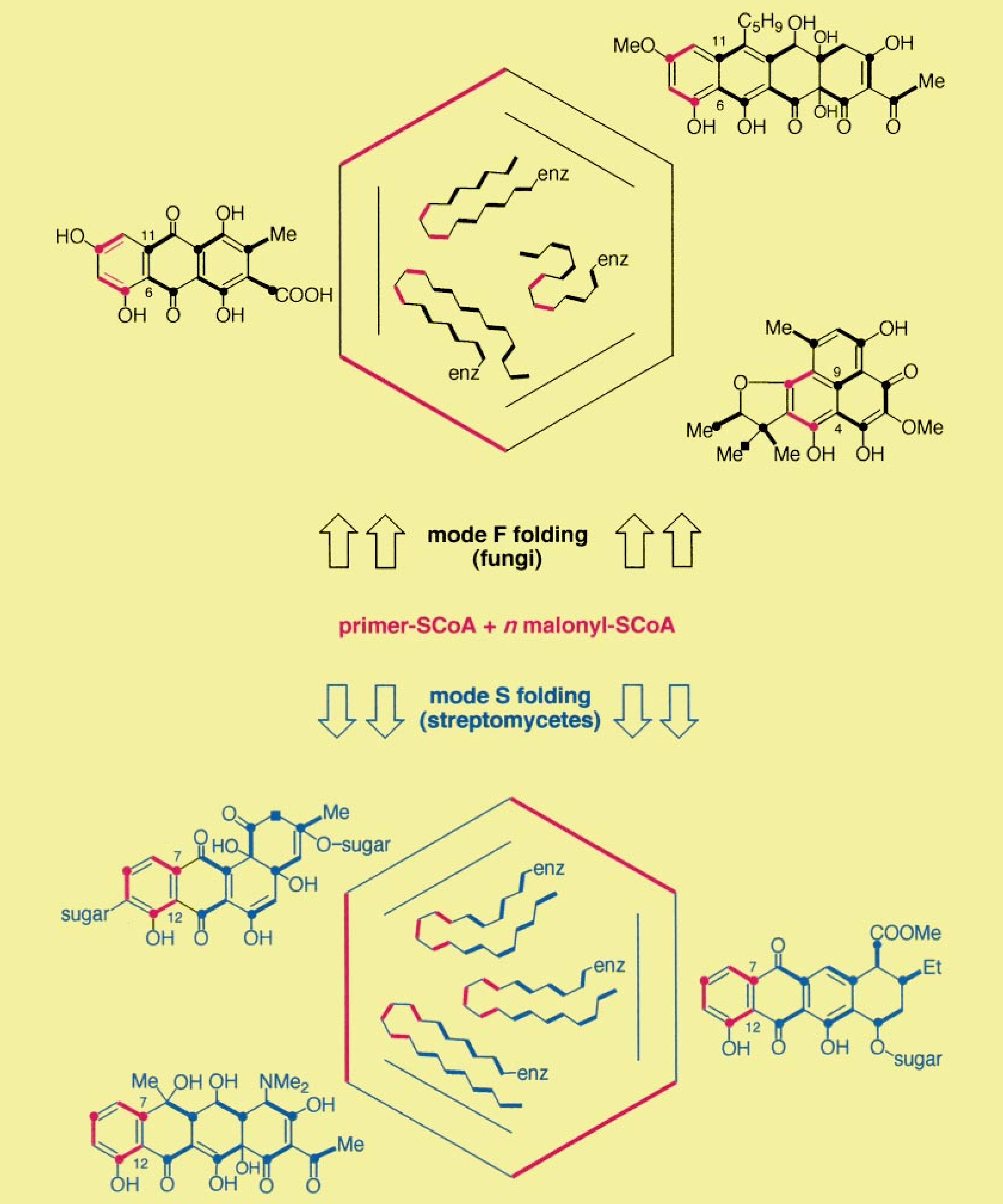
A biosynthetic whodunit–fungus or bacterium?



