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 How to cite: *Angew. Chem. Int. Ed.* **2025**, *64*, e202418613
 doi.org/10.1002/anie.202418613

Non-Canonical C₁₆ Homoterpene Biosynthesis Widespread in Actinobacteria

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Abstract: A novel biosynthetic pathway towards the rare and underexplored non-canonical family of homoterpenes was discovered in actinobacteria through targeted genome mining and enzymatic *in vitro* reconstitution. The pathway comprises initial methylation-induced double bond isomerization of farnesyl diphosphate (FPP) to (2*E*,7*E*)-6-methyl-farnesyl diphosphate, catalyzed by a novel family of methyltransferases with unique dual function. The resulting linear C₁₆ double bond isomer of FPP constitutes the specific substrate for a distinct family of type I terpene cyclases, catalyzing diverse cyclization reactions. Functional characterization of nine enzyme pairs led to discovery of five unprecedented homoterpene natural products. The enzymological novelty enables the development of novel biocatalytic and genetically programmable synthetic strategies towards methylated terpenoids with potentially unique properties (“magic methyl effect”).

ways lead to the formation of canonical terpenes with archetypical polyisoprenoid backbones. Terpenes deviating from the “biogenic isoprene rule”^[5] are exceptionally rare. Homoterpenes, which carry an additional carbon in their polyisoprene backbones, make up only a small fraction of the 180.000 known terpenoids and represent a highly underexplored natural product family.^[6] The two plant homoterpenes 4,8-dimethyl-1,3,7-nonatriene (DMNT, **1**, C₁₁) and 4,8,12-trimethyltrideca-1,3,7,11-tetraene (TMTT, **2**, C₁₆) are important chemical defense compounds and biosynthesized via oxidative removal of a C₄ unit from (*E*)-nerolidol and (*E,E*)-geranylinalool, respectively (Figure 1A).^[7] Until recently, the only examples from bacteria were the homomoterpene 2-methylisoborneol^[8] (**3**, C₁₁) and the homosesquiterpene sodorifen^[9] (**4**, C₁₆), which are generated via an alternative biosynthetic strategy entailing methyltransferase (MT)-mediated C₁ addition prior to scaffold assembly (Figure 1A).

Terpenes are ubiquitous natural products that stand out in terms of their impressive structural diversity and importance as medicinal drugs,^[1] aroma compounds,^[2] and alternative biofuels.^[3] Their carbon backbones are composed of integer multiples of methyl branched C₅-isoprene units, a structural feature directly linked to their common biosynthetic origin: dimethylallyl diphosphate (DMAPP) and isopentenyl diphosphate (IPP). DMAPP and IPP are assembled to oligoprenyl diphosphates by prenyltransferases that dictate the number of elongation steps, yielding geranyl diphosphate (GPP, C₁₀), farnesyl diphosphate (FPP, C₁₅), or geranylgeranyl diphosphate (GGPP, C₂₀). The linear precursors are subsequently converted by terpene cyclases (TCs), catalyzing scaffold formation via often complex carbocation cascade reactions.^[4] These biosynthetic path-

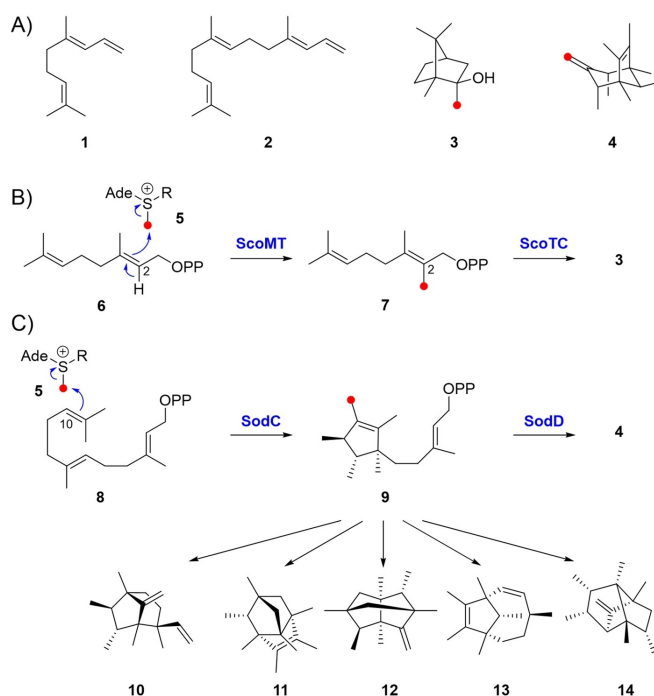


Figure 1. A) Structures of homoterpenes DMNT (**1**), TMTT (**2**), 2-methylisoborneol (**3**), and sodorifen (**4**). B) Biosynthetic pathway to **3**. C) Biosynthetic pathway to **4** and “Greek philosophers terpenes” **10**–**14**. Ade = adenosyl; R = 1-aminocarboxypropyl.

