Aromatic polyketide GTRI-02 is a previously unidentified product of the act gene cluster in Streptomyces coelicolor A3(2)

Wu, C.; Ichinose, K.; Choi, Y.H.; Wezel, G.P. van

ChemPubSoc Europe

DOI: 10.1002/cbic.201700107

CHEMBIOCHEM Full Papers

Aromatic Polyketide GTRI-02 is a Previously Unidentified Product of the *act* Gene Cluster in *Streptomyces coelicolor* A3(2)

Changsheng Wu,[a] Koji Ichinose,[b] Young Hae Choi,[c] and Gilles P. van Wezel*[a]

The biosynthesis of aromatic polyketides derived from type II polyketide synthases (PKSs) is complex, and it is not uncommon that highly similar gene clusters give rise to diverse structural architectures. The *act* biosynthetic gene cluster (BGC) of the model actinomycete *Streptomyces coelicolor* A3(2) is an archetypal type II PKS. Here we show that the *act* BGC also specifies the aromatic polyketide GTRI-02 (**1**) and propose a mechanism for the biogenesis of its 3,4-dihydronaphthalen-1(2*H*)-one backbone. Polyketide **1** was also produced by *Streptomyces* sp. MBT76 after activation of the *act*-like *qin* gene cluster by overexpression of the pathway-specific activator. Mining of this strain also identified dehydroxy-GTRI-02 (**2**), which most likely originated from dehydration of **1** during the isolation process. This work shows that even extensively studied model gene clusters such as *act* of *S. coelicolor* can still produce new chemistry, offering new perspectives for drug discovery.

Introduction

Polyketides are widespread in bacteria, fungi, and plants and represent a vast chemical diversity of natural products.[1,2] The unparalleled biological significance and commercial value of polyketides have triggered immense endeavors to uncover their underlying biosynthetic logic, because total chemical synthesis of the extraordinary architectures of polyketides is challenging. Despite their tremendous structural diversity, polyketides are universally assembled through successive Claisen decarboxylative condensation of simple building blocks such as acetate and propionate, catalyzed by polyketide synthases (PKSs).[3]

Three major types of PKS are known to date; of these, type II PKSs are mostly involved in the biosynthesis of aromatic polyketides. The hallmark for type II PKS gene clusters is a ketosynthase heterodimer (KS_α and KS_β) catalyzing iterative Claisen decarboxylative condensation between thioesters and malonyl-CoA units, together with an acyl carrier protein (ACP) that serves as an anchor for the nascent polyketide chain. Additional PKS subunits, including ketoreductases, cyclases, and aromatases, define the folding pattern of the poly-β-keto intermediate to form a specific aromatic backbone that can be further tailored by, among other enzymes, oxygenases and glycosyl- and methyltransferases.[4] The biosynthetic machinery of type II PKSs is difficult to study,[4] because 1) production of aromatic polyketides is carried out by enzyme complexes, 2) the determinants for the extension cycles are puzzling, and so the chain lengths of poly-β-keto intermediates are difficult to predict,[5,6] 3) poly-β-keto intermediates are highly unstable and prone to spontaneous cyclization, and 4) the initial backbones of aromatic compounds can be changed substantially in post-PKS tailoring steps. Consequently, it is not uncommon that a single type II PKS gene cluster gives rise to diverse chemical skeletons.[7]

Actinomycetes are mycelial Gram-positive bacteria with a complex multicellular lifestyle that culminates in sporulation.[8,9] These bacteria produce a plethora of natural products, including antibiotic, antifungal, and anticancer compounds.[10,11] Their biosynthetic potential is far from exhausted, despite problems with chemical redundancy and the consequent poor return of investment of high-throughput screening campaigns.[12–14]

Streptomyces coelicolor A3(2) is a model actinomycete, and has been investigated for more than five decades to study the genetic and biochemical basis for the production of bioactive metabolites.[15] Prior to the publication of its genome sequence, *S. coelicolor* had been reported to produce a broad spectrum of secondary metabolites, such as actinorhodin, hopanoids, prodiginines, and nonribosomal peptides. In 2002, though, its genome sequence revealed a far greater capacity to produce natural products than seen from classic bioactivity screens.[16] Genome mining of other model organisms again shed light on many silent gene clusters.[17–19] Bioinformatics algorithms have therefore been developed for the purposes of mining the vast genome sequence information efficiently and allowing the prediction of the chemical output,[2,20] whereas

[a] *Dr. C. Wu, Prof. Dr. G. P. van Wezel*
Molecular Biotechnology, Institute of Biology, Leiden University
Sylviusweg 72, 2333 BE Leiden (The Netherlands)
E-mail: g.wezel@biology.leidenuniv.nl

[b] *Prof. Dr. K. Ichinose*
Research Institute of Pharmaceutical Sciences, Musashino University
Shinmachi, Nishitokyo-shi, Tokyo 202–8585 (Japan)

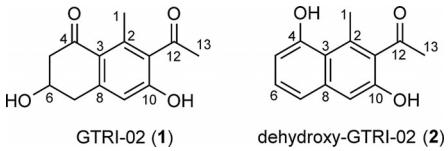
[c] *Dr. Y. H. Choi*
Natural Products Laboratory, Institute of Biology, Leiden University
Sylviusweg 72, 2333 BE Leiden (The Netherlands)

Supporting information and the ORCID identification numbers for the authors of this article can be found under https://doi.org/10.1002/cbic.201700107.

technologies to activate the expression of silent biosynthetic gene clusters (BGCs) have been developed.[21–23]

