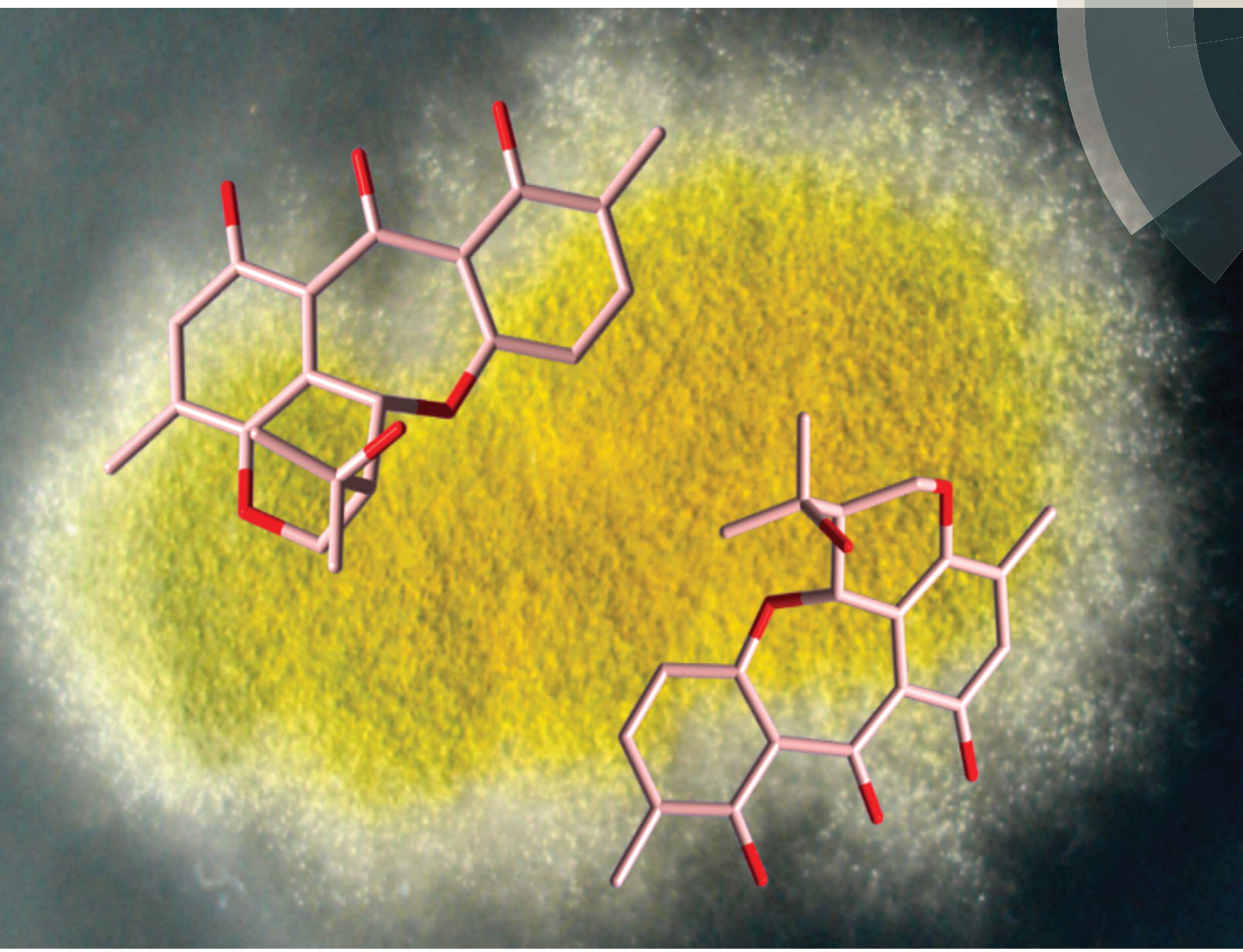
Volume 31 | Number 10 | October 2014 | Pages 1233–1490

NPR

Natural Product Reports

www.rsc.org/npr



ISSN 0265-0568

ROYAL SOCIETY OF CHEMISTRY

VIEWPOINT

Thomas J. Simpson

Fungal polyketide biosynthesis – a personal perspective

NPR

VIEWPOINT

View Article Online
View Journal | View Issue

Cite this: *Nat. Prod. Rep.*, 2014, **31**, 1247

Fungal polyketide biosynthesis – a personal perspective†

Thomas J. Simpson*

This Viewpoint article provides a personal viewpoint of research in the area of fungal polyketide biosynthesis. It spans the period from the first applications and subsequent development of modern stable-isotope labelling methods, the isolation, sequencing and expression of PKS genes, biochemical and structural characterisation of PKS domains, fungal genome sequencing, through to genetic engineering of whole pathways. This article forms part of a "trilogy" of related Viewpoints from three close contemporaries who have been privileged to share in these developments since their outset over forty years ago and to enjoy both professional and personal interactions throughout that time.