A Biosynthetic Classification of Fungal and Streptomycete Fused-Ring Aromatic Polyketides

Robert Thomas*[a]

Dedicated to the late Professor Harold Raistrick (F.R.S.) in recognition of his seminal contributions to the chemistry of fungi

Polyketides are one of the largest and most structurally diverse classes of naturally occurring compounds, ranging from simple aromatic metabolites to complex macrocyclic lactones. Fungi and filamentous bacteria, particularly the actinomycetes, are major sources of polycyclic aromatic structures, which include many clinically important antibiotics and other useful metabolites. These fused-ring polyketides are formed by the action of polyketide synthases (PKSs), which catalyse the assembly, folding and cross-linking of poly-β-ketoacyl intermediates. In view of the taxonomic gulf between the eukaryotic fungi and prokaryotic bacteria, it is not surprising that they are rarely found to produce structurally identical fused-ring metabolites. A review of [$^{13}C_2$]acetate incorporation data has revealed consistent differences in the reported cyclisation patterns, which require regiospecifically distinct cross-linking of otherwise identical linear polyketide precursors. This observation provides the basis for a structural and biosynthetic classification of microbial fused-ring polyketides, which has a number of useful ramifications.

KEYWORDS:

antibiotics · biosynthesis · fungi · polyketides · streptomycetes

1. Introduction

The first systematic study of fungal metabolites was initiated soon after World War I by Harold Raistrick, who in the course of the following four decades made a seminal contribution to the recognition of fungi as a major source of natural products.[1] The discovery that filamentous bacteria and especially the actinomycetes are an equally fertile source of novel compounds,[2] was an unanticipated bonus of the search for new antibiotics following the clinical and industrial development of penicillin during the World War II.

Not surprisingly, the taxonomic gulf between the eukaryotic fungi and prokaryotic actinomycetes is reflected in the structural and biosynthetic individuality of their respective secondary metabolites. Although both phyla produce a wide diversity of products which have provided many challenging synthetic targets,[3] they have rarely been shown to yield structurally identical compounds.

Pre-eminent among the metabolites of fungi and actinomycetes are the polyketides, pivotal to our understanding of the biosynthesis of which have been the landmark contributions of Birch[4] and Hopwood.[5] The major group of aromatic fused-ring polyketides include medicinally important substances of long-established utility, such as the tetracycline antibiotics and antitumour anthracyclines. These are formed by the action of polyketide synthases (PKSs) which catalyse the assembly and subsequent cyclisation of poly-β-ketoacyl intermediates through intramolecular aldol and Claisen condensations.

On the other hand, alicyclic fused-ring metabolites, such as the fungal hypocholesteraemic agents compactin and mevinolin (*syn*-lovastatin), form a separate group of polycyclic polyketides which appear to undergo partial fatty-acid-like reduction of intermediate keto groups prior to cyclisation. The absence of suitably juxtaposed latent bridgehead carbonyl and carbanion groups necessitates cross-linking by non-aldol mechanisms such as Diels – Alder cycloaddition or epoxide and endoperoxide ring opening.[6, 7]

The primary focus of this review concerns the relative modes of formation of the respective aromatic fused-ring polyketides of fungi and streptomycetes and, to a lesser extent, those of higher plants. Analysis of [$^{13}C_2$]acetate incorporation data indicates the consistent utilisation of different folding and cyclisation pathways in fungi and bacteria, giving rise to two structurally distinct groups of aromatic metabolites. These contrasting pathways provide the basis of a general rule for the biosynthetic classification of this major group of polyketides.

2. Distribution of intact C_2 units in fungal fused-ring polyketides

The distribution of intact acetate-derived C_2 units in cyclic polyketides, and hence the geometry of folding of linear intermediates, can be determined through precursor feeding studies using [$^{13}C_2$]acetate.[8, 9] This requires the unambiguous assignment of paired $^{13}C-^{13}C$ couplings in the ^{13}C NMR spectra of biosynthetically labelled metabolites.

