Zitierweise: *Angew. Chem. Int. Ed.* **2023**, *62*, e202303692
doi.org/10.1002/anie.202303692

Natural Products

Non-canonical Biosynthesis of the Brexane-Type Bishomosesquiterpene Chlororaphen through Two Consecutive Methylation Steps in *Pseudomonas chlororaphis* O6 and *Variovorax boronicumulans* PHE5-4

Nancy Magnus[+], Stephan H. von Reuss[+],* Freya Braack, Chi Zhang, Katja Baer, Arthur Koch, Philine L. Hampe, Sylvain Sutour, Feng Chen, and Birgit Piechulla*

In memory of Alex Nickon (1927–2021)

Abstract: A non-canonical biosynthetic pathway furnishing the first natural brexane-type bishomosesquiterpene (chlororaphen, $C_{17}H_{28}$) was elucidated in the γ-proteobacterium *Pseudomonas chlororaphis* O6. A combination of genome mining, pathway cloning, *in vitro* enzyme assays, and NMR spectroscopy revealed a three-step pathway initiated by C10 methylation of farnesyl pyrophosphate (FPP, C_{15}) along with cyclization and ring contraction to furnish monocyclic γ-presodorifen pyrophosphate (γ-PSPP, C_{16}). Subsequent *C*-methylation of γ-PSPP by a second *C*-methyltransferase furnishes the monocyclic α-prechlororaphen pyrophosphate (α-PCPP, C_{17}), serving as the substrate for the terpene synthase. The same biosynthetic pathway was characterized in the β-proteobacterium *Variovorax boronicumulans* PHE5-4, demonstrating that non-canonical homosesquiterpene biosynthesis is more widespread in the bacterial domain than previously anticipated.

[*] Dr. N. Magnus,[+] F. Braack, K. Baer, A. Koch, P. L. Hampe, Prof. Dr. B. Piechulla
Institute of Biological Sciences, University of Rostock
Albert-Einstein-Strasse 3, 18059 Rostock (Germany)
E-mail: nancy.magnus@uni-rostock.de

Prof. Dr. S. H. von Reuss[+]
Institute of Chemistry, University of Neuchatel
Avenue de Bellevaux 51, 2000 Neuchatel (Switzerland)
E-mail: stephan.vonreuss@unine.ch

Prof. Dr. S. H. von Reuss,[+] Dr. S. Sutour
Neuchatel Platform of Analytical Chemistry (NPAC), University of Neuchatel
Avenue de Bellevaux 51, 2000 Neuchatel (Switzerland)

Dr. C. Zhang, Prof. Dr. F. Chen
Department of Plant Sciences, University of Tennessee
2505 E J Chapman Drive, Knoxville, TN 37996 (USA)

[+] These authors contributed equally to this work.

Since the delineation of the biogenetic isoprene rule by Otto Wallach[1] and Leopold Ruzicka,[2] sesquiterpenoids are considered to be exclusively derived from cyclization of farnesyl pyrophosphate (FPP, **3a**)[3] as the canonical acyclic substrate for sesquiterpene synthases. However, we have recently shown that the biogenesis of the unique homosesquiterpene ($C_{16}H_{26}$) sodorifen (**1**), a polymethylated bicyclo-[3.2.1]octadiene hydrocarbon from the rhizobacterium *Serratia plymuthica* 4Rx13,[4] involves a non-canonical monocyclic intermediate, α-presodorifen pyrophosphate (α-PSPP, **2a**), derived from FPP (**3a**) through methylation and cyclization by a *S*-adenosyl-L-methionine (SAM)-dependent *C*-methyltransferase (*Sp*-FPP-MT, Figure 1a).[5] The cyclization mechanism of the *Sp*-FPP-MT has been studied by *in silico* modelling and site-directed mutagenesis, which revealed a peculiar ring-contraction step to the pentamethylcyclopentenyl moiety facilitated by a catalytic dyad.[6] The initial biosynthetic intermediates and a diversity of potential derailment products from cyclohexyl carbocations upstream of the ring-contraction step have been characterized by single-site mutation of *Sp*-FPP-MT.[7] Expression of the *S. plymuthica* sodorifen synthase (*Sp*-SodS) is upregulated in response to bacterial[8] and fungal[9] volatile organic compounds (VOCs), indicating that homosesquiterpenes such as sodorifen (**1**) might function in interspecies interactions.