Actinobacteria live in close association with other bacteria, as well as with fungi, nematodes, plants, and insects, and harnessing such interactions for the activation of BGCs is therefore an attractive strategy.[24–26] Indeed, microbial co-cultivations efficiently elicit the biosynthesis of previously unknown secondary metabolites, thus expanding the pre-existing chemical diversity.[27–29] The availability of genomic information has greatly accelerated progress in understanding the divergent biosynthetic logic of specific metabolites in *S. coelicolor*, including two model type II PKS gene clusters—*whiE* and *act*—for the production of the spore pigment and actinorhodin, respectively.[16] The ectopic expression of the minimal *whiE* in a recombinant *Streptomyces* strain generated a library of aromatic polyketides of varying chain lengths and cyclization patterns,[30] whereas the expression of subsets of the *act* genes was reported to synthesize disparate aryl scaffolds, such as pyranonaphthoquinone,[31,32] anthraquinones,[33,34] naphthoquinones,[35,36] and mutactin.[37]

In this study we report that the *act* BGC specifies the production of the aromatic polyketide GTRI-02 (**1**, Scheme 1), a molecule that had not previously been characterized in *S. coelicolor*. Moreover, the previously undescribed variant dehydroxy-GTRI-02 (**2**) was isolated from an ex-conjugant of *Streptomyces* sp. MBT76.



Scheme 1. Structures of GTRI-02 (**1**) and dehydroxy-GTRI-02 (**2**).

Results and Discussion

An NMR-based metabolomics study performed in our laboratory demonstrated that co-cultivation of the filamentous model microbes *S. coelicolor* A3(2) and *Aspergillus niger* N402 has a substantial impact on their mutual metabolism.[38] In response to challenge with *S. coelicolor*, *A. niger* shut down the production of the γ-pyrone derivatives carbonarone A and aurasperone B, but instead switched on phenylalanine metabolism, resulting in the production of cyclo(Phe-Phe), hydroxyacetic acid, and phenylacetic acid.[38]

Scrutiny of the ^{1}H NMR spectra (Figure 1) of three experimental groups—namely, *A. niger* monoculture, *S. coelicolor* monoculture, and a co-culture of *A. niger* with *S. coelicolor*—resulted in the detection of two low-abundance singlets at $\delta =$ 2.45 and 2.42 ppm that were exclusively present in the spectrum of the co-culture, but not in those of the monocultures. 2D NMR examination of co-culture crude extracts confirmed that these two singlets belong to the same compound. NMR-guided chromatographic fractionation was carried out to isolate the desired components.[39] Fractions showing the target signals ($\delta = 2.45$ and 2.42 ppm) were combined and further

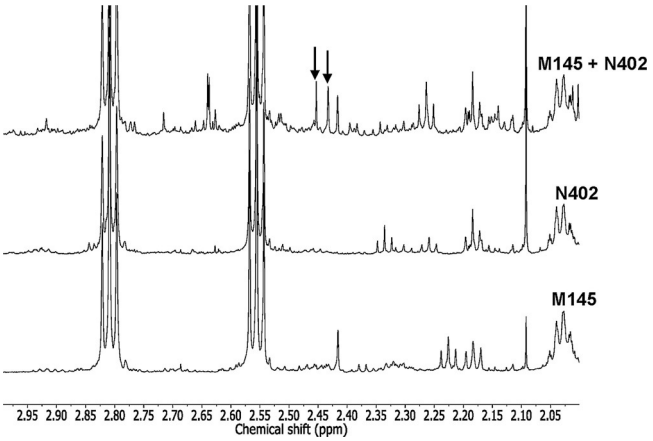


Figure 1. Comparison of ^{1}H NMR spectra of extracts obtained from *S. coelicolor* M145, *A. niger* N402, and their co-culture. Chemical shifts are shown for the non-oxygenated aliphatic region $\delta = 2.0$–3.0 ppm. Two singlets at $\delta = 2.45$ and 2.42 ppm highlighted by arrows were present in the spectrum from the co-culture group, but not in those from the two single cultures. M145: *S. coelicolor* M145 single culture. N402: *A. niger* N402 single culture. M145 + N402: co-culture of *S. coelicolor* M145 with *A. niger* N402.

analyzed by 2D NMR, including by COSY, ^{1}*J*-resolved, HSQC, and HMBC (Figures S1–S5 in the Supporting Information). This allowed the identification of the compound GTRI-02 (**1**, Scheme 1 and Table 1), which was further verified by a HRMS ion peak at *m/z* 235.0961 $[M+H]^+$ (calcd for $C_{13}H_{15}O_4$: 235.0965; Figure S6). This compound had never previously been characterized in cultures of either strain.

We then wondered which microbe was responsible for the production of GTRI-02 (**1**), or whether the molecule was the result of co-synthesis. The biosynthetic pathway for **1** is unknown, but its structure strongly suggests a polyketide bioassembly line. Actinomycete origin was suggested by the isolation of **1** from the soil actinomycete *Micromonospora* sp. SA246,[40] and more recently by recharacterization in *Streptomy-*

Table 1. ^{1}H and ^{13}C NMR data for GTRI-02 and dehydroxy-GTRI-02.[a]

	GTRI-02		Dehydroxy-GTRI-02	
	δC, type	δH (*J* [Hz])	δC, type	δH (*J* [Hz])
1	31.1, CH_3	2.41 (s)	19.0, CH_3	2.72 (s)
2	138.9, C		131.9, C	
3	123.4, C		118.7, C	
4	197.8, C		156.4, C	
5	48.7, CH_2	2.85 (dd, $J = 16.2$, 3.6 Hz) 2.60 (dd, $J = 16.2$, 7.2 Hz)	107.3, CH	6.58 (d, $J = 7.8$, 1.2 Hz)
6	65.4, CH	4.24 (m)	126.6, CH	7.11 (t, $J = 7.8$ Hz)
7	39.1, CH_2	3.15 (dd, $J = 16.2$, 3.6 Hz) 2.91 (dd, $J = 16.2$, 7.2 Hz)	117.7, CH	7.03 (d, $J = 7.8$, 1.2 Hz)
8	145.6, C		137.6, C	
9	113.2, CH	6.63 (s)	107.1, CH	6.87 (s)
10	157.4, C		150.4, C	
11	130.8, C		132.1, C	
12	206.9, C		208.9, C	
13	17.7, CH_3	2.45 (s)	31.4, CH_3	2.52 (s)