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For 2-methylisoborneol biosynthesis, ScoMT catalyzes initial methyl transfer from *S*-adenosyl-*L*-methionine (SAM, **5**) to GPP (**6**) yielding 2-methyl-GPP (**7**), the specific substrate for ScoTC (Figure 1B).^[10] Similarly, sodorifen biosynthesis is mediated via C₁ addition to FPP (**8**) by MT SodC and subsequent scaffold assembly by TC SodD (Figure 1C).^[11] Interestingly, SodC catalysis does not yield a linear methylated FPP derivative but precyclized intermediate **9**. In 2023, Kampranis discovered a series of biosynthetically related homoterpenes in proteobacteria (**10–14**, “Greek philosophers terpenes”) based on exploration of the functional diversity of SodD homologs (Figure 1C).^[12] Here we report the discovery of a novel general pathway to homoterpenes, widespread but specific to actinobacteria. Five unprecedented homoterpenes were identified, biosynthesized by a novel MT family (hMTs) with unique methyltransferase-isomerase activity and a distinct family of TCs (hTCs).

BLAST search in publicly available genome databases using bacterial TC I protein sequences as queries in combination with manual search for co-localized genes encoding for MTs led to the identification of a cryptic BGC in *Streptomyces varsoviensis*, which we termed *sva* (Figure 2A, Table S1). The *sva* BGC encodes for a type TC (SvaTC), a class I MT (SvaMT), and a cytochrome P450 monooxygenase (SvaCYP) adjacent to regulatory elements (*svaR1–R4*). SvaTC shares 35 % sequence identity (aaSI) with the diterpene cyclase spiroviolene synthase.^[13] SvaMT

attracted our attention, since the gene product was annotated as a mycolic acid cyclopropane synthase (MACS), catalyzing SAM-dependent cyclopropylation. However, SvaMT shares only 17 % aaSI with MACS (P9WBP2). Similarly, SvaMT exhibits merely 14 % and 15 % aaSI with SodC and ScoMT, respectively. BLAST search against the uniprot database revealed that the closest characterized homolog of SvaMT is Beza^[14] (37 % aaSI), a recently discovered C6-GPP MT involved in benzastatin biosynthesis.

To determine the function of SvaMT and SvaTC, the corresponding genes were amplified from genomic DNA and expressed in *Escherichia coli* BL21, with co-expression of chaperones GroES and GroEL to obtain SvaMT (Figure S1). Recombinant enzymes were incubated with GPP, FPP, and GGPP and subjected to gas chromatography-mass spectrometry (GC-MS) analysis after hexane extraction. Reactions with SvaTC did not yield any specific terpene products for either of the tested substrates (Figures S2). Next, SvaMT was incubated with GPP, FPP, and GGPP in the presence of SAM. To enable tracing of products containing diphosphate groups, samples were treated with calf intestinal phosphatase (CIP) to generate the corresponding alcohols and extracts derivatized with *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamid (MSTFA). Whereas no conversions of GPP or GGPP were detected (Figure S3), a specific product (**15a**) accumulated in the reaction with FPP (Figure 2B). Comparison of the mass spectrum of **15a** to an MSTFA-derivatized farnesol standard suggested the presence of an additional methyl group, judging from an increased molecular ion of +14 *m/z* (Figure S4). To test whether the SvaMT generated product is further converted by SvaTC, both enzymes were co-incubated with SAM and FPP. The reaction led to accumulation of a new product (**16**) with a molecular ion of 236 *m/z* indicating production of a methylated sesquiterpene alcohol or ether (Figure 2C, Figure S5).

To elucidate the molecular basis of enzymatic transformations, **15a** and **16** were isolated from preparative scale reactions and their structures determined by 1D and 2D nuclear magnetic resonance (NMR) spectroscopy. The structure of dephosphorylated SvaMT product **15a** was determined as (2*E*,7*E*)-6-methyl-farnesol and named prekantanol (Figure 2D, Table S2, Figure S6–S12). Prekantanol is an unprecedented homofarnesane terpene with an additional methyl group at C6. Unexpectedly, **15a** exhibits an *E*-configured double bond in position C7–C8 instead of the canonical C6–C7 location. The corresponding diphosphate SvaMT product **15b** (prekantanol diphosphate) thus represents the first natural methylated double bond isomer of FPP. Structure elucidation of **16** revealed a novel homoeudesmane terpene, which was named kantenol (Figure 2E, Table S3, Figure S13–S22, Table S4). Kantenol exhibits a 3-methyl-eudesm-7-en-4-ol scaffold with *trans*-configured decalin ring system, axial methyl group at C4, and an additional axially positioned methyl substitution at C3.