Herein, we describe the identification of chlororaphen (**6**), a bishomosesquiterpene hydrocarbon ($C_{17}H_{28}$) with an unprecedented brexane-type skeleton from *Pseudomonas chlororaphis* O6 and *Variovorax boronicumulans* PHE5-4. Chlororaphen biosynthesis depends on the consecutive action of a non-classical cyclizing FPP *C*-methyltransferase and a classical *C*-methyltransferase, followed by a terpene cyclase that exclusively utilizes non-canonical monocyclic C_{17} α-prechlororaphen pyrophosphate (α-PCPP, **5a**) as a substrate (Figure 1a). Our results demonstrate that non-canonical biosynthesis of bacterial homoterpenes is more complex and more widely distributed than previously anticipated, thus providing a first glance of a yet undisclosed part of the bacterial terpenome.

Genome mining highlighted the occurrence of a sodorifen-like biosynthetic gene cluster in the γ-proteobacterium

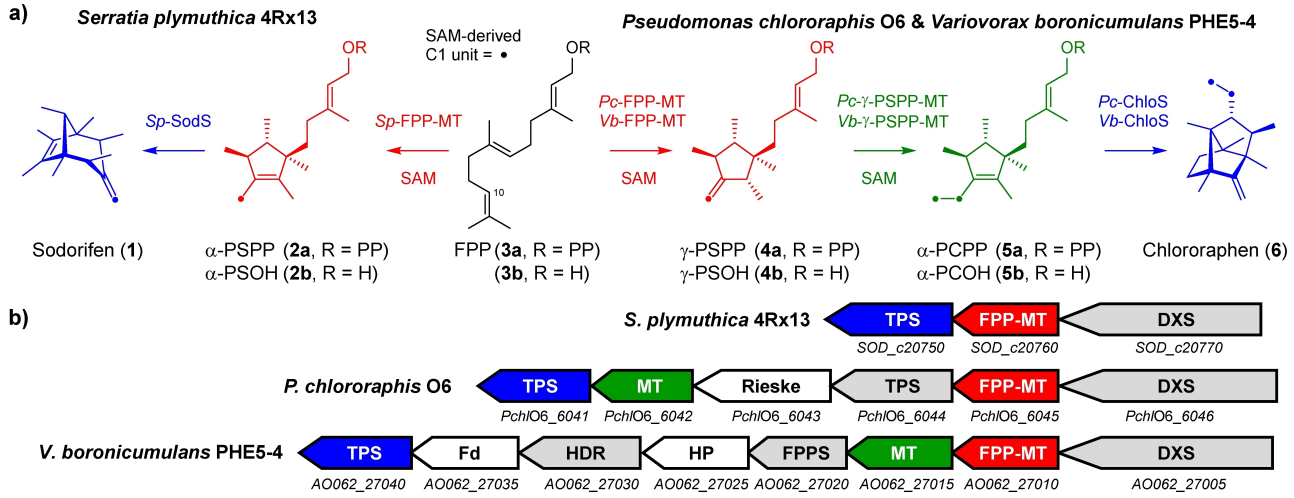


Figure 1. a) Non-canonical terpene biosynthesis of sodorifen (**1**) in *Serratia plymuthica* 4Rx13 and chlororaphen (**6**) in *Pseudomonas chlororaphis* O6 and *Variovorax boronicumulans* PHE5-4 via monocyclic α-PSPP (**2a**) and α-PCPP (**5a**) as substrates for the sodorifen synthase (*Sp*-SodS) and the chlororaphen synthases (*Pc*-ChloS and *Vb*-ChloS), respectively. b) Gene clusters for non-canonical homosesquiterpene biosynthesis by cyclizing FPP *C*-methyltransferases (FPP-MT), γ-PSPP *C*-methyltransferase (MT), and terpene synthases (TPS) in *S. plymuthica* 4Rx13, *P. chlororaphis* O6, and *V. boronicumulans* PHE5-4. DXS: DOXP synthase; Fd: ferredoxin; FPPS: FPP synthase; HDR: HMBPP reductase; HP: hypothetical protein; Rieske: Rieske protein.