[a] Recorded in CD_3OD. All chemical shift assignments were made on the basis of 1D and 2D NMR. The ^{1}H and ^{13}C NMR data for GTRI-02 are consistent with previously reported data.[40]

ces strain GW4184[41] and *Streptomyces* sp. ANK313,[42] whereas no fungal origin has been reported for GTRI-02. To test this, additional *Aspergillus*/*Streptomyces* co-cultivations were performed. *S. coelicolor* M145 was inoculated into submerged Minimal Medium (NMMP) cultures at pH 5.[38] After 72 h growth, spores from *A. niger* N402 were added, and co-cultivation was allowed to proceed for 72 h. The control experiments were M145 and N402 monocultures, corresponding to the cultivation time of each strain in the co-culture. The cultures were harvested by centrifugation and extracted with ethyl acetate (EtOAc). HPLC-diode array detection (DAD) analysis of the resulting extracts indicated the existence of **1** both in the co-culture and in the *S. coelicolor* M145 monoculture (Figure 2A). No product was seen in the *A. niger* single culture, thus strongly suggesting that **1** was produced by *S. coelicolor*. The production of **1** by *S. coelicolor* was further confirmed by UHPLC-DAD-ToF-MS analysis (Figure 2B). However, it should be noted that the yield of **1** was significantly lower than in the previous co-cultivation experiment, as quantified by ^{1}H NMR (data not shown). The poor reproducibility of the co-culture experiments might have been caused by major variations in the metabolome of *A. niger* between the different experiments. For instance, the major metabolite produced in previous *A. niger* monoculture was carbonarone A,[38] but here it predominantly synthesized TAN-1612, a premithramycinone-type polyketide,[43] under the same culture conditions (Figures S7, S8).

The observation that *S. coelicolor* produces GTRI-02 prompted further analysis of the genetic basis for its production. Interestingly, all actinomycetes that have been shown to produce GTRI-02 also produce anthraquinones, naphthoquinones, and/or pyranonaphthoquinones.[40–42,44–46] This suggested that in *S. coelicolor*, **1** might be a previously unidentified product of actinorhodin biosynthesis, similar to anthraquinones,[33,34] naphthoquinones,[35,36] and mutactin,[37] which also originate from the *act* BGC. To test this hypothesis, we analyzed the metabolome of *S. coelicolor* M1141, an *act*-BGC null mutant of *S. coelicolor* M145.[47] Although GTRI-02 was detected in *S. coelicolor* M145, both in monoculture and in co-culture with *A. niger*, the production of **1** could not be detected in its *act* null mutant M1141, grown either in monoculture or in co-culture (Figure 2). Further support for the notion that **1** originates from an *act*-like type II PKS was obtained from our investigations into *Streptomyces* sp. MBT76.[48] The *qin* BGC of *Streptomyces* sp. MBT76 shows high homology to the *act* BGC in the central type II PKS genes. Overexpression of the *qin* BGC was achieved by placing its pathway-specific regulatory gene *qin*ORF11 behind the strong and constitutive *ermE* promoter. Metabolic profiling in tandem with chromatographic isolation confirmed the production of **1** in the resultant ex-conjugant MBT76-1, but not in the parent (wild-type) strain. It is noteworthy that the titer of GTRI-02 was higher than that of the glycosylated pyranonaphthoquinones (qinimycins), whereas the production of nonglycosylated pyranonaphthoquinones and anthraquinones was below the detection limit of HPLC-DAD (Figure 3).[48] This suggests that under the given growth conditions, GTRI-02 is a major product of the *qin* gene cluster.

In this strain, a minor compound co-existed with **1** in a 1:9 ratio, as judged by either ^{1}H NMR (Figure S9) or HPLC-DAD analysis (Figure 3). 2D NMR (HMBC and HSQC, Figures S10, S11) measurements and HRMS in positive mode (Figures S12, S13) demonstrated that this compound was a dehydration product of **1** at C-6, giving the previously unknown polyketide dehydroxy-GTRI-02 (**2**, Table 1 and Figure S14). Similar dehydration of the 3,4-dihydronaphthalen-1(2*H*)-one motif has also been seen for the conversion of mutactin into dehydromutactin.[37] However, compound **2** was most likely an artifact of **1** due to the use of TFA during isolation, because **2** was not detected by UHPLC-DAD-ToF-MS in crude extracts obtained

