

Chemical Science

Accepted Manuscript

This article can be cited before page numbers have been issued, to do this please use: H. Heinemann, N. Gerlach, E. Kuhnert, J. Gerke, B. Verwaaijen, J. Kalinowski, M. Stadler and R. Cox, *Chem. Sci.*, 2026, DOI: 10.1039/D6SC00660D.



This is an Accepted Manuscript, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this Accepted Manuscript with the edited and formatted Advance Article as soon as it is available.

You can find more information about Accepted Manuscripts in the [Information for Authors](#).

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard [Terms & Conditions](#) and the [Ethical guidelines](#) still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this Accepted Manuscript or any consequences arising from the use of any information it contains.

Total and Engineered Biosynthesis of Cinatrin: Inhibitors of Phospholipase A₂ and Squalene Synthase

Article Online
DOI: 10.1039/D6SC00660D

Received 00th January 20XX,
Accepted 00th January 20XX

Henrike Heinemann,^a Nick Gerlach,^a Eric Kuhnert,^a Jennifer Gerke,^a Bart Verwaaijen,^b Jörn Kalinowski,^b Marc Stadler^c and Russell J. Cox^{a*}

DOI: 10.1039/x0XX00000x

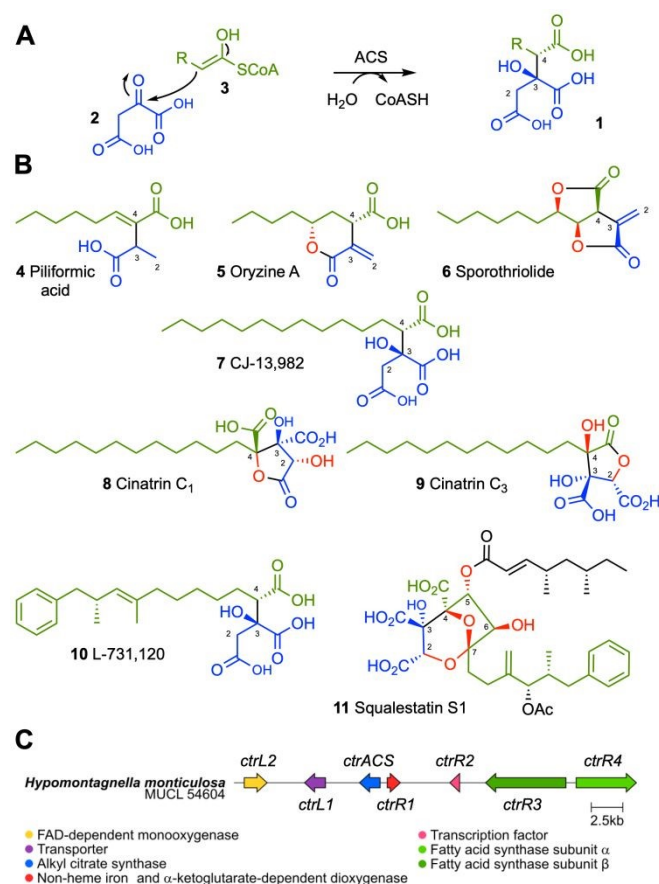
An alkyl citrate biosynthetic gene cluster (*ctr*) was identified from 47 members of the fungal family of *Hypoxylaceae* by genome mining. The *Hypomontagnella monticulosa* MUCL 54604 *ctr* cluster was deployed for the total biosynthesis of the known specialised metabolites CJ-13,982 and cinatrin C₁ and C₃. The required 2- and 4- oxygenations were catalysed by an α -ketoglutarate-dependent non-heme iron dioxxygenase that is homologous to known oxygenases including Mfr1 and Mfr2 from the squalestatin biosynthetic pathway. Combinatorial biosynthesis, and *in vitro* experiments including the oxygenases Mfr1 and Mfr2 from the squalestatin S1 biosynthetic gene cluster, were used for the synthesis of new natural products with bioactivity against phospholipase A₂ and squalene synthase.