P. chlororaphis O6 (Figure 1b).[9–12] GC-EIMS analysis of VOCs released by *P. chlororaphis* O6 showed a diversity of yet unidentified C_{16} hydrocarbons (Figure 2a) as well as a dominating compound **6** with *m*/*z* 232.2204 $[M]^{+\bullet}$ for the molecular formula $C_{17}H_{28}$ with four double-bond equivalents (Figure 3a), indicative of a potential bishomosesquiterpene that we have named chlororaphen (**6**). To confirm that the biosynthesis of chlororaphen (**6**) involves a non-canonical, cyclizing *C*-methyltransferase (*Pchl*O6_6045), the biosynthetic gene cluster from *P. chlororaphis* O6 (*Pchl*O6_6041–6045) was cloned into *E. coli*, which resulted in the production of chlororaphen (**6**) along with a number of minor isomers (Figure 2b). To unambiguously identify the genes involved in chlororaphen (**6**) biosynthesis, both putative *C*-methyltransferases (*Pchl*O6_6042 and *Pchl*O6_6045) and terpene synthases (*Pchl*O6_6041 and *Pchl*O6_6044) identified in the *P. chlororaphis* O6 gene cluster were heterologously expressed in *E. coli* and functionally characterized using *in vitro* assays with farnesyl pyrophosphate (FPP, **3a**) and *S*-adenosyl methionine (SAM). Systematic analysis of coupled enzyme assays demonstrated that only triple enzyme assays with *Pchl*O6_6045 (*Pc*-FPP-MT), *Pchl*O6_6042 (*Pc*-γ-PSPP-MT), and *Pchl*O6_6041 (*Pc*-TPS/ChloS) led to chlororaphen (**6**) synthesis (Figure 2e), indicating that two different SAM-dependent *C*-methyltransferases along with one terpene synthase (TPS) are required and sufficient for chlororaphen biosynthesis from FPP (**3a**). Single-enzyme assays of the terpene synthase *Pc*-TPS (*Pchl*O6_6041) with FPP (**3a**) did not produce any cyclized reaction product (Figure S1a), indicating the requirement of a non-canonical substrate derived from FPP (**3a**) by *C*-methyltransferase activities. None of the two *C*-methyltransferases produced any volatile organic product in single-enzyme assays when treated individually (or in combination) with FPP (**3a**) and SAM (Figure S1b,c,d), suggesting that their potential reaction products constitute non-volatile pyrophosphates. Of the two *C*-methyltransferases, only the *Pc*-FPP-MT (*Pchl*O6_6045) furnished a volatile reaction product in coupled enzyme assays with alkaline phosphatase (AP) (Figure 2f,g), demonstrating that *C*-methylation of FPP (**3a**) by *Pc*-FPP-MT represents the first step in chlororaphen (**6**) biosynthesis to produce a non-volatile pyrophosphate intermediate (γ-PSPP, **4a**). The mass spectrum of the corresponding hydrolysis product, a volatile C16 alcohol named γ-presodorifenol (γ-PSOH, **4b**), shows a molecular ion signal at *m*/*z* 236.2146 $[M]^{+\bullet}$ for $C_{16}H_{28}O$ (Figure 3b) in agreement with SAM-dependent *C*-methylation of FPP (**3a**) by *Pc*-FPP-MT and subsequent hydrolysis by AP. The base peak ion signal at *m*/*z* 137.1328 for $C_{10}H_{17}$ suggested a monocyclic structure, which implied that the SAM-dependent C-methylation of FPP (**3a**) by *Pc*-FPP-MT involves a non-classical cyclization reaction that is potentially similar to those of the α-presodorifen pyrophosphate (α-PSPP, **2a**) producing *Sp*-FPP-MT. Double-enzyme assays with *Pc*-FPP-MT (*Pchl*O6_6045) and *Pc*-TPS (*Pchl*O6_6041) did not furnish any volatile reaction product (apart from small quantities of the dehydration products; Figure S1e), indicating that γ-presodorifen pyrophosphate (γ-PSPP, **4a**) is not a suitable substrate for the terpene synthase, which suggests that a second *C*-methylation step catalyzed by *Pc*-γ-PSPP-MT is required. Triple-enzyme assays with *Pc*-FPP-MT (*Pchl*O6_6045), *Pc*-γ-PSPP-MT (*Pchl*O6_6042), and AP furnished an unprecedented C_{17} alcohol named α-prechlororaphenol (α-PCOH, **5b**) as the dominating cyclization product (Figure 2h), which exhibits a molecular ion signal at *m*/*z* 250.2309 $[M]^{+\bullet}$ corresponding to $C_{17}H_{30}O$ and a base peak ion signal at *m*/*z* 151.1492 for $C_{11}H_{19}$ (Figure 3c), in agreement with SAM-dependent