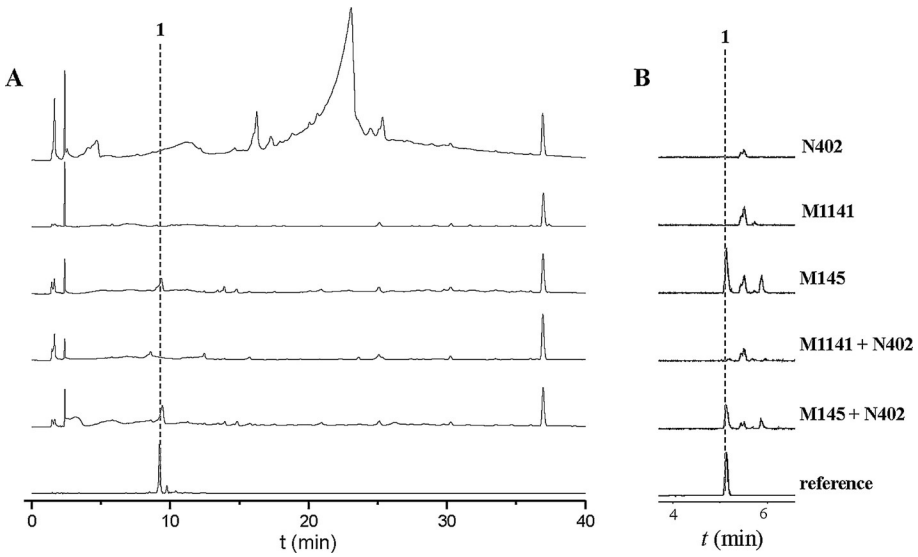


Figure 2. GTRI-02 (**1**) production by *S. coelicolor* M145 and its *act* null mutant M1141. A) HPLC-DAD analysis (detected at 280 nm) showed that **1** was produced by *S. coelicolor* M145 alone or in co-culture with *A. niger*, but not by *S. coelicolor* M1141 (neither alone nor in co-culture). The peak corresponding to **1** is highlighted by the dashed line. B) Extracted ion chromatography (*m/z* 235) of UHPLC-DAD-ToF-MS analysis further confirmed GTRI-02 production by *S. coelicolor* M145. The dashed line highlights the ion peak at *m/z* 235.0956 $[M+H]^+$ (calcd for $C_{13}H_{15}O_4$: 235.0965) for **1**. N402: *A. niger* N402. M145: *S. coelicolor* M145. M1141: *S. coelicolor* M1141 (which lacks the *act* BGC). Chromatographically purified GTRI-02 from *Streptomyces* sp. MBT76-1 was used as reference.