Received 19th May 2014

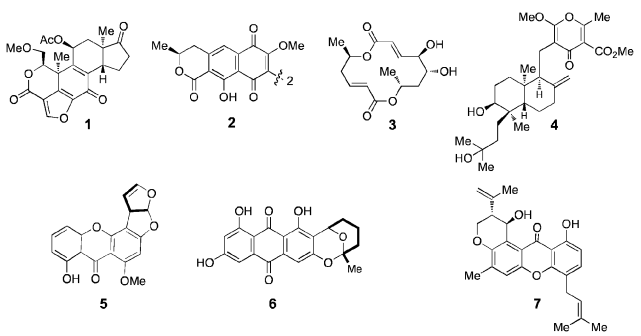
DOI: 10.1039/c4np00065j

www.rsc.org/npr

1. Introduction

Early work: my interest in fungal metabolites began in 1969 when I embarked on my graduate research with Jake MacMillan in Bristol. I studied the structures of wortmannin (**1**) a highly oxygenated steroidal triterpene from *Myrothecium roridum*,[1] a group of dimeric naphthoquinone–naphthol heptaketide pigments from *Aspergillus melleus*,[2] *e.g.* xanthomegnin (**2**), colletodiol (**3**) and related macrodiolides from *Colletotrichum capsici*[3] and colletotrichin (**4**) a mixed polyketide terpenoid metabolite of *C. capsici*.[4] The last was an early member of the meroterpenoid group, structural and in particular biosynthetic studies of which were to form part of a major collaboration with John Vederas (see below). Preliminary biosynthetic studies were also carried out on most of these compounds but it is noteworthy that the early 1970's proved to be a watershed that saw a resurgence in biosynthetic studies on fungal metabolites. Immediately prior to this, structure elucidation was carried out primarily by degradation studies, which provided a natural basis for subsequent biosynthetic studies with radioisotopes (*e.g.* ^{14}C). However as structure elucidation came to increasingly rely on spectroscopic (mainly ^{1}H NMR studies) the resulting disconnection between structure and biosynthetic methods severely hampered biosynthetic work. The advent of ^{13}C-NMR and in particular use of ^{13}C-labelled precursors, restored that essential link and resulted in a resurgence in biosynthetic studies using stable isotope labelling.[5] John Vederas, Craig Townsend and myself were in the vanguard of these studies.

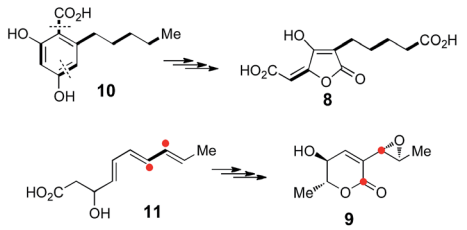
On moving to my first post-doctoral position with Stan Holker in Liverpool in 1973, while structure elucidation remained a continuing theme, ^{13}C based labelling studies became an increasingly important feature. Holker who never moved from Liverpool for a combination of personal and professional reasons is perhaps one of the greatest unsung, certainly under-sung, heroes of natural product chemistry in the UK. Among many other things his 1968 paper on incorporation of ^{14}C-acetate into the aflatoxin intermediate, sterigmatocystin (**5**) exemplified his rigorous approach.[6] He noted a small but significant difference (1%!) in the level of labelling in the branched C_4 side chain compared to the xanthone nucleus and suggested that this was due to a different biosynthetic origin. This insight was proved correct many years later when Townsend (see CAT Viewpoint) showed that [1-^{13}C]hexanoate was incorporated intact into the aflatoxin precursor averufin (**6**).[7]