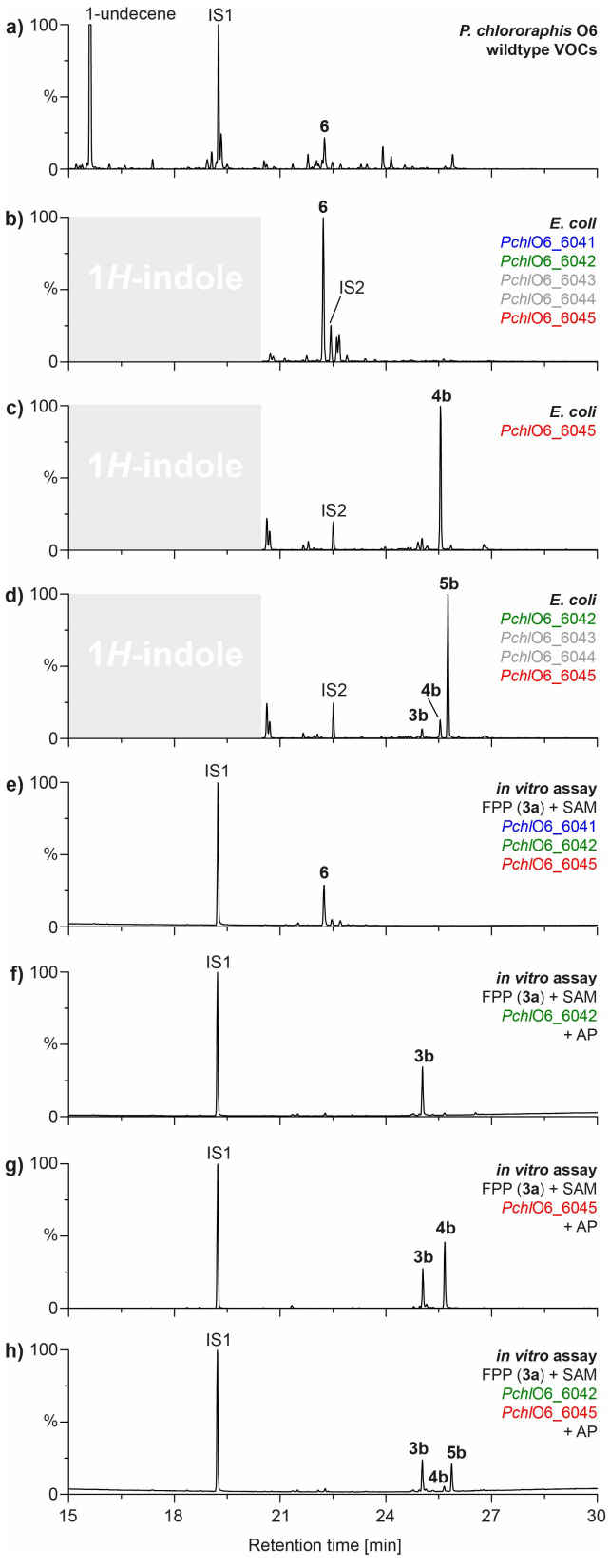


Figure 2. GC-EIMS chromatograms of VOCs collected from a) *Pseudomonas chlororaphis* O6; b)–d) pathway cloning in *E. coli*; e)–h) *in vitro* assays with FPP (**3a**), SAM, and heterologously expressed enzymes; showing farnesol (**3b**), γ-PSOH (**4b**), α-PCOH (**5b**) and chlororaphen (**6**). IS1 = nonyl acetate, IS2 = *cis*-nerolidol at 5 ng μL^{-1}; for negative controls, see Figure S1.