Introduction

Alkyl citrates **1** are a class of specialized metabolites derived from condensation of oxaloacetate **2** with fatty acyl or polyketide CoA thioesters **3**, catalyzed by alkyl citrate synthase (ACS) enzymes (Scheme 1A).¹ Alkyl citrates are common metabolites of fungi and examples of the class include piliformic acid **4**,² and oryzine A **5**.³ Many alkyl citrates including sporothriolide **6**,^{4,5} CJ-13,982 **7**,⁶ cinatrin C₁ **8** and C₃ **9**,^{7,8} possess interesting bioactivities (Scheme 1B). For example, CJ-13,982 **7** is a μ M inhibitor of human squalene synthase (SQS) with potential use as an anti-cholesterol compound, while the cinatrin are a family of inhibitors of phospholipase A₂ (PLA₂) with potential use as anti-inflammatory agents. Cinatrin were first identified in the fungal strain *Circinotrichum falcatisporum* RF-641 in 1992, but their biosynthetic origin has remained elusive.^{7,8} An interesting alkyl citrate metabolite is L-731,120 **10**^{9,10} that is a precursor of squalestatin S1 **11**. L-731,120 **10** possesses 0.26 μ M inhibitory activity vs rat SQS.¹¹ However, **11** is a very potent pM inhibitor of rat SQS due to its distinctive 4,8-dioxabicyclo-[3.2.1]octane core that arises due to selective oxygenation of **10** at carbons C-2, and C-4 to C-7, followed by cyclisation.

The useful bioactivity of alkyl citrates such as cinatrin has resulted in many reported total chemical synthesis campaigns for this class of compounds.^{12,13,14,15,16} However, chemical synthesis of cinatrin and related compounds is challenging due to the requirement for the selective construction of contiguous stereocenters. Moreover, generation of the highly oxidized triacid core usually requires sophisticated protecting group strategies, adding steps and reducing yields.¹⁷ For example the most recent chemical synthesis of **8** involves 15 linear chemical steps and *ca* 30 different reagents and solvents.¹⁶ Total chemical synthesis is also highly carbon-intensive.^{18,19} As the

requirement for all sectors of the economy to decarbonize becomes increasingly urgent,²⁰ it would be highly beneficial to find short, efficient and effective synthetic routes to new bioactive compounds such as alkyl citrates. Here we focus on the use of a biosynthetic system for the one-step syntheses of cinatrin and related compounds that are alkyl citrates with known bioactivities vs PLA₂ and SQS.



Scheme 1. Alkyl citrates: **A**, alkyl citrate formation catalyzed by ACS; **B**, fungal alkyl citrates. Structural parts derived from oxaloacetate **1** are shown in blue, the fatty acid or polyketide chains are shown in green, red atoms derive from O₂; **C**, the *ctr* BGC.

^a Institute of Organic Chemistry and BMWZ, Leibniz Universität Hannover, Schneiderberg 38, 30167, Hannover, Germany. russell.cox@oci.uni-hannover.de
^b CeBiTec, Universität Bielefeld, Universitätsstraße 27, D-33615 Bielefeld, Germany.
^c Helmholtz Zentrum für Infektionsforschung, Inhoffenstraße 7, 38124 Braunschweig, Germany
 Electronic Supplementary Information (ESI) available: including all experimental and characterisation details. See DOI: 10.1039/x0XX00000x



Results and Discussion

We previously identified a highly conserved cryptic alkyl citrate biosynthetic gene cluster (BGC) on the genomes of 13 fungi from the *Hypoxylaceae* family.²¹ The BGC was predicted to encode two fatty acid synthase (FAS) subunits, an ACS, a 2-oxoglutarate-dependent non-heme iron (NHI) dioxygenase and an FAD-dependent monooxygenase (FMO) as putative structural genes. However, no product could be linked to the BGC. The genome sequences of 34 additional *Hypoxylaceae* family members were obtained using combinations of Illumina with Oxford Nanopore or PacBio sequencing technologies. This afforded high-quality genome sequences for each additional organism (N50 values between 1.2 Mbp and 9.2 Mbp and a contig count ranging from 9 to 122).

The *Hypomontagnella monticulosa* ACS BGC identified in our previous study was used in BLAST searches vs the translated nucleotide database of the targeted organism to find homologous BGCs in the new genomes.²² A homologous BGC encoding the FAS α (*ctrR4*) and FAS β (*ctrR3*) components, the ACS (*ctrACS*) and the NHI (*ctrR1*) and FMO (*ctrL2*) oxygenases and putative transporter (*ctrL1*) and transcription factor (*ctrR2*) was detected in all 47 *Hypoxylaceae* genomes (ESI Fig. S1) and designated as the *ctr* BGC (Scheme 1C). Clunker analysis²³ revealed that the putative *ctr* biosynthetic genes are highly conserved within syntenic genomic regions (ESI Fig. S1). BLASTp analysis of the NHI enzyme CtrR1 showed significant homology between it and the Mfr1 (45 %) and Mfr2 (38 %) NHI oxygenases from the squalestatin S1 **10** biosynthetic pathway (ESI Fig. S2).¹⁰ None of the new *Hypoxylaceae* genomes contained the sporothriolide BGC cluster previously only found in the *Hypomontagnella* species.⁴