SvaMT-mediated formation of **15b** can be explained by methyl group transfer from SAM (**5**) to C6 of FPP (**8**) via electrophilic addition, producing intermediary cation **A**

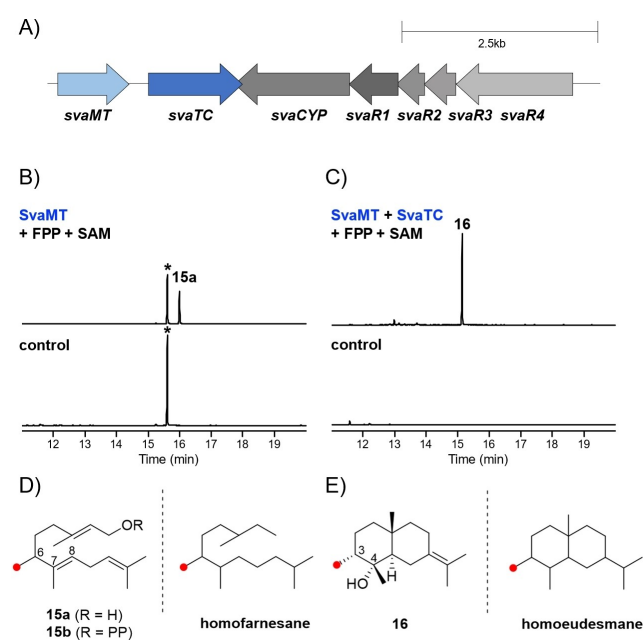


Figure 2. A) Depiction of *sva* BGC. B) Total ion chromatogram (TIC) of enzyme reaction SvaMT with FPP and SAM. Control (FPP + SAM without enzyme). Samples were treated with CIP and MSTFA. Peaks labelled with asterisks correspond to trimethylsilyl-farnesol. C) TIC of enzyme reaction SvaMT + SvaTC with FPP and SAM. Control (FPP + SAM without enzyme). D) Structure of SvaMT product and scaffold. Relative configurations are shown. E) Structure of SvaTC product and scaffold. Relative configurations are shown.

(Figure 3A). Instead of subsequent deprotonation from C6, which would result in formation of the thermodynamically more stable isomer with canonical double bond configuration, SvaMT specifically deprotonates from C8. SvaMT thus represents the first FPP MT with dual function of methyltransferase and double bond isomerase activity. The non-canonical oligoprenyl diphosphate **15b** is subsequently utilized as substrate for SvaTC, generating **16** (Figure 3B). Initial diphosphate abstraction is followed by C1,C10 ring closure and deprotonation from C10 to yield a neutral homo-*iso*-germacrene B intermediate (**17**). Upon reprotonation at C2, a second cyclization reaction is initiated, leading to C–C bond formation between C3 and C8. The cyclization cascade is terminated by addition of water to C7, yielding methyl eudesmane **16**. Interestingly, SvaTC thus mediates eudesmane backbone formation via an unprecedented cyclization mechanism. Eudesmanes, such as juniper camphor^[15] (**18**), the desmethyl analog of **16**, are a widespread family of sesquiterpenes.^[16] Backbone formation from FPP by canonical TCs proceeds via a conserved cyclization mode, entailing C1,C10 cyclization and subsequent C–C bond formation between C2 and C7 through protonation at C6 (Figure 3C).^[17] SvaTC catalysis, however, proceeds via an “inverse” cyclization reaction, resulting in an inverted orientation of the farnesyl chain in the eudesmane backbone (Figure 3B and 3C). To validate the proposed mechanisms, isotopic labelling experiments were conducted. Firstly, the SAM origin of the methyl group in position C16 was verified by incubation of (¹³C-methyl)-SAM and FPP with SvaMT and SvaTC. (¹³C-methyl)-SAM

was generated in situ from (¹³C-methyl)-L-methionine by co-incubation of methionine adenosyltransferase UuMAT.^[18] In line with the proposed mechanism, the ¹³C NMR spectrum showed a signal at $\delta_C=13.9$ ppm, corresponding to C16 (Figure 3D, Table S3).

Next, the cyclization mechanism of SvaTC was investigated by tracing of the reprotonation site (C2 vs. C6). SvaMT and SvaTC were incubated with FPP and SAM in reaction buffer prepared with ²H₂O and the obtained labelled kantenol (**16b**) analyzed by NMR spectroscopy. The ¹H,¹³C-HSQC spectrum showed selective deuterium incorporation in the pro-*S* proton at C2 judging from the absence of a signal at $\delta_{H,C}=1.34/45.3$ ppm, but presence at $\delta_{H,C}=1.16/45.3$ ppm (Figure 3E, Table S3, Figure S17). Accordingly, the ¹³C NMR signal for C2 is slightly shifted upfield compared to a chemical shift of $\delta_C=45.7$ ppm for C2 in unlabelled **16**, and appears as a triplet with a coupling constant of $^1J_{C,D}=18.8$ Hz.