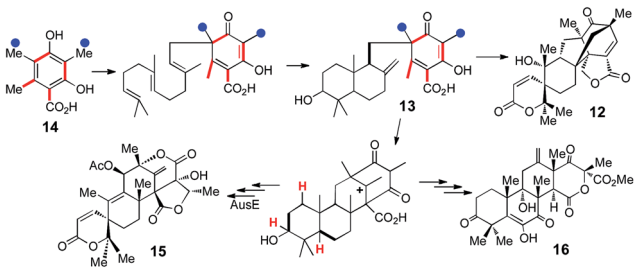
My work in Liverpool included elucidating the structures of the prenylated xanthone, shamixanthone (**7**) and related metabolites of *Aspergillus variecolor* and our work on incorporation of ^{13}C-acetates into shamixanthone was one of the first detailed ^{13}C-labelling studies.[8] Incorporation of doubly labelled [1,2-$^{13}C_2$]acetate into tetronic acids, *e.g.* multicolosic acid (**8**) in *Penicillium multicolor*[9] and aspyrone (**9**) in *A. melleus*[10] (Scheme 1) provided not only structural information but

School of Chemistry, University of Bristol, Cantock's Close, Bristol, BS8 1TS, UK. E-mail: tom.simpson@bristol.ac.uk

† This is dedicated to my PhD supervisor and lifelong mentor, Jake MacMillan FRS, who sadly died while this article was being prepared. He was a truly original scientist who pioneered what we now know as synthetic biology.



Scheme 1



Scheme 2

revealed the new power of "bond-labelling" (see JCV Viewpoint for further discussion) to reveal the biosynthesis of the tetronic acid moiety occurred *via* aromatic ring cleavage of a resorcylic acid (**10**) and detection of a two-bond coupling in aspyrone labelled from [1,2-$^{13}C_2$]acetate proved the involvement of a Favorskii-like rearrangement in formation of the branched hexaketide skeleton. Subsequent seminal studies by Staunton and Hill on aspyrone provided one of the most comprehensive and successful applications of feeding assembly intermediates (as SNAC thioesters) to define the mode of assembly of the hexaketide precursor (**11**).[11] This concept of "bond-labelling" was soon extended to the use of ^{2}H (Staunton)[12] and ^{18}O (Vederas)[13] labels in conjunction with ^{13}C detection. These applications with multiple stable-isotope labelling were to provide a quantum leap in our understanding of, in particular, polyketide biosynthesis as they gave us hitherto inaccessible information on the permitted oxidation levels of enzyme-bound intermediates, how they were folded prior to cyclisation, skeletal rearrangements and bond cleavages during biosynthesis. Importantly, they allowed the formulation of the idea that polyketide chain assembly occurred in a processive assembly akin to the process of fatty acid biosynthesis and very much paved the way for the next explosion in activity brought about by isolation and sequencing of PKS genes, initiated in bacterial polyketides but soon followed up in fungal systems.

Tom Simpson graduated from Edinburgh in 1969, and gained his PhD from the University of Bristol in 1973. After post-doctoral work in Liverpool and the Australian National University he was appointed to a Lectureship in Chemistry in Edinburgh in 1978. He moved to Professorships in Organic Chemistry in Leicester in 1988, and Bristol University in 1990. His research which covers all aspects of the chemistry and biosynthesis of microbial natural products has led to ca. *230 papers. He was elected Fellow of the Royal Society in 2001 and Fellow of the Royal Society of Edinburgh in 2006.*

2. Meroterpenes

Labelling studies[14] on the C_{25} metabolite of *Aspergillus varie-color*, andibenein B (**12**), initially thought to be a sesterterpene or degraded triterpene, revealed an unexpected biosynthesis *via* a key intermediate (**13**) formed by alkylation of 3,5-dimethylorsellinate (**14**), derived from a bis-C-methylated tetraketide, by farnesyl diphosphate, followed by a presumed intramolecular Diels–Alder to generate the bicyclo[2,2,2]ring system, and subsequent extensive oxidative modifications (Scheme 2). It immediately became apparent that several other fungal metabolites of uncertain biosynthetic origins, *e.g.* austin (**15**)[15] and terretonin (**16**)[16] could be formed *via* various rearrangements of the same key intermediate (**13**), which we were able to demonstrate *via* extensive labelling studies with both early and advanced[17] intermediates. We renamed this pathway the "meroterpenoid" pathway, resurrecting a term first coined by Cornforth for compounds of mixed, partly terpenoid origin. The number of members of the meroterpenoid family have subsequently grown greatly and represent a structurally highly diverse, but biosynthetically homogeneous family.[18]

It has been a pleasure to see our original proposed pathway and intermediates substantiated by much more recent genome-based work. The biosynthetic gene clusters for several of these compounds notably austin have been isolated as result of genome sequencing work and many of the genes expressed and their exact roles confirmed. Perhaps the most spectacular result is Abe's demonstration[19] that the single cytochrome P_{450} encoded by *ausE* is responsible for three separate oxidative steps (abstraction of axial **H**'s – see Scheme 2) in formation of the spiro-cyclic lactone system found in austin and several other of these metabolites (see Cox review herein for more detailed discussion).