We reasoned that strategic expression of genes from the *ctr* BGC and elsewhere could be used for the rational synthesis of both known and new alkyl citrates. In an initial experiment *ctrR4*, *ctrR3*, and *ctrACS* were each cloned downstream of strong fungal promoters in the Lazarus vector system.²⁴ These vectors were stably integrated into the genome of *A. oryzae* NSAR1,²⁵ and after selection and genetic validation transformants were grown in inducing media. After 7 days of growth the fungal cultures were extracted with EtOAc and the extracts interrogated by LCMS (Table 1, Exp1). These transformants produced CJ-13,982 **7** as the major metabolite (12.5 mg·L⁻¹), that was not produced by untransformed *A. oryzae* (Figs 1A-C).

Table 1. Synthesis of alkyl citrates *in vivo* and *in vitro* (shaded).

Exp	in vivo	in vitro	<i>ctrR3 ctrR4 ctrACS ctrR1 ctrL2 mfr1 mfr2</i>								Characterized Products	Data in Fig.	
			FASA	FASB	ACS	NHI	FMO	NHI	NHI	NHI			
1	✓	-	-	✓	✓	✓	-	-	-	-	-	7	S5
2a	✓	-	-	✓	✓	✓	✓	-	-	-	-	7, 8, 9, 12	S32
2b	✓	-	-	✓	✓	✓	✓	✓	-	-	-	7, 8, 9, 12	S33
3	-	✓	✓	-	-	-	✓	-	-	-	-	12	S46
4	-	✓	✓	-	-	-	-	-	✓	-	-	12	S48
5	-	✓	✓	-	-	-	-	-	-	✓	-	14	S49-51
6	-	✓	✓	-	-	-	-	✓	✓	-	-	Suggestions in ESI	S58
7	✓	-	-	✓	✓	✓	-	-	✓	-	-	7, 8, 9, 12	S59
8	✓	-	-	✓	✓	✓	-	-	-	✓	-	7, 14	S60
9	✓	-	-	✓	✓	✓	-	-	✓	✓	-	7, 12, 15	S61
10	✓	-	-	✓	✓	✓	✓	✓	✓	✓	-	7, 8, 9, 12, 15	S62

Compound **7** was purified and identified by full NMR, Analysis of optical rotation, and comparison to literature data identified **7** as (2S, 3S)-CJ-13,982 (Scheme 2, ESI Figs S6 - S12, Tables S12 - S13). A number of minor *A. oryzae* shunts were also purified and characterised. These were the 17-methyl ester of **7** (**16**, 0.5 mg·L⁻¹, ESI Figs S13 - S18, Table S14), an inseparable mixture of 13-, 14-, 15-hydroxy and 15-oxo-**7** (**17a-d**, 3 mg·L⁻¹, ESI Figs S19 - S25, Tables S15 - S19), and 14-oxo-**7** (**18**, 0.9 mg·L⁻¹, ESI Figs S26 - 31, Table S20).

We then coexpressed *ctrR1* encoding the putative NHI dioxygenase (Table 1, Exp2a, ESI Fig. S32) and, in another experiment, both *ctrR1* and *ctrL2* encoding the putative FAD-dependent monooxygenase (Exp2b, ESI Fig. S33). Transformants of Exp2a and Exp2b had identical chemotypes. CJ-13,982 **7** was again produced (5.9 mg·L⁻¹ [from Exp2b]), but in addition 4-hydroxy-CJ-13,982 **12** (1 mg·L⁻¹ [from Exp2b], Figs 1DE, ESI Figs S34 - S39, Table S21) was purified and its structure elucidated. We also attempted purification of compound **13**, but it isomerised to a mixture of cinatrin C₁ **8** (< 1mg·L⁻¹, Scheme 2, ESI Figs S40 - S45, Tables S22 - S23) and cinatrin C₃ **9** (identified by comparison to literature data, 1.3 mg·L⁻¹ [from Exp2b], Scheme 2, ESI Figs S40 - S45, Tables S22 - S23).^{8,12,13} Cinatrin C₁ **8** and C₃ **9** are known to be lactonised products of **13**.¹² The absolute stereochemistry for both cinatrin C₁ **8** and C₃ **9** is known to be 2S, 3S, 4R.¹³ The stereochemical relationships among these compounds suggest that **13** must therefore possess 2S stereochemistry.²⁶