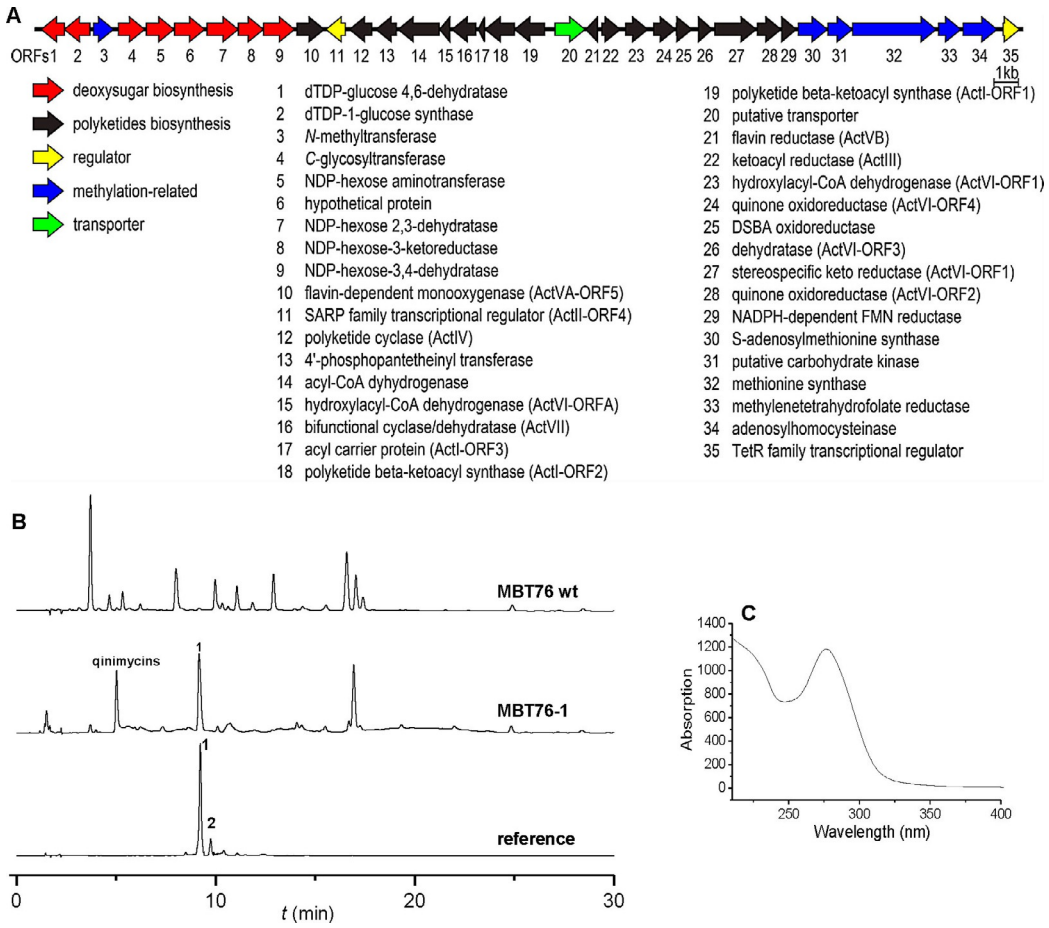


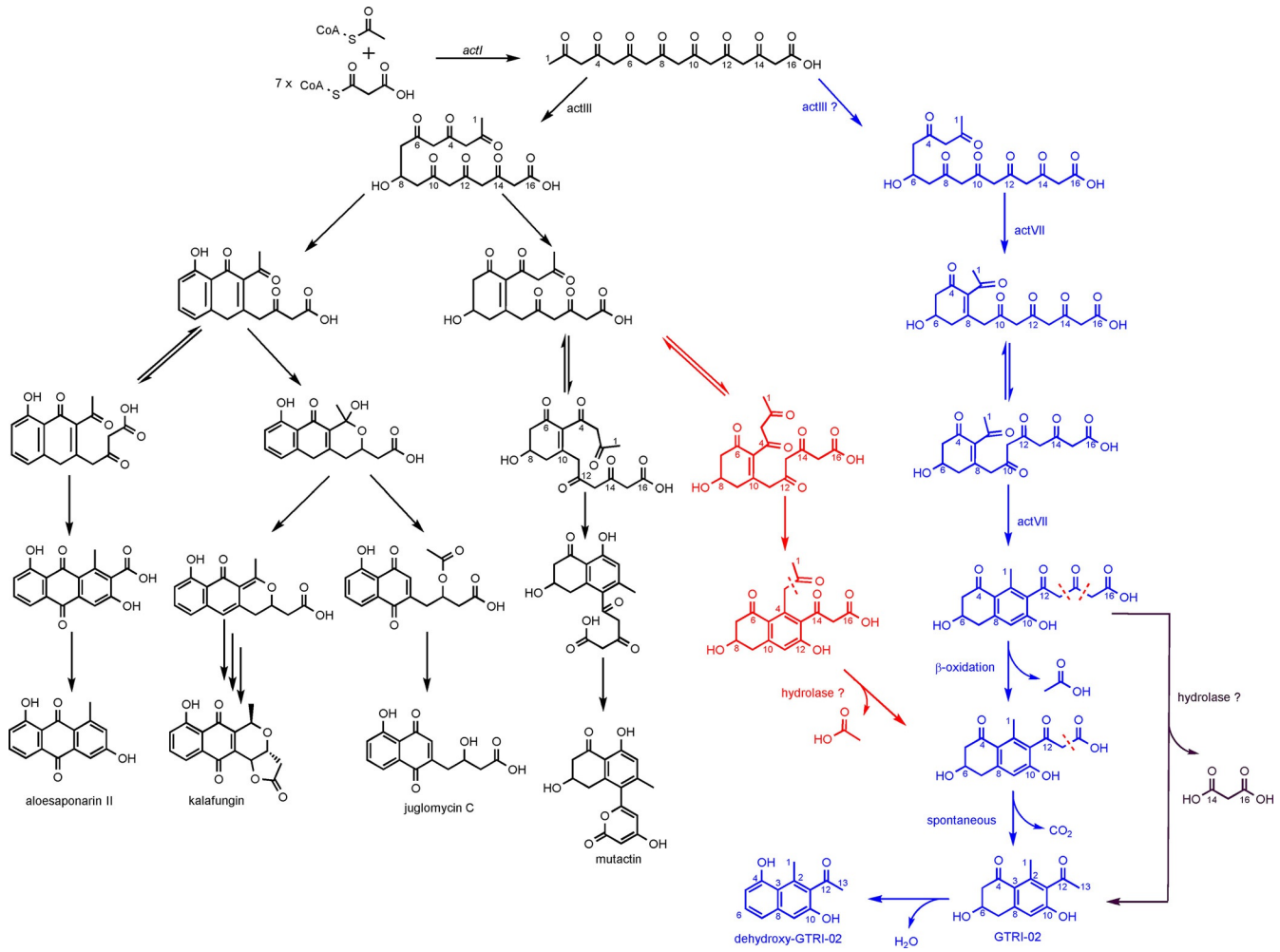
Figure 3. Production of GTRI-02 (**1**) by *Streptomyces* sp. MBT76-1. A) Type II PKS gene cluster (*qin*) was activated in *Streptomyces* sp. MBT76 through constitutive expression of the pathway-specific activator gene *qin*-ORF11.[48] Where relevant, homologous enzymes encoded by the *act* cluster are given in brackets. B) HPLC-DAD (detection at 280 nm) comparison of *qin*-overexpression ex-conjugant MBT76-1 with the parent strain harboring empty plasmid. The chromatographically isolated mixture containing **1** and **2** from *Streptomyces* sp. MBT76-1 was used as the reference. C) UV spectrum of compound **1**. The peak detected at 9.23 min in the chromatogram of MBT76-1 has the same UV spectrum as compound **1**.

from either the co-culture or the ex-conjugant of MBT76-1. In a previous study it was demonstrated that GTRI-02 acts as a lipid peroxidation inhibitor.[40] In line with this, neither **1** or **2** showed antimicrobial activity against *Bacillus subtilis* 168, *Escherichia coli* K12, *Pseudomonas aeruginosa* PAO1, *A. niger* N402, or *S. coelicolor* M145, as shown by a disc agar diffusion assay (Figure S15).

Plausible biosynthetic pathways for **1** are presented in Scheme 2. The minimal PKS catalyzes the iterative decarboxylative condensation of seven malonyl-CoA extenders with an acyl-CoA starter to form a nascent linear octaketide chain. This highly reactive poly-β-keto intermediate can undergo nonenzymatic spontaneous aldol reactions or enzymatic cyclization. The standard U-shaped folding at C-7/C-8 is known to give rise to the structural skeleton of anthraquinones (i.e., aloesaponarin II),[33,34] naphthoquinones (juglomycin C),[35,36] and mutactin,[37] whereby ActIII is responsible for the ketoreduction at C-8 (numbered from the methyl terminus). For GTRI-02 assembly, it is postulated that an irregular folding pattern at C-5/C-6 occurs in the octaketide chain and that the C-6 ketone is instead reduced by ActIII into the corresponding secondary alcohol. Two aldol reactions at C-2/C-11 and C-3/C-8 then close the bicyclic system to form a 3,4-dihydronaphthalen-1(2*H*)-one backbone, whereby the aromatase/cyclase ActVII might be involved in dehydration. The acetyl substituent in **1** could be generated either by β-oxidation of the C_2 acetate in tandem with spontaneous release of CO_2,[49] or by direct cleavage at C-13/C-14 to release the C_3 malonate. An alternative possible biosynthetic pathway of **1** is to follow the initial reaction steps of mutactin biosynthesis. The aldol reaction at C-4/C-13 instead of C-2/C-11 (which would give rise to mutactin) produces the 3,4-dihydronaphthalen-1(2*H*)-one intermediate, which then further undergoes shortening of the C_2 unit and spontaneous decarboxylation to generate **1**.

