crucial for the formation of biofilms.<sup>8</sup> In addition, it has been speculated that 8 was converted to an unidentified pigment.<sup>8</sup> Thus, we also analyzed the lipid fractions of *B. subtilis* cultured in the biofilm-inducing and the pigment-producing media. Although a trace amount of 8 (<2% less than that produced by *B. megaterium*) could be detected from both cultures, that of 11 could not be confirmed. This suggests that the SS was expressed under the biofilm-inducing and pigment-producing conditions, but the TC was not. The *B. subtilis* TC is probably not bifunctional in vivo.

In order to obtain further evidence on the bifunctionality of *B. megaterium* TC, the *B. megaterium* *tc* gene was introduced into the pColdTF vector, the recombinant *B. megaterium* TC was expressed in *E. coli*, and the activity cyclizing both 1 and 8, separately, was confirmed by using purified *B. megaterium* TC (Figure S5 and S6 (Supporting Information)). Furthermore, the products biosynthesized by incubating the *B. megaterium* TC with a mixture of the substrates 1 and 8 were analyzed by GC-MS. Since the amount of 8 was about 4-fold larger than that of 1 + 2 in *B. megaterium* cells (Figure 1E), concentrations of 1 and 8 were prepared as 40  $\mu$ M/160  $\mu$ M for 1/8. The products 3 and 11 were successfully detected in the reaction mixture (Figure S6). Therefore, it was demonstrated that cyclizations of both tetraprenyl- $\beta$ -curcumene and squalene occurred with a purified *B. megaterium* TC homologue in the same reaction mixture. It has recently been reported that changing the pH of the reaction dramatically changes the product profile of the sesquiterpene cyclase.<sup>9</sup> In contrast, the product selectivity of the *B. megaterium* TC was not influenced by the surrounding pH (Figures S7 and S8 (Supporting Information)). Ferns produce 11 in addition to other triterpenes. Although several squalene cyclases have been isolated from the fern,<sup>10</sup> the enzyme that forms 11 remains unknown.

This is therefore the first report describing the 8 $\alpha$ -hydroxypoly-poda-13,17,21-triene (11) synthase.

When it was cultured in sporulation medium, *B. megaterium* produced the TC products 3, 4, and 11 in addition to the TC substrates 1, 2, and 8 (Figure 1E), while the vegetative *B. megaterium* cells accumulated only the TC substrates 1, 2, and 8. This result is similar to the productivities of the sesquiterpenes in *B. subtilis*; the TC products 3 and 4 were only produced under the sporulation conditions.<sup>3</sup> Studies on *B. subtilis* have demonstrated that the expression of *tc* depends on the sporulation-specific RNA polymerase subunit  $\sigma^E$  that controls the expression of a number of genes during sporulation and that the intracellular localization of the TC was also sporulation specific.<sup>3</sup> Thus, the *B. megaterium* *tc* gene may also be expressed under sporulation conditions. It has been proposed that sporulenes, the thermally dehydrated compounds from 4, increase the resistance of spores to reactive oxygen species.<sup>3</sup> The compound 11 may therefore have a unique physiological function that acts only in the *B. megaterium* spore and not in other *Bacillus* species.

The TC family, one of which is the bifunctional triterpene/sesquiterpene cyclase in *B. megaterium*, constructs different cyclic scaffolds—6/6/6/6-fused tetracycle (3) and 6/6-fused bicycle (11)—from a head-to-tail type of 1 and a tail-to-tail type of 8, respectively (Scheme 3). The cyclization of 1 into the 6/6/6/6-fused 3, catalyzed by the TC, proceeds only via tertiary carbocations, whereas the cyclization of 8 into any compound containing a 6/6/6/6-fused ring, such as 9 and 10, accompanies two unstable secondary carbocations (anti-Markovnikov closure) at C-14 and C-18 (Scheme 3). It has been proposed that an aromatic  $\pi$  electron in Phe601 from the *A. acidocaldarius* SHC functions to stabilize the secondary carbocations through a cation– $\pi$  interaction at C-14 and C-18 in 8.<sup>2,11</sup> The corresponding residue for Phe601 in the SHC is Leu597 in the *B. subtilis* TC. Thus, the lack of stabilization at C-14 and C-18 may be one of the reasons the cyclization of 8 is quenched at the bicyclic intermediate stage. However, this hypothesis is incomplete in that it does not account for the cyclization mechanism, because F601A SHC produced no bicyclic compound other than the 6/6/5-fused tricycles, 6/6/6/5-fused tetracycles, and 6/6/6/6/5-fused pentacycles.<sup>2,11</sup> The methyl groups in 1 and 8 are found in different positions, at C-14 and C-18 in 1 versus C-15 and C-19 in 8 (Scheme 3). These distinct methyl positions may promote different binding conformations of 1/8 in the active site cavity in the TC, resulting in the different numbers of rings in the terpene compounds.