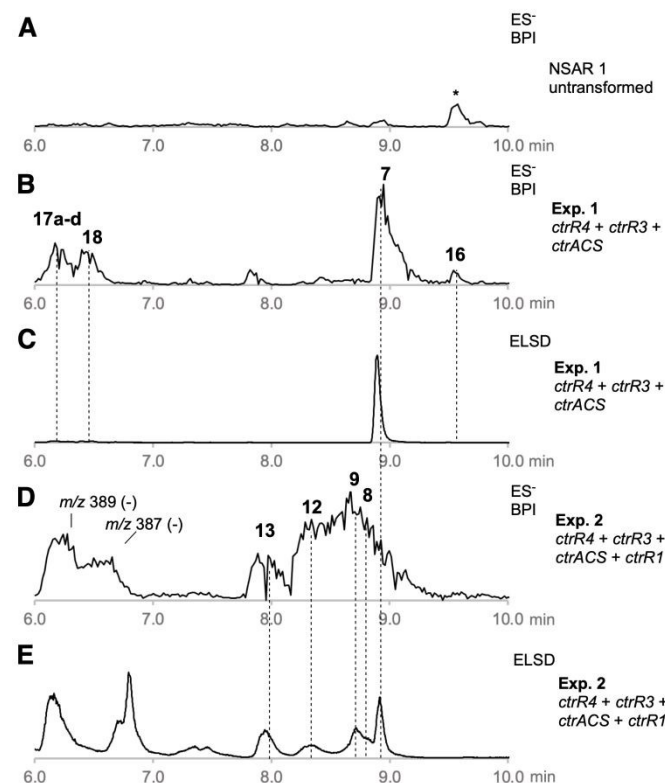
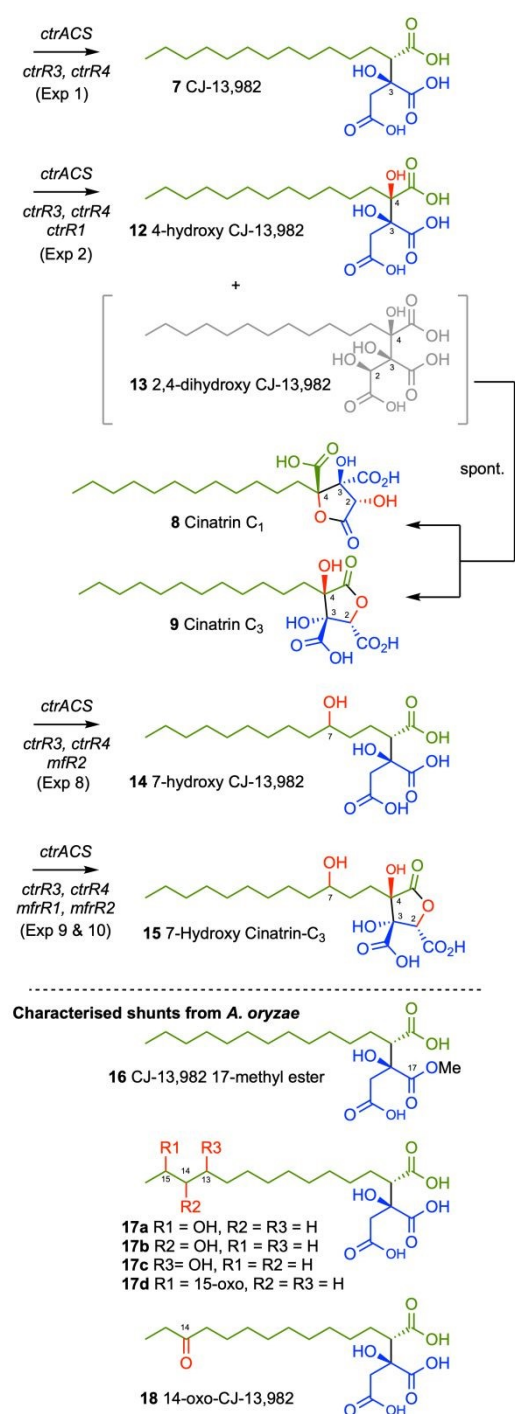


Figure 1. LCMS analysis of organic extracts from experiments 1 and 2: **A**, untransformed *A. oryzae* (ES⁺, BPI); **B**, *A. oryzae* + *ctrR3* + *ctrR4* + *ctrACS* (ES⁺, BPI); **C**, *A. oryzae* + *ctrR3* + *ctrR4* + *ctrACS* (ELSD); **D**, *A. oryzae* + *ctrR3* + *ctrR4* + *ctrACS* + *ctrR1* (ES⁺, BPI); **E**, *A. oryzae* + *ctrR3* + *ctrR4* + *ctrACS* + *ctrR1* (ELSD); * = unrelated compound.