3. PCR Based PKS probes and PKS classification

In collaboration with Colin Lazarus, and subsequently Russell Cox, we developed PCR-primer based probes for isolation of fungal PKSs. An interesting feature which soon emerged was that there were clear sequence differences that allowed two subclasses of fungal polyketide synthase genes to be distinguished[20] – so called WA and MSAS types – which we now classify as *non-reducing* (NR) and *partially reducing* (PR) PKSs

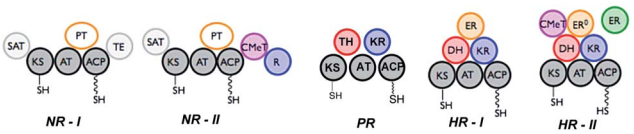


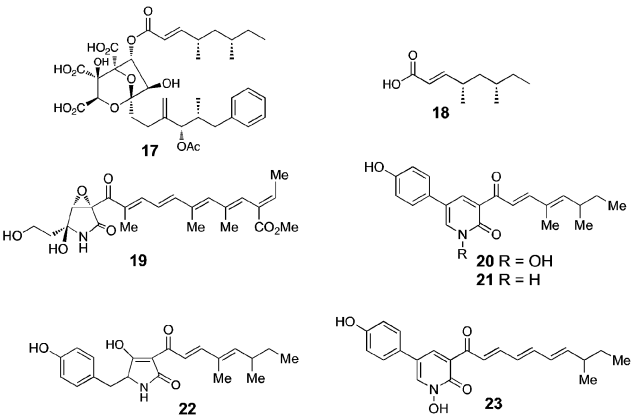
Fig. 1 Arrangement of domains in iterative fungal PKSs.

respectively. We were soon able to show that we could also design PCR primers selective for *highly reducing* (HR) PKSs, and for PKSs containing C-methyl transferase domains.[21] It is noteworthy that there were very few PKS sequences then known and so it has been satisfying to see how this hypothesis drawn up on the basis of very limited data has proved correct.

These functional types of PKS we now know are consistent with the distinctly different domain structures of the major classes of fungal PKS, perhaps best summarised in Russell Cox's seminal review.[22] These are shown in Fig. 1 and we can now distinguish two sub-sets of NR PKS, depending on the presence or absence of a C-MeT domain – interestingly (see Craig Townsend Viewpoint for more details), these contain distinct domains for starter unit selection (SAT),[23] and for control of chain length and folding: the product template (PT) domains.[24] PR-PKSs remain restricted to MSAS and lack the SAT and PT domains but have a domain originally assigned as a dehydratase (DH) but now known to be a thiolester hydrolase (TH) implicated in product release.[25] The HR PKSs are very similar to a mammalian FAS in both sequence, and presumably structure, in containing a full set of domains, KR, DH and ER necessary for modification and eventual deoxygenation of the β-carbonyl of the β-ketothioester product of the KS-catalysed condensation. In the HR-I series, an active ER domain is present, but in the HR-II series the ER domain is inactive and the functionality, when required, is supplied by a separate ER acting *in trans*. Heterologous expression studies of such HR-II PKSs have shown that when the *trans*-ER domain is absent, the polyketide chain assembly fidelity is impaired resulting in incorrectly assembled polyketides.[26,27]