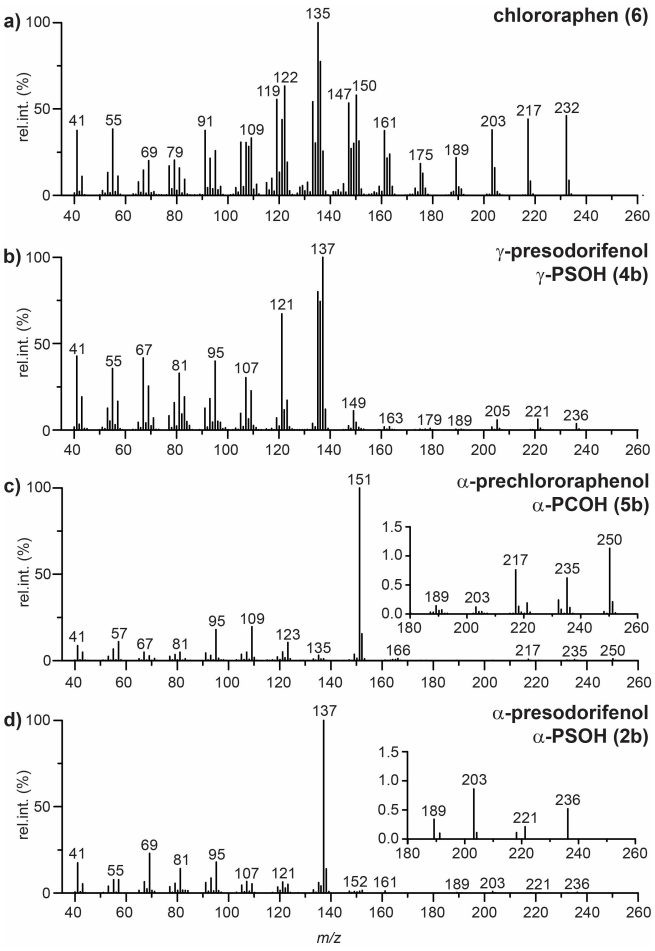


Figure 3. GC-EIMS spectra of a) chlororaphen (**6**), b) γ-presodorifenol (γ-PSOH, **4b**) and c) α-prechlororaphenol (α-PCOH, **5b**) from *P. chlororaphis* O6, and d) α-presodorifenol (α-PSOH, **2b**) from *S. plymuthica* 4Rx13.

methylation of γ-presodorifen pyrophosphate (γ-PSPP, **4a**) by *Pc*-γ-PSPP-MT.

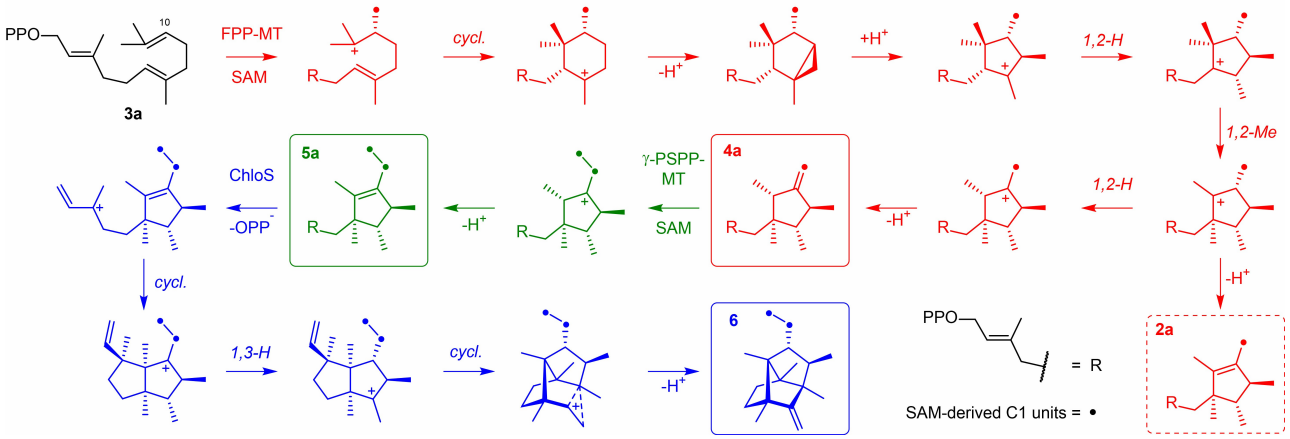
Consequently, chlororaphen (**6**) production in triple-enzyme assays with *Pc*-FPP-MT (*Pchl*O6_6045), *Pc*-γ-PSPP-MT (*Pchl*O6_6042), and *Pc*-TPS (*Pchl*O6_6041; Figure 2e) indicated that α-prechlororaphen pyrophosphate (α-PCPP, **5a**) is the substrate for chlororaphen biosynthesis by the chlororaphen synthase (ChloS). Taken together, these results reveal an unprecedented biosynthetic pathway for the bishomosesquiterpene chlororaphen (**6**) in which FPP (**3a**) is *C*-methylated and cyclized by *Pc*-FPP-MT (*Pchl*O6_6045) to furnish the ring-contracted γ-presodorifen pyrophosphate (γ-PSPP (C_{16}), **4a**), which is subsequently *C*-methylated by *Pc*-γ-PSPP-MT (*Pchl*O6_6042) to yield α-prechlororaphen pyrophosphate (α-PCPP (C_{17}), **5a**), which ultimately serves as a substrate for cyclization by *Pc*-ChloS (*Pchl*O6_6041) to give chlororaphen (**6**). Both methyltransferases share only 41.25 % sequence identity on the amino acid level, indicating their different roles during biosynthesis (Figure S10).