Scheme 2. Synthesis of alkyl citrates by heterologous expression of *ctr* and *mfg* genes in *A. oryzae* NSAR1 or reaction *in vitro*. Oxygenation positions shown in red.

We further probed the potential function of the FMO encoded by *ctrL2* in different experimental setups *in vivo* and *in vitro*. However, we did not observe any new compounds in the presence of CtrL2, and thus the function of the FMO remains elusive.

To assess the possibility of using the NHI oxygenase CtrR1 *in vitro*, reactions with recombinant enzyme were conducted (Table 1, Exp3). CtrR1 was expressed in *E. coli* as a hexahistidine-tagged protein and purified by Ni-NTA chromatography (ESI section 3 for details). Incubation of purified CtrR1 with CJ-

13,982 **7**, FeSO₄, α-ketoglutarate, and ascorbate at 28 °C for 2 h led to the synthesis of **12** and **13** with **8** and **9** detected as minor components. The addition of more enzyme over the course of the reaction resulted in the complete consumption of intermediate 4-hydroxy-CJ-13,982 **12** after 2 hours, forming 2,4-dihydroxy-CJ-13,982 **13** (ESI Fig S47).

Squalenstatin S1 **11** is an alkyl citrate derived from the condensation of a hexaketide with oxaloacetate that produces the key intermediate **10**. Two NHI oxygenases Mfr1 and Mfr2 that are homologous to CtrR1 are known to be involved in the sequential oxygenation of **10** that ultimately leads to the 4,8-dioxabicyclo-[3.2.1]octane core of **11** that is a pM inhibitor of rat squalene synthase compared to the μM activity of **7**.^{6,11} Since **7** is structurally analogous to **10** we were interested to test whether Mfr1 and Mfr2 could be used to convert **7** to a potentially more potent squalene synthase inhibitor. In previous studies, soluble Mfr1 and Mfr2 could not be produced in *E. coli*.¹⁰ For this reason, both enzymes were here expressed and purified as thioredoxin-fusion proteins²⁷ (TrxA-Mfr1 and TrxA-Mfr2) that improves their solubility and activity *in vitro*. Incubation of purified TrxA-Mfr1 with CJ-13,982 **7**, FeSO₄, α-ketoglutarate, and ascorbate at 28 °C for 2 h resulted in the formation of **12** and **13** as observed for CtrR1 (Table 1, Exp4). However, *in vitro* reaction using TrxA-Mfr2, FeSO₄, α-ketoglutarate, and ascorbate at 28 °C (Exp5) resulted in synthesis of several new compounds that are consistent with the suggested oxidative modifications in the biosynthesis of **11**. From a large scale *in vitro* reaction of 5 mg of substrate **7** with TrxA-Mfr2, compound **14** (0.6 mg) was obtained and fully characterized (Scheme 2, Table 1 Exp5, Table S24 and Figs S52-S57).

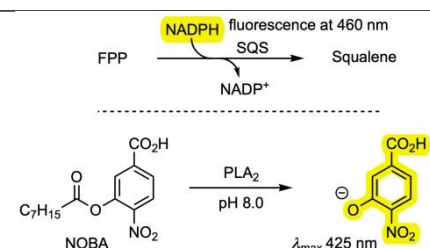
Combined use of TrxA-Mfr1 and TrxA-Mfr2 with **7** as the substrate was also attempted (Table 1, Exp6). Several oxygenated products were observed by LCMS that are consistent with the oxidative pathway towards the 4,8-dioxabicyclo-[3.2.1]octane motif (see ESI section 17 for details), but **14** remained the only isolated and fully characterised compound.