4. Isolation and expression of HR-PKSs

The HR PKSs often contain an additional C-MeT domain. We were able to use our C-MeT domain based PCR primers to isolate the tetraketide synthase (SQTKS) responsible for assembly of the side chain of squalestatin (**17**)[28] and on heterologous expression using the *Aspergillus oryzae* system developed by Fujii and Ebizuka,[29] the side chain (**18**) could be isolated in high yields (up to 80 mgL^{-1}). We extended this work to investigate the isolation of the PKS involved in biosynthesis of the mycotoxin fusarin C (**19**), a metabolite of several *Fusarium* spp., a subsidiary aim being to identify the origin of the nitrogen functionality in the pyrrolidinone ring.[30] This became apparent when we found that the HR-II PKS was fused to a single NRPS module containing condensation (C), adenylation (A) and thiolation (T) domains terminating with an apparent reductase. We were subsequently able to demonstrate (with Chris Willis) that the NRPS activated and incorporated homoserine as the non-PKS component of fusarin C.[31] This was the first example of a fused PKS-NRPS from a fungus, but other work, and in particular analysis of the fungal genome sequences which were then appearing revealed that these are very common, *e.g. Aspergillus nidulans* contains in addition to 32 PKSs and 28 NRPSs, at least 2 PKS-NRPS fusions.[32] This soon led to the isolation of the PKS-NRPS gene *tenS* (see Fig. 1), involved in the biosynthesis of the pyridone tenellin (**20**) in *Beauvaria bassiana*, this time the amino acid being tyrosine.[33] Heterologous expression of *tenS* along with the *trans*-ER encoded by *tenC*, gave a tetramic acid pretenellin A (**22**) as the major product,[34] and further expression with the cytochrome P_{450} genes *tenA* and *tenB* gave ring expansion to provide pretenellin B (**21**) followed by *N*-hydroxylation to tenellin.[35] It also showed that the apparent reductase domain at the end of the NRPS module in fact functions as a Dieckmann cyclase (DKC). Interestingly, the lovastatin LNKS terminates with the C-domain of an NRPS module.[36]



5. Domain swaps in HR-PKSs

Without doubt, in my view at least, one of the great remaining problems in natural product biosynthesis is understanding at molecular and mechanistic levels how programming of HR-PKSs is controlled. We have gone a small way to solving this for one system at least by carrying out a series of systematic domain swaps between TENS and DMBS.[37] The latter is the PKS-NRPS responsible for the biosynthesis of desmethylbassianin (**23**) which differs from tenellin in being formed from a *mono*-methylated *hexa*-ketide, in contrast to tenellin's *di*-methylated *penta*-ketide, all else being the same. The high homology between the two genes has allowed us to make systematic domain swaps in which we progressively replaced DNA encoding tenellin domains with the equivalent DNA from desmethybassianin. Following that, selective domain swaps were made which showed unequivocally that the C-MeT domain controlled the degree of methylation and, at least at first surprisingly, that it was the KR domains that controlled chain length. Thus expression of an engineered gene which contained the DMBS KR in a tenellin background (Fig. 2) gave a mixture of mono and

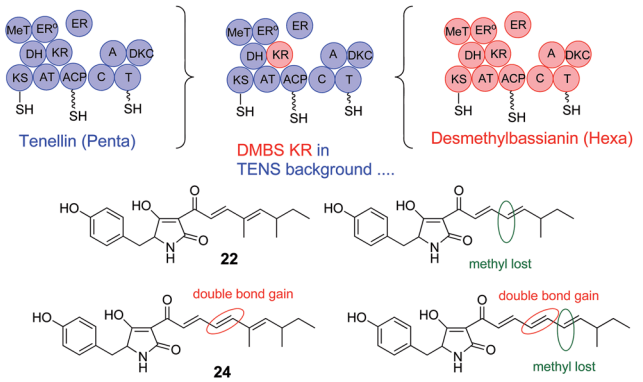
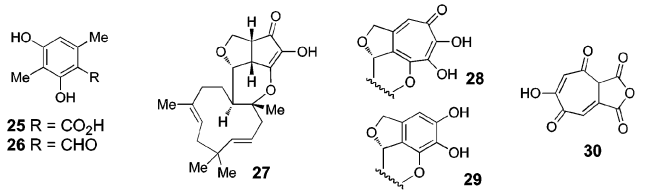


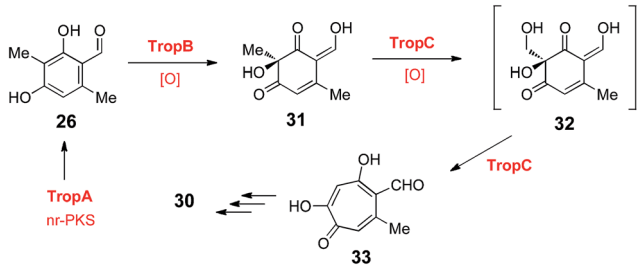
Fig. 2 Products isolated from substituting the TENS (tenellin, blue) KR domain with the DMBS (desmethylbassianin, red) KR domain.

dimethylated penta- and hexa-ketide products but the predominant products were prebassianin A (**24**) which now contained a *di*-methylated *hexa*-ketide, along with a smaller amount of the monomethylated product. Thus, we have increased the chain length by two carbons in the first successful controlled modification of polyketide chain length. This work has a long way to go of course before we will have achieved our aim of a full understanding of programming of these highly complex molecular machines.