The substrates **1** and **8** were also incubated separately with the SHC, which was purified by the methods described in our previous report (Figure 1).<sup>12</sup> The SHC, which cyclized the natural substrates **8** into their usual products **9** and **10** (Figure 1D and Scheme 2), biosynthesized no product from **1** (Figure 1B and Scheme 3). Compound **2**, which was detected in the chromatogram displayed in Figure 1B, was synthesized by the autoxidation of **1** (Scheme 1).<sup>14</sup> The nonreactivity of **1** with the SHC can be interpreted as the repulsive interaction between the active site cavity in the SHC, and the methyl groups at C-14 and C-18 in **1** may be preventing the terminal double bond of **1** from being located to take part in the initial protonation. This assumption is consistent with our previous reports describing that the introduction of a methyl group at C-14 on the squalene backbone interrupted the cyclization of **8** by the SHC.<sup>13</sup> Studies on the bifunctional catalytic mechanism of the TC using a 3D structure of the TC, site-directed mutageneses, and analyses of products biosynthesized from substrate analogues, which can be compared with similar studies on the SHC, will be attractive projects for the future.

In conclusion, this study has demonstrated that the TC, which was originally identified as a sesquiterpene cyclase converting the head-to-tail type of monocyclic **1** into the pentacyclic **3** (Scheme 1), also cyclized the tail-to-tail type of linear triterpene **8** into the bicyclic **11** (Scheme 3). The compound **11** was found to be a natural triterpene from the *B. megaterium* cells in addition to **3**, and it was demonstrated that cyclizations of both **1** and **8** occurred with a purified *B. megaterium* TC homologue in the same reaction mixture. These results suggest that the TC would be bifunctional, cyclizing both **1** and **8** in vivo. Although two nearly identical nerolidol/linalool synthases are known to be bifunctional terpene synthases, these enzymes are not cyclases and biosynthesize two linear compounds, nerolidol and linalool, from farnesyl diphosphate (C<sub>15</sub>) and geranyl diphosphate (C<sub>10</sub>), respectively, as natural products in the plant.<sup>14</sup> In addition, it has been reported that the many terpene cyclases have broad substrate specificities, and other classes of terpenes have been created in vitro.<sup>9,13b,15</sup> However, the bifunctionality of these terpene cyclases in vivo has not been hypothesized or demonstrated.<sup>9,13b,15</sup> Thus, no bifunctional terpene cyclase, which biosynthesizes two classes of cyclic terpenes with different numbers of carbons as natural products in vivo, has been reported to date.<sup>2,16</sup> Combinational studies determining enzymatic products biosynthesized from unusual substrates and the analysis of genome sequences will lead to the further discovery of additional functions of these enzymes in the organism and will also reveal additional novel natural terpenoids functioning in their producers. Furthermore, it has been reported that many kinds of mutated SHCs create various natural and unnatural triprepenes from **8** in addition to **9** and **10**.<sup>2</sup> However, compound **11** has never been formed by them. This study has also revealed that the SHC was not able to utilize **1** as a substrate (Scheme 3). The TC should prove to be a promising tool for the production of novel terpenes from the various substrates that are able to react with the SHC and those that cannot. The finding of novel bifunctional terpene cyclase will help us to expand our understanding of the terpenoids and to create novel compounds.

## ■ ASSOCIATED CONTENT

**S** Supporting Information. Text, figures, and tables giving experimental details, sequence data of enzymes, MS spectra, and NMR spectra. This material is available free of charge via the Internet at <http://pubs.acs.org>.

## ■ AUTHOR INFORMATION

### Corresponding Author

satot@agr.niigata-u.ac.jp.

## ■ ACKNOWLEDGMENT

This work was supported by a Grant-in-Aid for Young Scientists (B) from the Japan Society for Promotion of Science (JSPS) (21780109 and 23780114 to T.S.) and the Agricultural Chemical Research Foundation (T.S.). We are grateful to Dr. Masahiro Fujihashi of Kyoto University for helpful discussions.