In order to produce further new compounds in a single process, heterologous expression in *A. oryzae* was again used. Four different gene combinations were created. Coexpression of *mfr1* with *ctrR4*, *ctrR3* and *ctrACS* (Table 1, Exp7) generated **7** as the major product together with small amounts of 4-hydroxy-CJ-13,982 **12** and 2,4-dihydroxy CJ-13,982 **13** and the usual shunt compounds. Coexpression of *mfr2* with *ctrR4*, *ctrR3*, and *ctrACS* (Table 1, Exp8) resulted in production of **7** and 7-hydroxy-CJ-13,982 **14**. In extracts of transformants containing *ctrR4*, *ctrR3*, *ctrACS* and both, *mfr1* and *mfr2* we identified **7** and 4-hydroxy-CJ-13,982 **12** (Table 1, Exp9), and a minor compound **15**. To improve yields further, we additionally co-expressed *ctrR1* that appears to be a more effective 2,4-hydroxylase than Mfr1 (Table 1, Exp10). This resulted in increased production of compound **15** (5.7 mg·L⁻¹) and allowed its identification as 7-hydroxy-cinatrin C₃ (Scheme 2, ESI Figure S63 - S68, Table S25).



Finally, the new compounds were tested as inhibitors of PLA₂ and SQS. An *in vitro* SQS assay was established based on the consumption of NADPH.²⁸ *A. oryzae* SQS was expressed in *E. coli* and purified. The assay involved incubation of farnesyl diphosphate (FPP) and SQS with NADPH, and the consumption of NADPH was observed via fluorescence with an absorption wavelength of 360 and an emission wavelength of 460 nm in the presence and absence of inhibitors. Phospholipase A₂ (PLA₂) from porcine pancreas (Sigma-Aldrich) was assayed by measuring the rate of hydrolysis of 4-nitro-3-(octanoyloxy)benzoic acid (NOBA) and detecting the formation of 4-nitro-3-hydroxybenzoic acid (NHBA) spectrophotometrically at 425 nm in the presence and absence of inhibitors. Measured IC₅₀ values of the compounds **6**, **7**, **8/9**, **11**, **14**, and **15** are shown in Table 2 and raw data is included in the ESI.

Table 2. IC₅₀ values vs SQS and PLA₂ experimentally observed for alkyl citrates described in this study. n.t. = not tested. * lit. value 95.5 ± 13.6 nM vs *T. elongatus* SQS;²⁹ † lit. value 70 μM vs rat platelet PLA₂;⁷ ‡ lit. value 1.1 μM vs human SQS.⁶



Compound	IC ₅₀ / μM vs		PLA ₂ /SQS Selectivity
	SQS	PLA ₂	
Sporothriolide 6	n.t.	> 1000.	-
CJ-13,982 7	33 ± 12 [‡]	293 ± 78	8.9
Cinatrins C ₁ 8 and C ₃ 9 mixture	18 ± 3.0	145 ± 10 [†]	8.1
Squalestatin S1 11	0.092 [*]	n.t.	-
7-Hydroxy-CJ-13982 14	24 ± 6	> 1000	> 41.6
7-Hydroxy-Cinatrins C ₃ 15	57 ± 12	> 1000	> 17.5

Conclusion

The biosynthesis of cinatrins has previously been suggested to follow the same pattern as the squalestatins.¹⁶ Here we showed that the *ctr* BGC, widely present in *Hypoxylon* species, encodes a pathway that does indeed follow the squalestatin model. Myristoyl CoA (C14:0) is likely to be the product of the FAS components (CtrR3 & CtrR4) and is condensed with oxaloacetate by CtrACS to create CJ-13,982 **7**. CtrR1 then sequentially hydroxylates C-4 and then C-2 to create **13** that is the precursor to cinatrins C₁ **8** and C₃ **9**.

CJ-13,982 **7**, cinatrins C₁ **8** and C₃ **9** and intermediate **12** are produced by total biosynthesis in *A. oryzae* in titers that allow isolation, full characterisation, and bio-testing after a single fermentation. This compares with 15-steps by total chemical synthesis.¹⁶ Furthermore, implementation of oxygenase steps from the squalestatin pathway allow the synthesis of new hybrid metabolites **14** and **15** for the first time. Thus, expression of the biosynthetic genes in *A. oryzae* provides a highly effective platform for the total biosynthesis of new and known alkyl citrates in a single step. None of the products of the *ctr* pathway have ever been reported as metabolites of the *Hypoxylaceae*, either from wild-collected material or from laboratory

fermentations. Total biosynthesis is thus also an effective way to activate a silent and previously cryptic BGC.

The synthesis of **12** from **7** can also be achieved in a single step *in vitro* using purified CtrR1. Additionally, *in vitro* conversion of **7** by Mfr2 resulted in synthesis of new hybrid metabolite **14**, again in yields sufficient for purification and characterisation. However, the *in vitro* synthesis is less convenient than total biosynthesis as it requires laborious purification of the biosynthetic proteins themselves and access to **7**.