To identify the molecular structure of the bishomosesquiterpene chlororaphen (**6**), the *P. chlororaphis* O6 gene

cluster, including both *C*-methyltransferases (*Pchl*O6_6045 and *Pchl*O6_6042), and the *Pc*-ChloS (*Pchl*O6_6041; along with *Pchl*O6_6044 and *Pchl*O6_6043), was cloned into an expression vector for heterologous expression in *E. coli*. VOCs released by transformed *E. coli* (Figure 2b) were collected on PorapakQ and eluted with C_6D_6 for direct NMR analysis. ^{1}H NMR spectroscopy showed *E. coli* derived 1*H*-indole as the dominating component (99 % of the total volatiles) along with minor amounts of a complex mixture of hydrocarbons (1 %) that exhibit signals at δ_H = 0.5–6.0 ppm. The dominating bishomosesquiterpene chlororaphen (**6**) is characterized by one exocyclic methylene group (δ_H = 4.57 and 4.91 ppm) and four singlet methyl groups (δ_H = 0.66, 0.76, 0.84, and 0.92 ppm). Inspection of the *dqf*-COSY spectrum revealed an unsubstituted and a 1-ethyl-2-methyl-substituted ethylene bridge, which implied the presence of four quaternary carbon centers and a tricyclic structure (Figure S5). Non-uniform sampling (NUS) facilitated the acquisition of HSQC and HMBC spectra, which provided relevant heteronuclear H,C-correlations (Figure S5). These data revealed a helical tricyclo[4.3.0.0^{3,7}]nonane skeleton comprised of two partially superimposed norbornyl units known as brexane (in reference to the bridge involving an exo-norbornyl bond).[13] Brexanes have previously been explored as models for non-classical carbocations,[14–21] but have never been observed as natural products. The 5-ethyl-2-methylene-1,3,4,6,7-pentamethylbrexane skeleton of chlororaphen (**6**) corresponds to an EIMS fragmentation pattern that shows characteristic fragment ions at *m*/*z* 150 for $C_{11}H_{18}$ from neutral loss of C_6H_{10}, and *m*/*z* 122 for C_9H_{14} from neutral loss of C_8H_{14} (Figure 3a). The relative configuration was deduced from the NOESY spectrum (Figure S6) and H,H-coupling constants (Table S3). The molecular structures of the biosynthetic intermediates in chlororaphen (**6**) biosynthesis, the pyrophosphates γ-PSPP (**4a**) and α-PCPP (**5a**), were deduced by NMR analysis of their hydrolysis products γ-PSOH (**4b**) and α-PCOH (**5b**), respectively, which are released by transformed *E. coli* along with dominating 1*H*-indole. γ-Presodorifenol (γ-PSOH, **4b**) was obtained from *E. coli* expressing the cyclizing *C*-methyltransferase *Pc*-FPP-MT (*Pchl*O6_6045; Figure 2c). ^{1}H NMR spectroscopy revealed signals reminiscent of those of α-presodorifenol (α-PSOH, **2b**), previously identified from the *S. plymuthica* SodS knockout mutant (Table S3),[5] whereas the endocyclic double bond of the pentamethylcyclopentenyl unit in **2b** is shifted to an exocyclic position in **4b**. Inspection of the *dqf*-COSY spectrum showed long-range H,H-coupling between the exocyclic methylene and two allylic methine protons at δ_H = 2.23 and 1.97 ppm, which facilitated the localization of the double bond (Figure S5). Structure assignment was confirmed by H,C-correlations derived from HSQC and HMBC spectra (Figure S5), whereas the relative configuration was deduced from the NOESY spectrum (Figures S7 and S9). α-Prechlororaphenol (α-PCOH, **5b**) was obtained from *E. coli* expressing a *P. chlororaphis* gene cluster, including both SAM-dependent *C*-methyltransferases *Pc*-FPP-MT (*Pchl*O6_6045) and *Pc*-γ-PSPP-MT (*Pchl*O6_6042; along with *Pchl*O6_6044 and *Pchl*O6_6043; Figure 2d). ^{1}H NMR analysis showed signals similar to those of α-presodorifenol (α-PSOH, **2b**) from the *S. plymuthica* SodS knockout mutant (Table S3),[5] whereas one of the olefinic methyl groups is replaced by an ethyl group in **5b**, the position of which was deduced from HSQC, HMBC, and NOESY spectra (Figures S5, S8, and S9).