6. Troplones

In 1997, we showed by simple ^{13}C-labelling studies, that the cyclopentenone ring in xenovulene (**27**) in *Acremonium strictum* must be formed *via* tetraketide-derived phenol, at the time thought to be 3-methylorsellinic acid (**25**), which ring expands to give a tropolone (**28**) followed amazingly by two ring contractions, back to a phenol (**29**) and further to the cyclopentenone.[38] Although such ring expansion and contractions were known to take place individually, their combination in a single pathway was unprecedented. This triggered an interest in tropolone biosynthesis. Tropolones have of course long posed classic problems in structure, aromaticity and biosynthesis. Again making use of our C-MeT domain probes we were able to isolate a NR-II PKS gene (MOS) which terminates with a thiolester-reductase (R) domain from *A. strictum*. Heterologous expression of MOS produced high titres of 3-methyl-orcinaldehyde (**26**) rather than 3-methylorsellinic acid (**25**).[39] Interestingly deletion of the R domain led to slightly lower titres of (**25**) presumably resulting from spontaneous hydrolysis of the thiolester linkage to the ACP domain.[40] This suggests that in a recent experiment where a thiolesterase (TE) domain was engineered in place of the R domain to produce an orsellinate carboxylic acid rather than the aldehyde, the TE is





Scheme 3

superfluous.[41] Other parts of the xenovulene gene cluster were sequenced but we had problems carrying out further genetic anipulations, so we turned to the classical stipitatonic acid (**30**) producer, *Talaromyces stipitatum*.

The gene cluster responsible for biosynthesis of (**30**) was identified by homology to MOS and other *A. strictum* genes, and we were soon able by gene KO, heterologous expression and *in vitro* enzymology to demonstrate that tropolone biosynthesis required a NR-PKS, TropA, to produce 3-methyorcinaldehyde (**26**), which was ring hydroxylated (TropB) to give (**31**), methyl hydroxylation (TropC) then gives the un-isolated intermediate (**32**) which undergoes further TropC-mediated ring expansion to produce stipitaldehyde (**33**) as the first tropolone metabolite on the pathway.[42] Further recent work has allowed us to completely define the genetics and pathway of tropolone metabolism in *T. stipitatum*.[43] So I finish this update on our contributions (to date!) to fungal polyketide biosynthesis with a revisiting of one of the oldest biosynthetic problems in fungal natural products (Scheme 3).

7. Future prospects

There are many problems left to address. Full understanding of programming and control of assembly in all iterative PKSs is something that will pose major challenges in bringing protein expression, structural biology and biochemical assays together. Rapid genome sequencing is of course revolutionizing our area as much as any other. Many, many orphan gene clusters are being found as we realize that the real biosynthetic potential of these fascinating organisms far exceeds what we would have guessed in our wildest dreams. Great efforts are being made in unravelling the full polyketome of well characterized organisms such as *A. nidulans*, notably by the groups of Wang and Keller[44] and Larsen and Mortensen[45] using combinations of classical and molecular biology based approaches and switching on of silent clusters by manipulation of transcription factors[46] and other tricks[47] notably by the Hertweck and Brakhage groups.

One caveat is that while BLAST predictions of biosynthetic gene clusters can give tremendously useful information, it is vital to keep basic chemical and biochemical mechanisms (see CAT Viewpoint!) to the fore when predicting pathways based on them. Simple classical biosynthetic labelling and other experiments can give definitive answers so when that information is available it should be not be ignored or facts reinterpreted to fit convenient gene-based speculations.[48] An exciting prospect only

beginning to be tackled successfully is heterologous expression of whole fungal gene clusters (see Lazarus, Williams and Bailey) review herein. I for one look forward to many and as yet unforeseeable and exciting developments in fungal polyketide biosynthesis in the years and decades to come. Natural product chemistry can truly never have been at a more exciting stage.

Acknowledgements

My work over the last 40 years has been successful due to the sustained efforts of many brilliant graduate students who are too many to name individually, but whose contributions are gratefully acknowledged. In addition to the guidance and inspiration provided by my senior mentors Jake MacMillan, Stan Holker and Arthur Birch, I have been most fortunate in working with several senior colleagues, in particular Ian Sadler, Chris Willis, John Vederas, Russell Cox and Colin Lazarus, all of whom have contributed immeasurably not only to the success but also the fun of fungal polyketide biosynthesis. Finally, my thanks to John Vederas and Craig Townsend for their help and co-operation in this latest venture and all the other enjoyable interactions over too many years!