The total biosynthesis platform also offers a convenient way of screening oxygenases related to CtrR1. For example, we showed that Mfr2 acts as a 7-hydroxylase, and Mfr1 acts as a 2,4-dihydroxylase in this system.³⁰ Mfr2 appears to catalyse further oxygenations of the fatty-acid-derived chain, but a fully-developed 4,8-dioxabicyclo-[3.2.1]octane core could not be isolated. Never-the-less, new hybrid metabolites **14** and **15** were synthesised, characterised, and shown to inhibit SQS. Interestingly, the 7-hydroxylation improves selectivity for SQS vs PLA₂ (Table 2), but does not increase potency vs SQS itself. Further oxidative modifications are clearly required to evolve the potency of **14** and **15** towards the nM potency of **11** vs SQS. It is hypothesised that biosynthetic gene clusters may evolve by gain or loss of biosynthetic genes.³¹ Our results support this idea because introduction of 7-hydroxylation by Mfr2 alters the spectrum of enzyme inhibition of the pathway product and sets the scene for the eventual development of much more potent inhibition as observed for **11** itself.

In conclusion, our results show that total biosynthesis can be used as an effective platform for the synthesis of known and new specialised metabolites with bioactive properties. Total biosynthesis dramatically out-competes total chemical synthesis in terms of step-count. While total biosynthesis currently lacks the flexibility of total chemical synthesis, the ability to rationally combine biosynthetic genes from different pathways for the synthesis of *new* bioactive metabolites such as **14** and **15** illustrates that total biosynthesis offers a practical and effective route for the synthesis of known and new bioactive compounds. Since BGCs encoding very many alkyl citrates and related oxygenases³² are known it should now be possible to synthesise a wide range of related metabolites without the need for total chemical synthesis.

Author contributions

HH conceptualization, investigation, supervision, writing – original draft; NG investigation, writing – review & editing; EK and JG, informatics and visualization; BW and JK, genome sequence, assembly and annotation; EK, MS, JK, & RJC funding acquisition, resources. RJC, supervision, writing – review & editing.

Conflicts of interest

There are no conflicts to declare.



Data availability

The data supporting this article have been included as part of the Supplementary Information including NMR spectra, LCMS data and further experimental details. See DOI: [URL – format <https://doi.org/DOI>].

Acknowledgements

This work was funded by the DFG priority program “Taxon-Omics” (SPP1991), specifically CO 1328/4-2 and STA1282/4-1. The bio-informatics support of the BMBF-funded project ‘Bielefeld-Gießen Center for Microbial Bioinformatics; BiGi (Grant number O31A533)’ within the German Network for Bioinformatics Infrastructure (de.NBI) is gratefully acknowledged.