To address the question whether the newly discovered pathway to **16** via **15b** represents a general route towards homoterpenes, *sua*-like BGCs were searched through genome mining. In total over 150 BGCs encoding for homologous SvaMT and SvaTC pairs were identified in the JGI genome database, which were found to be exclusively encoded in actinobacterial genomes. Phylogenetic analysis of SvaMT and homologs with related MTs^[10b,11b,12,14,19] indicates a distinct and monophyletic origin for the discovered FPP methyltransferase/isomerase family (Figure 4A). Similarly, SvaTC and homologs form a distinct branch in the

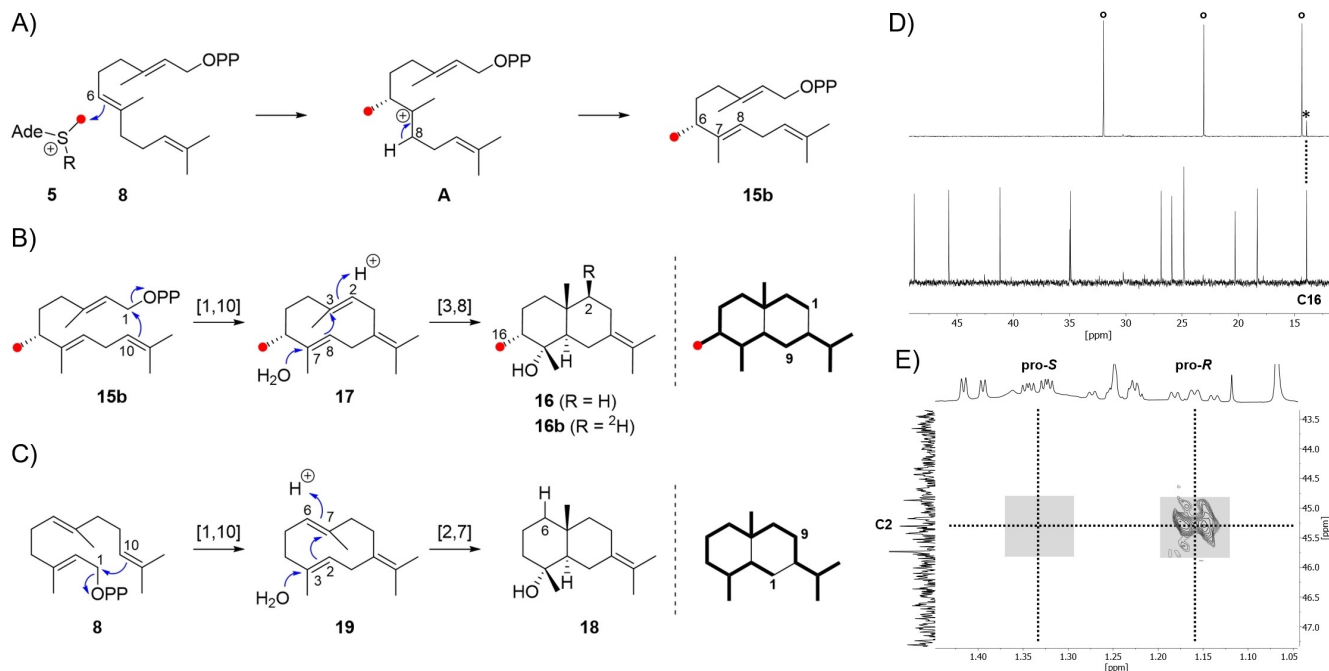


Figure 3. A) Proposed mechanism of SvaMT-mediated catalysis. B) Proposed mechanism of SvaTC-mediated catalysis. Farnesyl chain is highlighted in bold bonds. C) Exemplified canonical mechanism towards the eudesmane backbone. D) ¹³C NMR data of **16** from incubation of SvaMT + SvaTC with FPP and (¹³C-methyl)-SAM (upper spectrum), and unlabelled **16** (lower spectrum). Circles indicate signals corresponding to hexane. Asterisk indicates signal corresponding to C16. E) ¹H,¹³C-HSQC data of **16b** obtained from incubation of SvaMT + SvaTC with FPP and SAM in ²H₂O. ¹H NMR data of unlabelled **16** is shown in f2 dimension.