In conclusion, the combination of genome mining and *in vitro* enzyme assays along with NMR analysis of VOCs released by transformed *E. coli* demonstrated that *C*-methyltransferases from both *S. plymuthica* (*Sp*-FPP-MT) and *P. chlororaphis* (*Pc*-FPP-MT) represent members of a novel class of non-canonical FPP-cyclizing *C*-methyltransferases,[22] which produce isomeric α-PSPP (**2a**) and γ-PSPP (**4a**), respectively. Alignment of the amino acid sequences revealed that the active site of *Sp*-FPP-MT[6] is largely conserved in *Pc*-FPP-MT (Figure S11).

The proposed reaction mechanism (Scheme 1), initiated by SAM-dependent *C*-methylation of FPP (**3a**) at C10, followed by cyclization and ring contraction,[5,6] is identical for α-PSPP (**2a**; from *Sp*-FPP-MT) and γ-PSPP (**4a**; from *Pc*-FPP-MT) but requires one additional 1,2-*H* rearrange-



Scheme 1. Proposed biosynthetic pathway from FPP (**3a**) to chlororaphen (**6**) via γ-presodorifen pyrophosphate (γ-PSPP, **4a**) and prechlororaphen pyrophosphate (α-PCPP, **5a**) as catalyzed by FPP-MT, γ-PSPP-MT, and ChloS.

ment step to furnish the exocyclic double bond of γ-PSPP (**4a**) in agreement with its relative configuration as derived from the NOESY spectrum of γ-PSOH (**4b**). Subsequently, the second SAM-dependent *C*-methylation of γ-PSPP (**4a**) by *Pc*-γ-PSPP-MT involves the exocyclic methylene carbon, resulting in the formation of α-PCPP (**5a**), in which both SAM-derived C_1 units are directly connected to form the ethyl moiety. The monocyclic bishomoterpene precursor α-PCPP (**5a**) represents the substrate for the chlororaphen synthase *Pc*-ChloS, the second example for a bacterial terpene synthase that exclusively accepts non-canonical monocyclic precursors. Cross-enzyme assays of FPP (**3a**) and SAM with *Sp*-FPP-MT and *Pc*-ChloS furnished a yet unidentified C_{16} hydrocarbon (Figure S2), indicating that both α-PSPP (**2a**) and α-PCPP (**5a**) are accepted by *Pc*-ChloS. As expected for terpene cyclases resulting in different products, the amino acid sequence identities between *Pc*-ChloS and *Sp*-SodS are limited to 17.66 % (Figure S12). The second putative TPS within the *P. chlororaphis* O6 gene cluster (*Pchl*O6_6044) is more similar to sodorifen synthase (*Sp*-SodS; 41.96 % identity; Figure S13), but did not produce any cyclization product with FPP (**3a**; or GPP) or α-PSPP (**2a**) upon coupled enzyme assays with *Sp*-FPP-MT, or γ-PSPP (**4a**) and α-PCPP (**5a**) when co-expressed with both *C*-methyltransferases from *P. chlororaphis* O6 (Figure S3), and is considered to be nonfunctional.

Biosynthesis of chlororaphen (**6**) from FPP (**3a**) via γ-PSPP (**4a**) and α-PCPP (**5a**; Scheme 1) was also characterized in the β-proteobacterium *Variovorax boronicumulans* PHE5-4 (Burkholderiales)[23] (Figure 1b, Figure S4). Alignment of the amino acid sequences of the *C*-methyltransferases (FPP-MT and γ-PSPP-MT) and chlororaphen synthases (ChloS) from *P. chlororaphis* and *V. boronicumulans* revealed a large degree of sequence identity in agreement with their common enzymatic functions (Figures S14–S16). Genome mining revealed additional putative non-canonical cyclizing FPP *C*-methyltransferase and terpene cyclase couples in 70 different bacterial genomes, which demonstrates that homosesquiterpene biosynthesis via monocyclic pyrophosphate intermediates is not unique for *S. plymuthica*, but more widespread among bacteria. Additional research will be required to elucidate the structural diversity, distribution, and biological functions of non-canonical homosesquiterpenes in bacteria.