Conclusion

The *act* BGC of *S. coelicolor* serves as a model for type II PKSs. The discovery of the polyketide GTRI-02 (**1**), with a 3,4-dihydronaphthalen-1(2*H*)-one backbone, in *S. coelicolor* surprisingly unveiled additional chemistry based on the *act* BGC. This provides new insights into the versatile folding mode and/or modification of the nascent polyketide chains derived from type II PKSs. The divergent biosynthesis in which a single gene



Scheme 2. Proposed biosynthetic pathway for GTRI-02. GTRI-02 is a previously undetected side product of the *act* and *qin*[48] BGCs. So far, these BGCs have mainly been known for the production of the pyranonaphthoquinones kalafungin and actinorhodin. Besides GTRI-02, anthraquinones (i.e., aloesaponarin II),[33,34] naphthoquinones (juglomycin C),[35,36] and mutactin[37] are known side products of the *act* BGC. The alternative pathways for GTRI-02 biosynthesis are presented in red, blue, and brown, respectively.

cluster orchestrates architecturally disparate skeletons might be more common than previously thought. In view of the importance of secondary metabolites in nature, such divergent biosynthesis likely offers an evolutionary advantage to microorganisms, allowing them to modify their biosynthetic arsenal depending on the environmental challenges they face.

Experimental Section

General experimental procedures: NMR spectra were recorded in CD_3OD with a Bruker 600 MHz instrument calibrated to the residual CD_3OD (3.30 ppm) signal. The UHPLC-DAD-ToF-MS analyses were performed with an UltiMate 3000 UHPLC system (Thermo Fisher Scientific) coupled to a micro-ToF-2Q mass spectrometer (Bruker Daltonics) with an electrospray (ESI) interface.[50] HPLC analysis was performed with an Agilent 1200 series HPLC apparatus (Agilent) and use of a 150×4.6 mm Luna 5 µm C_{18} (2) 100 Å column equipped with a guard column containing C_{18} 4×3 mm cartridges (Phenomenex). Semipreparative HPLC separation was performed with a reversed-phase column [Phenomenex Luna 5 µm C_{18} (2) 100 Å column, 250×10 mm]. Silica gel 60 F_{254} (Merck) was used for TLC analysis, with elution with $CHCl_3/CH_3OH$ (10:1) and visualization with anisaldehyde/sulfuric acid reagent. All organic solvents and chemicals were of analytical or HPLC grade, depending on the experiment.

Bacterial strains and culturing conditions: *S. coelicolor* A3(2) M145 was obtained from the John Innes Centre (Norwich, UK) strain collection, and *Streptomyces* sp. MBT76, *B. subtilis* 168, *E. coli* K12, *P. aeruginosa* PAO1 and *A. niger* N402 from the Leiden University strain collection. *A. niger* N402 (*cspA1*) is a derivative of *A. niger* ATCC 9029. Co-cultivation of *A. niger* N402 with *S. coelicolor* M145 or its *act* null mutant M1141[47] was carried out by our previously published method.[38] *Streptomyces* sp. MBT76-1, which overexpresses the *qin* biosynthetic gene cluster, was described in ref. [48]. *Streptomyces* strains were cultured as described[51] without PEG 6000 but in the presence of glycerol (1%, *w/v*) and mannitol (0.5%, *w/v*) as the carbon sources, further supplemented with Bacto peptone (0.8%, *w/v*).

Metabolic profiling: The metabolites in the culture broths were extracted with ethyl acetate (EtOAc). Metabolite analysis, including HPLC-DAD, UHPLC-DAD-ToF-MS, and ^1H NMR measurement, were performed as previously described.[38,48]

Purification of GTRI-02 and dehydroxy-GTRI-02: *Streptomyces* sp. MBT76-1 was grown in modified NMMP (50 mL) with glycerol (1%, *w/v*) and mannitol (0.5%, *w/v*) as the carbon sources, further supplemented with bacto peptone (0.8%, *w/v*); 15 replicates of cultures (750 mL in total) were pooled and further subjected to EtOAc extraction. The resulting crude extract (0.26 g) was partitioned between methanol and *n*-hexane to remove the lipids. The resolved methanol fraction was subsequently separated by semipreparative reversed-phase HPLC [Phenomenex Luna 5 μm C_{18} (2) 100 Å column, 250×10 mm] with an Agilent 1200 series HPLC (Agilent), eluted with a gradient of MeCN in H_2O adjusted with TFA (0.1%) from 20 to 60% at a flow rate of 2 mL min^{-1} over 40 min; 16 fractions (Fr1–Fr16), numbered in ascending order of retention time, were manually collected by peak detection at 254 nm. After rotary evaporation at 42 °C under vacuum, these 16 factions were further subjected to ^{1}H NMR profiling and UHPLC-DAD-ToF-MS analysis. GTRI-02 (**1**) and dehydroxy-GTRI-02 (**2**) were contained as a mixture in Fr8 (2.5 mg) of HPLC-DAD isolation, at retention time 15.51 min. In UHPLC-DAD-ToF-MS analysis, compounds **1** and **2** were separable at retention times 5.72 min and 8.43 min, respectively (Figures S6 and S7).