Notes and references

- R. Fujii, Y. Matsu, A. Minami, S. Nagamine, I. Takeuchi, K. Gomi and H. Oikawa, *Org. Lett.*, 2015, **17**, 5658–5661.
- J. R. Anderson, R. L. Edwards and A. J. S. Whalley, *J. Chem. Soc., Perkin Trans. 1*, 1985, **34**, 1481.
- Z. Wasil, E. Kuhnert, T. J. Simpson and R. J. Cox, *J. Fungi*, 2018, **4**, 96.
- D. S. Tian, E. Kuhnert, J. Ouazzani, D. Wibberg, J. Kalinowski and R. J. Cox, *Chem. Sci.*, 2020, **11**, 12477–12484.
- H. Heinemann, K. Becker, H. Schrey, H. Zeng, M. Stadler and R. J. Cox, *RSC Adv.*, 2023, **13**, 29768–29772.
- S. Watanabe, H. Hirai, T. Kambara, Y. Kojima, N. Hiroyuki, S. Ayumu, Y. Yuji, Y. Nobuji, J. H. James, Harwood, H. Liang, Hsuang, et al., *J. Antibiot.*, 2001, **54**, 1025–1030.
- K. Tanaka, H. Itazaki and T. Yoshida, *J. Antibiot.*, 1992, **45**, 50–55.
- H. Itazaki, K. Nagashima, Y. Kawamura, K. Matsumoto, H. Nakai and Y. Terui, *J. Antibiot.*, 1992, **45**, 38–49.
- P. J. Sidebottom, R. M. Highcock, S. J. Lane, P. A. Procopiou and N. S. Watson, *J. Antibiot.*, 1992, **45**, 648–658.
- K. E. Lebe and R. J. Cox, *Chem. Sci.*, 2019, **10**, 1227–1231.
- G. H. Harris, C. Dufresne, H. Joshua, L. A. Koch, D. L. Zink, P. M. Salmon, K. E. Göklen, M. M. Kurtz, D. J. Rew, J. D. Bergstrom, et al., *Bioorg. Med. Chem. Lett.*, 1995, **5**, 2403–2408.
- A. N. Cuzzupe, R. Di Florio, J. M. White and M. A. Rizzacasa, *Org. Biomol. Chem.*, 2003, **1**, 3572–3577.
- D. A. Evans, B. W. Trotter and J. C. Barrow, *Tetrahedron*, 1997, **53**, 8779–8794.
- F. Urabe, S. Nagashima, K. Takahashi, J. Ishihara and S. Hatakeyama, *J. Org. Chem.*, 2013, **78**, 3847–3857.
- T. Yakura, A. Ozono, K. Matsui, M. Yamashita and T. Fujiwara, *Synlett*, 2013, **24**, 65–68.
- N. P. Rossouw, Z. Chen, J. M. White and M. A. Rizzacasa, *Org. Lett.*, 2023, **25**, 8010–8015.
- N. P. Rossouw and M. A. Rizzacasa, *Aust. J. Chem.*, 2024, **77**, 1–13.
- R. J. Cox, *Nat. Rev. Chem.*, 2024, **8**, 61–78.
- D.-S. Tian, X. Zhang and R. J. Cox, *Nat. Prod. Rep.*, 2025, **42**, 720–738.
- IPCC, 2023: Synthesis Report. Contribution of Working Groups I, II and III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, H. Lee and J. Romero (eds.)]. IPCC, Geneva,

Switzerland, pp. 35–115, doi: [10.59327/IPCC/AR6-9789291691647](https://doi.org/10.59327/IPCC/AR6-9789291691647)

- E. Kuhnert, J. C. Navarro-Muñoz, K. Becker, M. Stadler, J. Collemare and R. J. Cox, *Studies in Mycology*, 2021, **99**, 1–43.
- S. F. Altschul, T. L. Madden, A. A. Schäffer, J. Zhang, Z. Zhang, W. Miller and D. J. Lipman, *Nuc. Acids Res.*, 1997, **25**, 3389–3402.
- C. L. M. Gilchrist and Y. H. Chooi, *Bioinformatics*, 2021, **37**, 2473–2475.
- K. A. K. Pahirulzaman, K. Williams and C. M. Lazarus, *Meth. Enzymol.*, 2012, **517**, 241–60.
- F. J. Jin, J. Maruyama, P. R. Juvvadi, M. Arioka and K. Kitamoto, *FEMS Microbiol. Lett.*, 2004, **239**, 79–85.
- S. Yin, S. Friedrich, V. Hrupins and R. J. Cox, *RSC Adv.*, 2021, **11**, 14922–14931.
- E. R. LaVallie, E. A. DiBlasio, S. Kovacic, K. L. Grant, P. F. Schendel and J. M. McCoy, *BioTechnol.*, 1993, **11**, 187–193.
- A. D. Napper and S. Sivendran, *Curr. Protoc. Chem. Biol.*, 2011, **3**, 81–97.
- S. Lee and C. D. Poulter, *J. Bacteriol.*, 2008, **190**, 3808–3816.
- See ESI for a discussion of the differences in concluded regioselectivity from ref 10.
- A. Rokas, M. E. Mead, J. L. Steenwyk, H. A. Raja and N. H. Oberlies, *Nat. Prod. Rep.*, 2020, **37**, 868–878.
- C. Xiao, M. Long, X. Sun, D. Yi, M. S. Miah, S. Yang, H. Ma, D. Tian, R. J. Cox, X. Zhang and B. Zhang, *Bioresour. Technol.*, 2025, 133787.

View Article Online

DOI: [10.1039/C5PY00660G](https://doi.org/10.1039/C5PY00660G)

Data Availability Statement

View Article Online
DOI: 10.1039/D6SC00660D

Total Biosynthesis and Engineering of Cinatrins: Inhibitors of Phospholipase A₂ and Squalene Synthase

Henrike Heinemann,^a Nick Gerlach,^a Eric Kuhnert,^a Jennifer Gerke,^a Bart Verwaaijen,^b Jörn Kalinowski,^b Marc Stadler^c and Russell J. Cox^{a*}

The data supporting this article have been included as part of the Supplementary Information.