References

1 J. MacMillan, T. J. Simpson, A. E. Vanstone and S. K. Yeboah, *J. Chem. Soc., Perkin Trans. 1*, 1972, 2892–2898.
2 R. C. Durley, J. MacMillan, T. J. Simpson, A. T. Glen and W. B. Turner, *J. Chem. Soc., Perkin Trans. 1*, 1975, 163–169.
3 J. MacMillan and T. J. Simpson, *J. Chem. Soc., Perkin Trans. 1*, 1973, 1487–1493.
4 R. Goddard, I. K. Hatton, J. A. K. Howard, J. MacMillan, T. J. Simpson and C. J. Gilmore, *J. Chem. Soc., Perkin Trans. 1*, 1979, 1494–1498.
5 T. J. Simpson, *Chem. Soc. Rev.*, 1975, **4**, 497–522; J. C. Vederas, *Nat. Prod. Rep.*, 1987, **4**, 277–337.
6 J. S. E. Holker and L. J. Mulheirn, *Chem. Commun.*, 1968, 1576–1577.
7 C. A. Townsend, S. B. Christensen and K. Trautwein, *J. Am. Chem. Soc.*, 1984, **106**, 3869–3970.
8 J. S. E. Holker, R. D. Lapper and T. J. Simpson, *J. Chem. Soc., Perkin Trans. 1*, 1974, 2135–2140.
9 J. A. Gudgeon, J. S. E. Holker and T. J. Simpson, *J. Chem. Soc., Chem. Commun.*, 1974, 636–638.
10 T. J. Simpson and J. S. E. Holker, *Tetrahedron Lett.*, 1975, 4693–4696.
11 A. M. Hill and J. Staunton, *J. Chem. Soc., Chem. Commun.*, 1975, 861–862.
12 C. Abell and J. Staunton, *J. Chem. Soc., Chem. Commun.*, 1981, 856–858.
13 J. C. Vederas and T. T. Nakashima, *J. Chem. Soc., Chem. Commun.*, 1980, 183–185.
14 J. S. E. Holker and T. J. Simpson, *J. Chem. Soc., Chem. Commun.*, 1978, 626–627.
15 T. J. Simpson and D. J. Stenzel, *J. Chem. Soc., Chem. Commun.*, 1981, 1042–1043.
16 C. R. McIntyre and T. J. Simpson, *J. Chem. Soc., Chem. Commun.*, 1981, 1043–1044.
17 F. E. Scott, T. J. Simpson, L. A. Trimble and J. C. Vederas, *J. Chem. Soc., Chem. Commun.*, 1986, 214–215.
18 R. Geris and T. J. Simpson, *Nat. Prod. Rep.*, 2009, **26**, 1063–1094.
19 Y. Matsuda, T. Akagawa, T. Wakimoto and I. Abe, *J. Am. Chem. Soc.*, 2013, **135**, 10962–10965.
20 L. E. H. Bingle, T. J. Simpson and C. M. Lazarus, *Fungal Genet. Biol.*, 1999, **26**, 209–223.
21 T. P. Nicholson, C. M. Lazarus, B. A. M. Rudd, M. J. Dawson, T. J. Simpson and R. J. Cox, *Chem. Biol.*, 2001, **8**, 151–178.
22 R. J. Cox, *Org. Biomol. Chem.*, 2007, **5**, 2010–2016.
23 J. M. Crawford, B. C. R. Dancy, A. E. Hill, D. W. Udwary and C. A. Townsend, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 16728–16733.
24 J. M. Crawford, P. M. Thomas, J. R. Scheerer, A. L. Vagstad, N. L. Kelleher and C. A. Townsend, *Science*, 2008, **320**, 243–246.
25 T. Moriguchi, Y. Kezuka, T. Nonaka, Y. Ebizuka and I. Fujii, *J. Biol. Chem.*, 2010, **285**, 15637–15643.
26 J. Kennedy, K. Auclair, S. G. Kendrew, C. Park, J. C. Vederas and C. R. Hutchinson, *Science*, 1999, **284**, 1368–1372.
27 M. N. Heneghan, A. A. Yakasai, K. Williams, K. A. Kadir, W. Bakeer, K. M. Fisch, Z. Wasil, A. M. Bailey, T. J. Simpson, R. J. Cox and C. M. Lazarus, *Chem. Sci.*, 2011, **2**, 972–979.
28 R. J. Cox, F. Glod, D. Hurley, C. M. Lazarus, T. P. Nicholson, B. A. M. Rudd, T. J. Simpson, B. Wilkinson and Y. Zhang, *Chem. Commun.*, 2004, 2260–2261.
29 I. Fujii, Y. Ono, H. Yada, Y. Ebizuka and U. Sankawa, *Mol. Gen. Genet.*, 1996, **253**, 1–10.
30 Z. Song, R. J. Cox, C. M. Lazarus and T. J. Simpson, *ChemBioChem*, 2004, **5**, 1196–1203.
31 D. O. Rees, N. Bushby, R. J. Cox, J. R. Harding, T. J. Simpson and C. L. Willis, *ChemBioChem*, 2007, **8**, 46–50.
32 M. R. Andersen, J. B. Nielsen, A. Klitgaard, L. M. Petersena, M. Zachariasen, T. J. Hansen, L. H. Blicher, C. H. Gotfredsen, T. O. Larsen, K. F. Nielsen and U. H. Mortensen, *Proc. Natl. Acad. Sci. U. S. A.*, 2010, **110**, 24–25.
33 K. L. Eley, L. M. Halo, Z. Song, H. Powles, R. J. Cox, A. M. Bailey, C. M. Lazarus and T. J. Simpson, *ChemBioChem*, 2007, **8**, 289–297.
34 L. M. Halo, J. W. Marshall, A. A. Yakasi, Z. Song, C. P. Butts, M. P. Crump, M. Heneghan, A. M. Bailey, T. J. Simpson, C. M. Lazarus and R. J. Cox, *ChemBioChem*, 2008, **9**, 585–594.
35 M. N. Henegan, A. A. Yakasai, L. M. Halo, Z. Song, A. M. Bailey, R. J. Cox, T. J. Simpson and C. M. Lazarus, *ChemBioChem*, 2010, **11**, 1508–1512.
36 S. M. Ma, J. W.-H. Li, J. W. Choi, H. Zhou, K. K. M. Lee, V. A. Moorthie, X. Xie, J. T. Kealey, N. A. Da Silva, J. C. Vederas and Y. Tang, *Science*, 2009, **326**, 589–592.
37 K. M. Fisch, W. Bakeer, A. A. Yakasai, Z. Song, J. Pedrick, Z. Wasil, A. M. Bailey, C. M. Lazarus, T. J. Simpson and R. J. Cox, *J. Am. Chem. Soc.*, 2011, **133**, 16635–16641.