Acknowledgements

C.S.W. was supported by a grant from the China Scholarship Council.

Conflict of Interest

The authors declare no conflict of interest.

Keywords: antibiotics · biosynthesis · NMR profiling · polyketides · type II PKSs

[1] C. Hertweck, *Angew. Chem. Int. Ed.* **2009**, *48*, 4688 – 4716; *Angew. Chem.* **2009**, *121*, 4782 – 4811.
[2] M. H. Medema, R. Kottmann, P. Yilmaz, M. Cummings, J. B. Biggins, K. Blin, I. de Bruijn, Y. H. Chooi, J. Claesen, R. C. Coates, P. Cruz-Morales, S. Duddela, S. Düsterhus, D. J Edwards, D. P Fewer, N. Garg, C. Geiger, J. P. Gomez-Escribano, A. Greule, M. Hadjithomas, et al., *Nat. Chem. Biol.* **2015**, *11*, 625 – 631.
[3] J. Staunton, K. J. Weissman, *Nat. Prod. Rep.* **2001**, *18*, 380 – 416.
[4] C. Hertweck, A. Luzhetskyy, Y. Rebets, A. Bechthold, *Nat. Prod. Rep.* **2007**, *24*, 162 – 190.
[5] Y. Tang, S. C. Tsai, C. Khosla, *J. Am. Chem. Soc.* **2003**, *125*, 12708 – 12709.
[6] P. H. Szu, S. Govindarajan, M. J. Meehan, A. Das, D. D. Nguyen, P. C. Dorrestein, J. Minshull, C. Khosla, *Chem. Biol.* **2011**, *18*, 1021 – 1031.
[7] H. Zhou, Y. Li, Y. Tang, *Nat. Prod. Rep.* **2010**, *27*, 839 – 868.
[8] D. Claessen, D. E. Rozen, O. P. Kuipers, L. Søgaard-Andersen, G. P. van Wezel, *Nat. Rev. Microbiol.* **2014**, *12*, 115 – 124.
[9] K. Flärdh, M. J. Buttner, *Nat. Rev. Microbiol.* **2009**, *7*, 36 – 49.
[10] D. A. Hopwood, *Streptomyces in Nature and Medicine: The Antibiotic Makers*, Oxford University Press, New York, **2007**.
[11] E. A. Barka, P. Vatsa, L. Sanchez, N. Gaveau-Vaillant, C. Jacquard, H. Klenk, C. Clément, Y. Ouhdouch, G. P. van Wezel, *Microbiol. Mol. Biol. Rev.* **2016**, *80*, 1 – 43.
[12] M. A. Cooper, D. Shlaes, *Nature* **2011**, *472*, 32.
[13] R. Kolter, G. P. van Wezel, *Nat. Microbiol.* **2016**, *1*, 15020.
[14] D. J. Payne, M. N. Gwynn, D. J. Holmes, D. L. Pompliano, *Nat. Rev. Drug Discovery* **2007**, *6*, 29 – 40.
[15] G. L. Challis, *J. Ind. Microbiol. Biotechnol.* **2014**, *41*, 219 – 232.
[16] S. D. Bentley, K. F. Chater, A.-M. Cerdeño-Tárraga, G. L. Challis, N. R. Thomson, K. D. James, D. E. Harris, M. A. Quail, H. Kieser, D. Harper, A. Bateman, S. Brown, G. Chandra, C. W. Chen, M. Collins, A. Cronin, A. Fraser, A. Goble, J. Hidalgo, T. Hornsby, et al., *Nature* **2002**, *417*, 141 – 147.
[17] P. Cruz-Morales, E. Vijgenboom, F. Iruegas-Bocardo, G. Girard, L. A. Yáñez-Guerra, H. E. Ramos-Aboites, J.-L. Pernodet, J. Anné, G. P. van Wezel, F. Barona-Gómez, *Genome Biol. Evol.* **2013**, *5*, 1165 – 1175.
[18] H. Ikeda, J. Ishikawa, A. Hanamoto, M. Shinose, H. Kikuchi, T. Shiba, Y. Sakaki, M. Hattori, S. Omura, *Nat. Biotechnol.* **2003**, *21*, 526 – 531.
[19] Y. Ohnishi, J. Ishikawa, H. Hara, H. Suzuki, M. Ikenoya, H. Ikeda, A. Yamashita, M. Hattori, S. Horinouchi, *J. Bacteriol.* **2008**, *190*, 4050 – 4060.
[20] N. D. Fedorova, V. Moktali, M. H. Medema, *Methods Mol. Biol.* **2012**, 23 – 45.
[21] P. J. Rutledge, G. L. Challis, *Nat. Rev. Microbiol.* **2015**, *13*, 509 – 523.
[22] H. Zhu, S. K. Sandiford, G. P. van Wezel, *J. Ind. Microbiol. Biotechnol.* **2014**, *41*, 371 – 386.
[23] J. S. Zarins-Tutt, T. T. Barberi, H. Gao, A. Mearns-Spragg, L. Zhang, D. J. Newman, R. J. M. Goss, *Nat. Prod. Rep.* **2016**, *33*, 54 – 72.
[24] R. F. Seipke, M. Kaltenpoth, M. I. Hutchings, *FEMS Microbiol. Rev.* **2012**, *36*, 862 – 876.
[25] E. A. Shank, R. Kolter, *Curr. Opin. Microbiol.* **2009**, *12*, 205 – 214.
[26] A. van der Meij, S. F. Worsley, M. I. Hutchings, G. P. van Wezel, *FEMS Microbiol. Rev.* **2017**, *41*, 392 – 416.