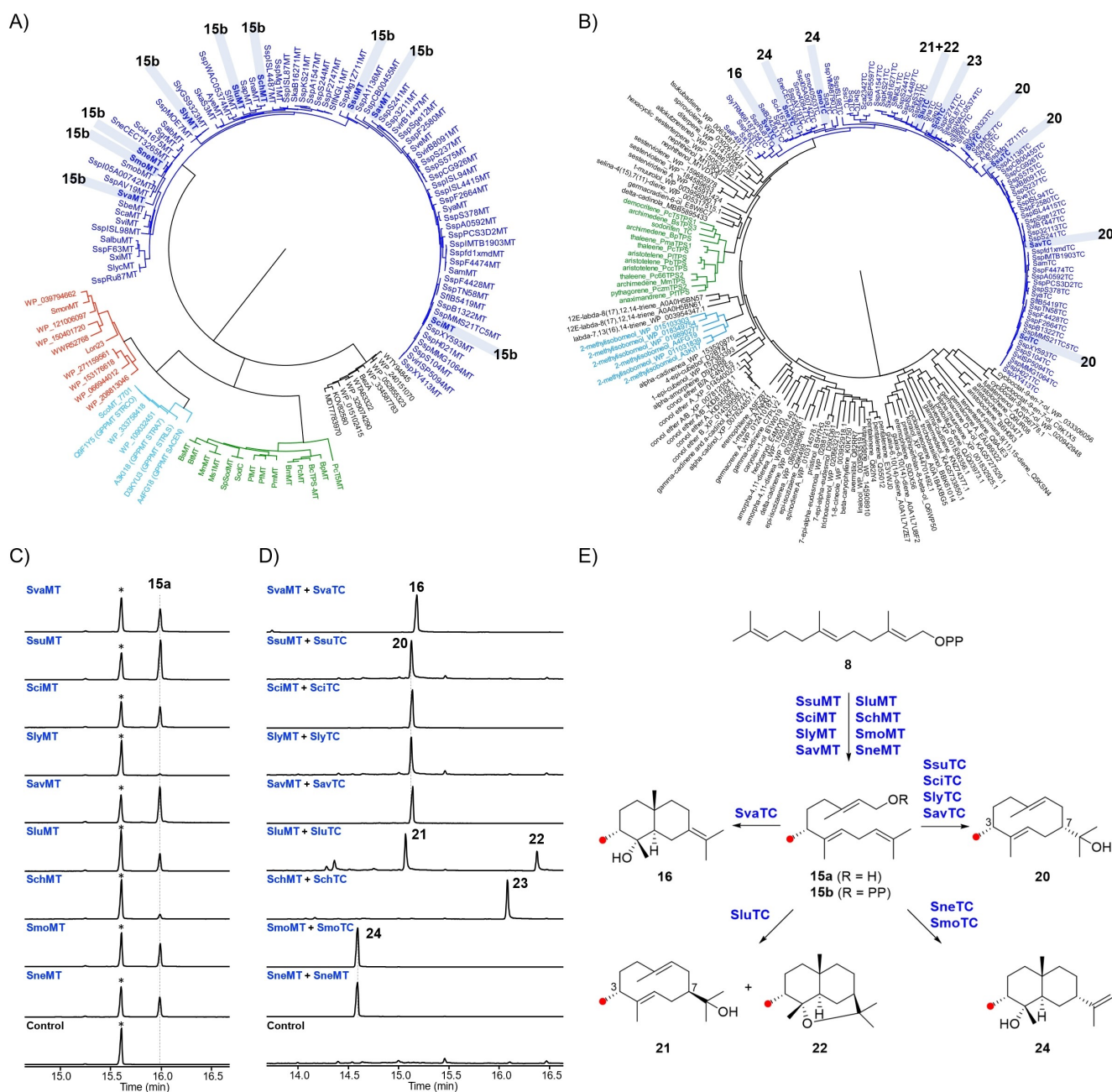


Figure 4. A) Phylogenetic analysis of SvaMT (blue), Beza (black), SodC (green), ScoMT (light blue), Lon23 (red), and homologs. Investigated enzymes are highlighted in boxes and products assigned. B) Phylogenetic analysis of SvaTC (blue), SodD (green), ScoTC (light blue), and homologs with canonical microbial type I TCs (black). C) TICs of enzyme reactions of SvaMT and homologs with FPP and SAM. Control (FPP + SAM without enzyme). Samples were treated with CIP and MSTFA. Peaks labelled with asterisks correspond to trimethylsilyl-farnesol. D) TICs of enzyme reactions of SvaMT + SvaTC and homologous pairs with FPP and SAM. Control (FPP + SAM without enzyme). E) Discovered pathways and structures of kantenol (**16**), weberol (**20**), *epi*-weberol (**21**), hegelenether (**22**), and jaegerol (**24**). Relative configurations are shown.

phylogenetic analysis compared to ScoTC,^[10a] SodD,^[11a,12] and canonical microbial type I TCs^[4c] (Figure 4B).