Acknowledgements

This work was funded by the Deutsche Forschungsgemeinschaft (DFG, Pi153/36-1/2). GC-HRMS measurements by Pierre-Marie Allard (University of Fribourg, Switzerland) are gratefully acknowledged as well as technical assistance by Dörte Warber and Claudia Dinse. 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: Brexanes · Homoterpenes · Natural Products · Non-Canonical Pathway · Structure Elucidation

[1] O. Wallach, *Justus Liebigs Ann. Chem.* **1887**, *238*, 78–89.
[2] L. Ruzicka, *Experientia* **1953**, *9*, 357–367.
[3] F. Lynen, H. Eggerer, U. Henning, I. Kessel, *Angew. Chem.* **1958**, *70*, 738–742.
[4] S. H. von Reuß, M. Kai, B. Piechulla, W. Francke, *Angew. Chem. Int. Ed.* **2010**, *49*, 2009–2010.
[5] S. H. von Reuß, D. Domik, M. C. Lemfack, N. Magnus, M. Kai, T. Weise, B. Piechulla, *J. Am. Chem. Soc.* **2018**, *140*, 11855–11862.
[6] 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.
[7] C. Ignea, M. H. Raadam, A. Koutsaviti, Y. Zhao, Y.-T. Duan, M. Harizani, K. Miettinen, P. Georgantea, M. Rosenfeldt, S. E. Viejo-Ledesma, M. A. Petersen, W. L. P. Bredie, D. Staerk, V. Roussis, E. Ioannou, S. C. Kampranis, *Nat. Commun.* **2022**, *13*, 5188.
[8] M. Kai, B. Piechulla, *FEMS Microbiol. Lett.* **2018**, *365*, fny253.
[9] R. Schmidt, V. de Jager, D. Zühlke, C. Wolff, J. Bernhardt, K. Cankar, J. Beekwilder, W. van Ijcken, F. Sleutels, W. de Boer, K. Riedel, P. Garbeva, *Sci. Rep.* **2017**, *7*, 862.
[10] D. Domik, N. Magnus, B. Piechulla, *FEMS Microbiol. Lett.* **2016**, *363*, fnw139.
[11] D. Domik, A. Thürmer, T. Weise, W. Brandt, R. Daniel, B. Piechulla, *Front. Microbiol.* **2016**, *7*, 737.
[12] E. R. Duell, P. M. D'Agostino, N. Shapiro, T. Woyke, T. M. Fuchs, T. A. M. Gulder, *Microb. Cell Fact.* **2019**, *18*, 32.
[13] A. Nickon, H. Kwasnik, T. Swartz, R. O. Williams, J. B. DiGiorgio, *J. Am. Chem. Soc.* **1965**, *87*, 1613–1615.
[14] A. Nickon, R. C. Weglein, *J. Am. Chem. Soc.* **1975**, *97*, 1271–1273.
[15] M. Nakazaki, K. Naemura, H. Kadowaki, *J. Org. Chem.* **1976**, *41*, 3725–3730.
[16] R. S. Bly, R. K. Bly, J. B. Hamilton, J. N. C. Hsu, P. K. Lillis, *J. Am. Chem. Soc.* **1977**, *99*, 216–223.
[17] A. Nickon, H. R. Kwasnik, C. T. Mathew, T. D. Swartz, R. O. Williams, J. B. DiGiorgio, *J. Org. Chem.* **1978**, *43*, 3904–3916.
[18] C. J. Collins, *J. Am. Chem. Soc.* **1979**, *101*, 1878–1880.
[19] A. Nickon, R. C. Weglein, C. T. Mathew, *Can. J. Chem.* **1981**, *59*, 302–313.
[20] A. Nickon, R. C. Weglein, *Tetrahedron Lett.* **1986**, *27*, 2675–2678.
[21] A. Nickon, T. D. Swartz, D. M. Sainsbury, B. R. Toth, *J. Org. Chem.* **1986**, *51*, 3736–3738.
[22] B. Piechulla, C. Zhang, D. Eisenschmidt-Bönn, F. Chen, N. Magnus, *FEMS Microbiol. Rev.* **2021**, *45*, fuab024.
[23] H. Miwa, I. Ahmed, J. Yoon, A. Yokota, T. Fujiwara, *Int. J. Syst. Evol. Microbiol.* **2008**, *58*, 286–289.

Manuscript received: March 13, 2023
Accepted manuscript online: May 3, 2023
Version of record online: June 12, 2023