[27] M. F. Traxler, J. D. Watrous, T. Alexandrov, P. C. Dorrestein, R. Kolter, *MBio* **2013**, *4*, 1 – 12.
[28] J. Watrous, P. Roach, T. Alexandrov, B. S. Heath, J. Y. Yang, R. D. Kersten, M. van der Voort, K. Pogliano, H. Gross, J. M. Raaijmakers, B. S. Moore, J. Laskin, N. Bandeira, P. C. Dorrestein *Proc. Natl. Acad. Sci. USA* **2012**, *109*, E1743 – E1752.
[29] J. Pérez, J. Munoz-Dorado, A. F. Brana, L. J. Shimkets, L. Sevillano, R. I. Santamaría, *J. Microb. Biotechnol.* **2011**, *4*, 175 – 183.
[30] Y. Shen, P. Yoon, T. W. Yu, H. G. Floss, D. Hopwood, B. S. Moore, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 3622 – 3627.
[31] S. Okamoto, T. Taguchi, K. Ochi, K. Ichinose, *Chem. Biol.* **2009**, *16*, 226 – 236.
[32] M. Metsä-Ketelä, T. Oja, T. Taguchi, S. Okamoto, K. Ichinose, *Curr. Opin. Chem. Biol.* **2013**, *17*, 562 – 570.
[33] T. Taguchi, K. Itou, Y. Ebizuka, F. Malpartida, D. A. Hopwood, C. M. Surti, K. I. Booker-Milburn, G. R. Stephenson, K. Ichinose, *J. Antibiot.* **2000**, *53*, 144 – 152.
[34] P. L. Bartel, C. B. Zhu, J. S. Lampel, D. C. Dosch, N. C. Connors, W. R. Strohl, J. M. Beale, H. G. Floss, *J. Bacteriol.* **1990**, *172*, 4816 – 4826.
[35] J. Gubbens, H. Zhu, G. Girard, L. Song, B. I. Florea, P. Aston, K. Ichinose, D. V. Filippov, Y. H. Choi, H. S. Overkleeft, G. L. Challis, G. P. van Wezel, *Chem. Biol.* **2014**, *21*, 707 – 718.
[36] T. Taguchi, Y. Ebizuka, D. A. Hopwood, K. Ichinose, *Tetrahedron Lett.* **2000**, *41*, 5253 – 5256.
[37] R. McDaniel, S. Ebert-Khosla, H. Fu, D. A. Hopwood, C. Khosla, *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 11542 – 11546.
[38] C. Wu, B. Zacchetti, A. F. J. Ram, G. P. van Wezel, D. Claessen, Y. H. Choi, *Sci. Rep.* **2015**, *5*, 10868.
[39] C. Wu, C. Du, J. Gubbens, Y. H. Choi, G. P. van Wezel, *J. Nat. Prod.* **2015**, *78*, 2355 – 2363.
[40] W. Yeo, B.-S. Yun, S.-S. Kim, E.-K. Park, Y.-H. Kim, I.-D. Yoo, S.-H. Yu, *J. Antibiot.* **1998**, *51*, 952 – 953.
[41] R. P. Maskey, S. Fotso, H. Lessmann, H. Lackner, H. Laatsch, *Z. Naturforsch. B* **2005**, *60*, 183 – 188.
[42] M. A. Abdalla, H. Y. Win, M. T. Islam, A. von Tiedemann, A. Schüffler, H. Laatsch, *J. Antibiot.* **2011**, *64*, 655 – 659.
[43] Y.-Z. Shu, J. Q. Cutrone, S. E. Klohr, S. Huang, *J. Antibiot.* **1995**, *48*, 1060 – 1065.
[44] W.-H. Yeo, O.-K. Lee, B.-S. Yun, J.-S. Woo, Y.-K. Kim, E.-K. Park, S.-S. Kim, Y.-H. Kim, S.-K. Kim, I.-D. Yoo, K.-S. Whang, S.-H. Yu, *J. Antibiot.* **1998**, *51*, 82 – 84.
[45] W. H. Yeo, B. S. Yun, N. I. Back, Y. H. Kim, S. S. Kim, E. K. Park, K. S. Whang, S. H. Yu, *J. Antibiot.* **1997**, *50*, 546 – 550.
[46] W. H. Yeo, B. S. Yun, Y. S. Kim, S. H. Yu, H. M. Kim, I. D. Yoo, Y. H. Kim, *J. Antibiot.* **2002**, *55*, 511 – 515.
[47] J. P. Gomez-Escribano, M. J. Bibb, *J. Microb. Biotechnol.* **2011**, *4*, 207 – 215.
[48] C. Wu, C. Du, K. Ichinose, Y. H. Choi, G. P. van Wezel, *J. Nat. Prod.* **2017**, *80*, 269 – 277.

[49] P. Le Maréchal, P. Decottignies, C. H. Marchand, J. Degrouard, D. Jaillard, T. Dulermo, M. Froissard, A. Smirnov, V. Chapuis, M. J. Virolle, *Appl. Environ. Microbiol.* **2013**, *79*, 5907 – 5917.

[50] N. Li, C.-L. Kuo, G. Paniagua, H. van den Elst, M. Verdoes, L. I. Willems, W. A. van der Linden, M. Ruben, E. van Genderen, J. Gubbens, G. P. van Wezel, H. S. Overkleeft, B. I. Florea, *Nat. Protoc.* **2013**, *8*, 1155 – 1168.

[51] H. D. Kieser T, Bibb M, Buttner M, Chater K, *Practical Streptomyces Genetics*, John Innes Foundation, Norwich, **2000**.

Manuscript received: February 28, 2017
Accepted manuscript online: April 30, 2017
Version of record online: June 7, 2017