To determine whether SvaMT and SvaTC homologs exhibit conserved or divergent catalytic functions, eight pairs were selected for further analysis based on phylogenetic considerations and aaSI to SvaMT or SvaTC (Table S5, Figure S23, Figure 4A and 4B). Selected enzyme pairs are genetically encoded in BGCs with similar architecture as the

sva cluster (Figure S24). Targeted enzymes were cloned from genomic DNA and expressed in *E. coli* BL21, with co-expression of chaperones GroES and GroEL to obtain SvaMT homologs (Figure S1). Incubation of recombinant SvaMT homologs with FPP and SAM led to formation of **15b** for all reactions, judging from identical retention times and mass spectra of accumulating products (Figure 4C, Figure S25). The results indicate a conserved function for

the discovered hMT family and accordingly suggest that SvaTC homologs specifically utilize **15b** as substrate, instead of canonical TC substrates. To investigate the product specificity, targeted hTCs were incubated with their native SvaMT homolog in the presence of FPP and SAM. Surprisingly, five homosesquiterpene products (**20–24**) distinct to **16** were detected (Figure 4D, Figure S26). Their structures were subsequently elucidated by NMR spectroscopy after isolation from preparative scale reactions.

The structure of **20**, produced by SsuTC, SciTC, SlyTC, and SavTC, was determined as 3-methyl-(9(19)*E*,4(5)*E*)-germacradien-11-ol and named weberol (Figure 4E, Table S6, Figures S27–S33). Weberol is a methylated double bond isomer of the central sesquiterpene hedycaryol^[17c] and exhibits *syn* oriented C3 methyl and C7 isopropyl ring substituents. The C3-desmethyl analog, neohedycaryol,^[20] is not a known natural product but has been chemically synthesized. SluTC generated two products (**21**, **22**) in a ratio of approx. 2:1 (Figure 4D). The major product (**21**) was elucidated as the C7 epimer of **20** and named *epi*-weberol (Figure 4E, Figures S34–S42). In contrast to **20** and neohedycaryol, which exist as single conformers (extended chair^[20]) in solution, **21** adopts two major (**21a**, **21b**) and one minor conformation (**21c**) in a ratio of approx. 2:1:0.1 (**21a**:**21b**:**21c**). NMR signals could be differentiated and assigned to the major conformer **21a** (extended chair, Table S7, Figure S34) and **21b** (extended boat, Table S8, Figure S35). The findings support an *anti* orientation of C3 methyl and C7 isopropyl substituents, likely destabilizing the extended chair conformation since the methyl group is forced into an axial position, compared to the equatorial orientation in **20** (Figure S27 and S34). The minor SluTC product **22**, which was named hegelener, exhibits an unprecedented tricyclic homosesquiterpene skeleton with a rare cyclic ether functionality (Figure 4E, Table S9, Figures S43–S59). No corresponding desmethyl analogue has been reported. Likely, **21** represents a biosynthetic intermediate to **22** and is prematurely released from the enzyme active site. Despite several attempts, SchTC product **23** could not be isolated in sufficient purity due to apparent instability. The structure of **24**, produced by SneTC and SmoTC, was determined as a novel homoeudesmane terpene and named jaegerol (Figure 4E, Table S10, Figure S60–S66). Jaegerol exhibits a 3-methyl-eudesm-11-en-4-ol structure with a *trans*-configured decalin ring system. The desmethyl analogue, intermedeol is a known natural product.^[21] Cyclization mechanisms for the generation of **20**, **21**, **22**, and **24** from **15b** are in line with the observed inverse mechanism catalyzed by SvaTC (Figure S67). Absolute configurations of isolated homoterpenes need to be clarified in future studies.

Taken together, a novel biosynthetic pathway towards homoterpenes was discovered in actinobacteria. The pathway differs from the recently identified route towards “Greek philosophers terpenes” in proteobacteria, demonstrating parallel evolution of homoterpene biosynthesis in bacteria. The widespread occurrence suggests a potentially overlooked biological significance for this underexplored family of non-canonical terpenes. The enzymological basis

has been elucidated, leading to discovery of a monophyletic FPP-specific MT family with a unique dual methyltransferase-isomerase function. Furthermore, a functionally divergent family of type I TCs has been discovered, specifically utilizing non-canonical substrate **15b**. hTCs catalyze formation of methyl analogs of widespread and central germacrane and eudesmane sesquiterpenes. Scaffold formation, however, differs from the canonical mechanism. The findings raise the question, if and how variations in methylation patterns influence biological activity, given the frequent observation that methylations have dramatic impact on pharmaceutical properties of bioactive compounds (“magic methyl effect”^[22]). Kirschning et al. recently reported that the synthetic desmethyl analog of **15b** can be converted by canonical TCs,^[23] indicating that the here reported novel enzymology might serve as a promising starting point for genetically programmable synthesis of methylated terpenoids and exploration of untapped chemical space.

Supporting Information

Supplementary Figures and Tables, experimental procedures, and compound characterization data is provided.