38 M. E. Raggatt, T. J. Simpson and M. I. Chicarelli-Robinson, *Chem. Commun.*, 1997, 2245–2246.
39 A. M. Bailey, R. J. Cox, K. Harley, C. M. Lazarus, T. J. Simpson and E. Skellam, *Chem. Commun.*, 2007, 4053–4055.
40 K. M. Fisch, E. Skellam, D. Ivison, R. J. Cox, A. M. Bailey, C. M. Lazarus and T. J. Simpson, *Chem. Commun.*, 2010, **46**, 5331–5333.
41 H.-H. Ye, S.-L. Chang, Y. M. Chiang, K. S. Bruno, B. R. Oakley, T.-K. Wu and C. C. C. Wang, *Org. Lett.*, 2013, **15**, 756–759.
42 J. Davison, A. al Fahad, M. Cai, Z. Song, S. y. Yehia, C. M. Lazarus, A. M. Bailey, T. J. Simpson and R. J. Cox, *Proc. Natl. Acad. Sci. U. S. A.*, 2012, **109**, 7642–7647.
43 A. al Fahad, A. Abood, T. J. Simpson and R. J. Cox, *Angew. Chem., Int. Ed.*, 2014, **53**, 7519–7523.
44 Y.-M. Chiang, E. Szewczyk, A. D. Davidson, R. Entwistle, N. P. Keller, C. C. C. Wang and B. R. Oakley, *Appl. Environ. Microbiol.*, 2010, **76**, 2067–2074.
45 M. L. Nielsen, J. B. Nielsen, C. Rank, M. L. Klejnstrup, D. K. Holm, K. H. Broggard, B. J. Hansen, J. C. Frisvad, T. O. Larsen and U. H. Mortensen, *FEMS Microbiol. Lett.*, 2011, **321**, 157–166.
46 S. Beergmann, J. Schumann, K. Scherlach, C. Lange, A. A. Brakhage and C. Hertweck, *Nat. Chem. Biol.*, 2007, **3**, 213–217.
47 A. A. Brakhage, *Nat. Rev. Microbiol.*, 2013, **11**, 21–32.
48 T. J. Simpson, *ChemBioChem*, 2012, **13**, 1680–1688.