## ■ REFERENCES

- (1) Sato, T.; Yoshida, S.; Hoshino, H.; Tanno, M.; Nakajima, M.; Hoshino, T. *J. Am. Chem. Soc.* **2011**, *133*, 9734–9737.
- (2) (a) Hoshino, T.; Sato, T. *Chem. Commun.* **2002**, 291–301. (b) Christianson, D. W. *Chem. Rev.* **2006**, *106*, 3412–3442. (c) Abe, I. *Nat. Prod. Rep.* **2007**, *24*, 1311–1331. (d) Siedenburg, G.; Jendrosseck, D. *Appl. Environ. Microbiol.* **2011**, *77*, 3905–3915.
- (3) (a) Bosak, T.; Losick, R. M.; Pearson, A. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 6725–6729. (b) Kontnik, R.; Bosak, T.; Butcher, R.; Brocks, J. J.; Losick, R.; Clardy, J.; Pearson, A. *Org. Lett.* **2008**, *10*, 3551–3554.
- (4) Takigawa, H.; Sugiyama, M.; Shibuya, Y. *J. Nat. Prod.* **2010**, *73*, 204–207.
- (5) Arai, Y.; Hirohara, M.; Ageta, H.; Hsu, H. Y. *Tetrahedron Lett.* **1992**, *33*, 1325–1328.
- (6) (a) Robinson, G. W.; Tsay, Y. H.; Kienzie, B. K.; Smith-Monroy, C. A.; Bishop, R. W. *Mol. Cell. Biol.* **1993**, *13*, 2706–2717. (b) Lee, S.; Poulter, C. D. *J. Bacteriol.* **2008**, *190*, 3808–3816. (c) Chamovitz, D.; Misawa, N.; Sandmann, G.; Hirschberg, J. *FEBS Lett.* **1992**, *296*, 305–310.
- (7) Clejan, S.; Krulwich, T. A.; Mondrus, K. R.; Seto-Young, D. *J. Bacteriol.* **1986**, *168*, 334–340.
- (8) Lopez, D.; Kolter, R. *Genes Dev.* **2010**, *24*, 1893–1902.
- (9) Lopez-Gallego, F.; Agger, S. A.; Pella, D. A.; Distefano, M. D.; Schmidt-Dannert, C. *ChemBioChem* **2010**, *11*, 1093–1106.
- (10) (a) Shinozaki, J.; Shibuya, M.; Masuda, K.; Ebizuka, Y. *FEBS Lett.* **2008**, *582*, 310–318. (b) Shinozaki, J.; Shibuya, M.; Masuda, K.; Ebizuka, Y. *Phytochemistry* **2008**, *69*, 2559–2564. (c) Shinozaki, J.; Shibuya, M.; Takahata, K.; Masuda, K.; Ebizuka, Y. *ChemBioChem* **2010**, *11*, 426–433.
- (11) Hoshino, T.; Kouda, M.; Abe, T.; Ohashi, S. *Biosci. Biotechnol. Biochem.* **1999**, *634*, 2038–2041.
- (12) Morikubo, N.; Fukuda, Y.; Ohtake, K.; Shinya, N.; Kiga, D.; Sakamoto, K.; Asanuma, M.; Hirota, H.; Yokoyama, S.; Hoshino, T. *J. Am. Chem. Soc.* **2006**, *128*, 13184–13194.
- (13) (a) Nakano, S.; Ohashi, S.; Hoshino, T. *Org. Biomol. Chem.* **2004**, *2*, 2012–2022. (b) Hoshino, T.; Kumai, Y.; Kudo, I.; Nakano, S.; Ohashi, S. *Org. Biomol. Chem.* **2004**, *2*, 2650–2657. (c) Hoshino, T.; Kumai, Y.; Sato, T. *Chem. Eur. J.* **2009**, *15*, 2091–2100.
- (14) Nagegowda, D. A.; Gutensohn, M.; Wilkerson, C. G.; Dudareva, N. *Plant J.* **2008**, *55*, 224–239.
- (15) For examples, see: (a) Xiong, Q.; Zhu, X.; Wilson, W. K.; Ganesan, A.; Matsuda, S. P. T. *J. Am. Chem. Soc.* **2003**, *125*, 9002–9003. (b) Crock, J.; Wildung, M.; Croteau, R. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 12833–12838. (c) Bohlmann, J.; Crock, J.; Jetter, R.; Croteau, R. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 6756–6761. (d) Kollner, T. G.; Schnee, C.; Gershenzon, J.; Degenhardt, J. *Plant Cell* **2004**, *16*, 1115–1131.
- (16) *Isoprenoids Including Carotenoids and Steroids*; Cane, D. E., Ed.; Elsevier: Oxford, U.K., 1999; Comprehensive Natural Products Chemistry, Vol. 2.