Acknowledgements

We gratefully acknowledge funding by Deutsche Forschungsgemeinschaft (DFG) through an Emmy Noether fellowship to LB (513519240) and funding of FOR project 510974120. We thank the Carl-Zeiss-Stiftung for financial support and the Funds of the Chemical Industry (FCI) Germany for a Kekulé Fellowship to DMH. We thank Prof. Dr. Dieter Spittler for providing GC-MS, HPLC instrumentation, and interesting discussions, Prof. Dr. Jennifer Andexer for providing UuMAT plasmid, and Dr. Immo Burkhardt for critical reading of the manuscript. We gratefully acknowledge Anke Friemel and Ulrich Haunz for technical assistance with NMR measurement. Open Access funding enabled and organized by Projekt DEAL.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords: actinobacteria · biosynthesis · enzyme discovery · non-canonical terpenoids · terpenoids

- [1] a) P. K. Ajikumar, W. H. Xiao, K. E. J. Tyo, Y. Wang, F. Simeon, E. Leonard, O. Mucha, T. H. Phon, B. Pfeifer, G. Stephanopoulos, *Science* **2010**, *330*, 70; b) C. J. Paddon, P. J. Westfall, D. J. Pitera, K. Benjamin, K. Fisher, D. McPhee, M. D. Leavell, A. Tai, A. Main, D. Eng, D. R. Polichuk, K. H. Teoh, D. W. Reed, T. Treynor, J. Lenihan, H. Jiang, M. Fleck, S. Bajad, G. Dang, D. Dengrove, D. Diola, G. Dorin, K. W. Ellens, S. Fickes, J. Galazzo, S. P. Gaucher, T. Geistlinger, R. Henry, M. Hepp, T. Horning, T. Iqbal, L. Kizer, B. Lieu, D. Melis, N. Moss, R. Regentin, S. Secrest, H. Tsuruta, R. Vazquez, L. F. Westblade, L. Xu, M. Yu, Y. Zhang, L. Zhao, J. Lievens, P. S. Covelto, J. D. Keasling, K. K. Reiling, N. S. Renninger, J. D. Newman, *Nature* **2013**, *496*, 528–532; c) X. Liu, J. Xin, Y. Sun, F. Zhao, C. Niu, S. Liu, *Mar. Drugs* **2024**, *22*, 347; d) J. S. Câmara, R. Perestrelo, R. Ferreira, C. V. Berenguer, J. A. M. Pereira, P. C. Castilho, *Molecules* **2024**, *29*, 3861.
- [2] a) S. A. Goff, H. J. Klee, *Science* **2006**, *311*, 815; b) F. M. Schempp, L. Drummond, M. Buchhaupt, J. Schrader, *J. Agric. Food Chem.* **2018**, *66*, 2247.
- [3] a) P. P. Peralta-Yahya, F. Zhang, S. B. del Cardayre, J. D. Keasling, *Nature* **2012**, *488*, 320; b) J. Keasling, H. G. Martin, T. S. Lee, A. Mukhopadhyay, S. W. Singer, E. Sundstrom, *Nat. Rev. Microbiol.* **2021**, *19*, 701.
- [4] a) C. T. Walsh, Y. Tang, *Natural Product Biosynthesis*, RSC, London, **2017**; b) D. W. Christianson, *Chem. Rev.* **2017**, *117*, 11570; c) J. S. Dickschat, *Nat. Prod. Rep.* **2016**, *33*, 87.
- [5] a) O. Wallach, *Liebigs Ann.* **1885**, *227*, 277; b) L. Ruzicka, *Experientia* **1953**, *9*, 357.
- [6] a) T. Zeng, Z. Liu, J. Zhuang, Y. Jiang, W. He, H. Diao, N. Lv, Y. Jian, D. Liang, Y. Qiu, R. Zhang, F. Zhang, X. Tang, R. Wu, *J. Chem. Inf. Model.* **2020**, *60*, 2082; b) J. D. Rudolf, C. Y. Chang, *Nat. Prod. Rep.* **2020**, *37*, 425; c) M. Li, H. Tao, *Beilstein J. Org. Chem.* **2024**, *20*, 959.
- [7] a) G. I. Arimura, R. Ozawa, T. Shimoda, T. Nishloka, W. Boland, J. Takabayashi, *Nature* **2000**, *406*, 512; b) A. Richter, C. Schaff, Z. Zhang, A. E. Lipka, F. Tian, T. G. Köllner, C. Schnee, S. Preiß, S. Irmisch, G. Jander, W. Boland, J. Gershenzon, E. S. Buckler, J. Degenhardt, *Plant Cell* **2016**, *28*, 2651.
- [8] a) M. Komatsu, M. Tsuda, S. Omura, H. Oikawa, H. Ikeda, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 7422; b) C. M. Wang, D. E. Cane, *J. Am. Chem. Soc.* **2008**, *130*, 8908; c) S. Giglio, W. K. W. Chou, H. Ikeda, D. E. Cane, P. T. Monis, *Environ. Sci. Technol.* **2011**, *45*, 992; d) J. S. Dickschat, T. Nawrath, V. Thiel, B. Kunze, R. Müller, S. Schulz, *Angew. Chem. Int. Ed.* **2007**, *46*, 8287.
- [9] S. H. von Reuß, M. Kai, B. Piechulla, W. Francke, *Angew. Chem. Int. Ed.* **2010**, *49*, 2009.
- [10] a) M. Köksal, W. K. W. Chou, D. E. Cane, D. W. Christianson, *Biochemistry* **2012**, *51*, 3003; b) M. Köksal, W. K. W. Chou, D. E. Cane, D. W. Christianson, *Biochemistry* **2012**, *51*, 3011.
- [11] a) S. von Reuss, D. Domik, M. C. Lemfack, N. Magnus, M. Kai, T. Weise, B. Piechulla, *J. Am. Chem. Soc.* **2018**, *140*, 11855; b) M. C. Lemfack, W. Brandt, K. Krüger, A. Gurowietz, J. Djifack, J. P. Jung, M. Hopf, H. Noack, B. Junker, S. von Reuß, B. Piechulla, *Sci. Rep.* **2021**, *11*, 3182; c) E. R. Duell, P. M. D'Agostino, N. Shapiro, T. Woyke, T. M. Fuchs, T. A. M. Gulder, *Microb. Cell Fact.* **2019**, *18*, 32; d) H. Xu, L. Lauterbach, B. Goldfuss, G. Schnakenburg, J. S. Dickschat, *Nat. Chem.* **2023**, *15*, 1164.
- [12] Y. T. Duan, A. Koutsaviti, M. Harizani, C. Ignea, V. Roussis, Y. Zhao, E. Ioannou, S. C. Kampranis, *Nat. Chem. Biol.* **2023**, *19*, 1532.
- [13] P. Rabe, J. Rinkel, E. Dolja, T. Schmitz, B. Nubbemeyer, T. H. Luu, J. S. Dickschat, *Angew. Chem. Int. Ed.* **2017**, *56*, 2776.
- [14] H. Tsutsumi, Y. Moriwaki, T. Terada, K. Shimizu, K. Shin-ya, Y. Katsuyama, Y. Ohnishi, *Angew. Chem. Int. Ed.* **2022**, *61*, e202111217.
- [15] G. L. Chetty, V. B. Zalkow, L. H. Zalkow, *Tetrahedron Lett.* **1968**, *28*, 3223.
- [16] E. Breitmaier, *Terpenes*, Wiley-VCH, **2006**.
- [17] a) H. Xu, J. S. Dickschat, *Beilstein J. Org. Chem.* **2023**, *19*, 186; b) H. Xu, J. S. Dickschat, *Chem. Eur. J.* **2020**, *26*, 17318; c) H. Xu, J. S. Dickschat, *Chem. Eur. J.* **2022**, *28*, e202200405.
- [18] D. Kleiner, F. Shmulevich, R. Zarivach, A. Shahar, M. Sharon, G. Ben-Nissan, S. Bershtein, *J. Mol. Biol.* **2019**, *431*, 4796.
- [19] a) L. Drummond, M. J. Kschowak, J. Breitenbach, H. Wolff, Y.-M. Shi, J. Schrader, H. B. Bode, G. Sandmann, M. Buchhaupt, *ACS Synth. Biol.* **2019**, *8*, 1303; b) T. Ozaki, S. S. Shinde, L. Gao, R. Okuizumi, C. Liu, Y. Ogasawara, X. Lei, T. Dairi, A. Minami, H. Oikawa, *Angew. Chem. Int. Ed.* **2018**, *57*, 6629.
- [20] A. J. Minnaard, G. A. Stork, J. B. P. A. Wijnberg, A. de Groot, *J. Org. Chem.* **1997**, *62*, 2344.
- [21] a) H. Itokawa, H. Morita, T. Kobayashi, K. Watanabe, Y. Iitaka, *Chem. Pharm. Bull.* **1987**, *35*, 2860; b) I. Burkhardt, N. Kreuzenbeck, C. Beemelmans, J. S. Dickschat, *Org. Biomol. Chem.* **2019**, *17*, 3348; c) P. Rabe, J. Rinkel, T. A. Klapschinski, L. Barra, J. S. Dickschat, *Org. Biomol. Chem.* **2016**, *14*, 158.
- [22] a) K. Feng, R. E. Quevedo, J. T. Kohrt, M. S. Oderinde, U. Reilly, M. C. White, *Nature* **2020**, *580*, 621; b) H. Schönherr, T. Cernak, *Angew. Chem. Int. Ed.* **2013**, *52*, 12256.
- [23] H. Struwe, J. Droste, D. Dhar, M. D. Davari, A. Kirschning, *ChemBioChem* **2024**, *25*, e202300599.

Manuscript received: September 26, 2024

Accepted manuscript online: November 22, 2024

Version of record online: December 17, 2024