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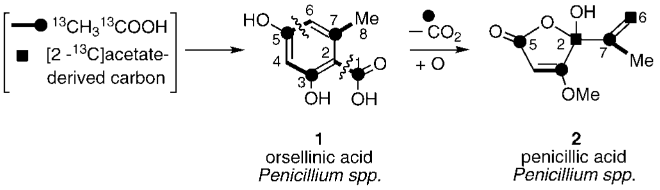
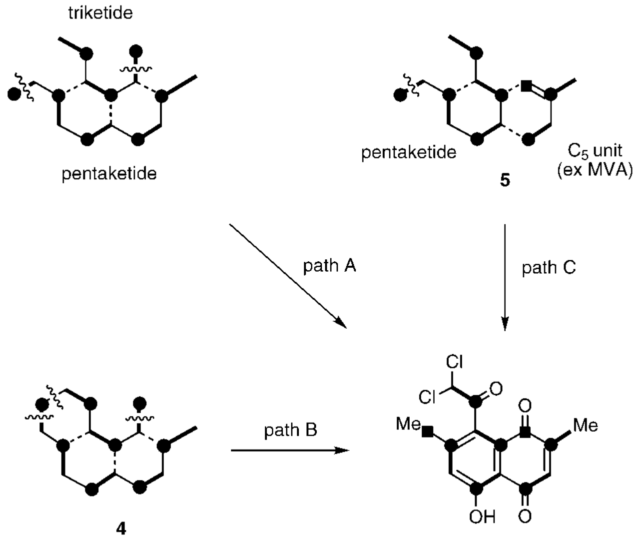
In one of the earliest applications of this method, Seto, Carey and Tanabe[10] determined the mode of cleavage of the fungal monocyclic tetraketide orsellinic acid (**1**) leading to penicillic acid (PA, **2**) (Scheme 1). The observed couplings of [$^{13}C_2$]acetate-derived PA require a regiospecific ring fission, in agreement with earlier studies based on the chemical degradation of [^{14}C]acetate-labelled PA[11, 12] and ^{3}H NMR spectroscopic assignments of [^{3}H]acetate-labelled PA.[13]



1
orsellinic acid
Penicillium spp.

2
penicillic acid
Penicillium spp.

Scheme 1. *Incorporation of [$^{13}C_2$]acetate into penicillic acid. The numbering of the carbon atoms of these and all subsequent structures corresponds to that of the parent polyketide, unless otherwise stated.*



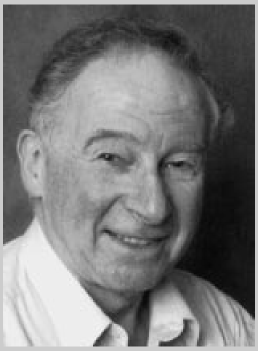
5

4
octaketide

3
mollisin
Mollisia caesia

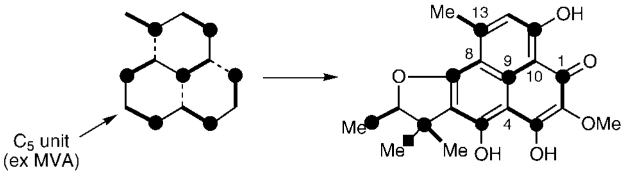
Scheme 2. *Incorporation of [$^{13}C_2$]acetate into mollisin (**3**).*

These authors also described the first application of this method to the investigation of an aromatic fused-ring metabolite, namely mollisin (**3**),[8] a structurally atypical naphthoquinone constituent of *Mollisia caesia*. The observed $^{13}C-{}^{13}C$ couplings were interpreted as favouring a branched-chain intermediate derived from a triketide and a pentaketide (Scheme 2, path A). This, however, does not preclude alternative single-chain polyketide pathways, either through ring cleavage of a phenanthrene-like octaketide **4**[9, 14] (Scheme 2, path B), or via an isopentenylated skeleton **5** derived from a pentaketide and mevalonate (MVA)[15] as in path C of Scheme 2.



Robert Thomas,
born in 1927, was awarded a PhD for research on the chemistry of fungal metabolites under Harold Raistrick at the London School of Hygiene and Tropical Medicine (LSHTM). Between 1951 and 1956, he undertook investigations of fungal cellulases initially with the CSIRO in Melbourne and then the NRC in Ottawa, following which he returned to the LSHTM to initiate studies on the biosynthesis of polyketides. He joined the Squibb Institute for Medical Research in New Jersey in 1959 and investigated the biosynthesis of various antibiotics and also indole alkaloids. In 1963, he was appointed to a senior lectureship in the Biochemistry Department headed by Sir Ernst Chain at Imperial College in London, where he directed a programme of biosynthetic studies. In 1969, he moved to the University of Surrey, where he was a Research Professor of Chemistry and subsequently Founding Director of the Biotechnology Unit. He launched Biotics Limited in 1983 and since 1987 has been based at the University of Sussex, where he is also a Honorary Professor.

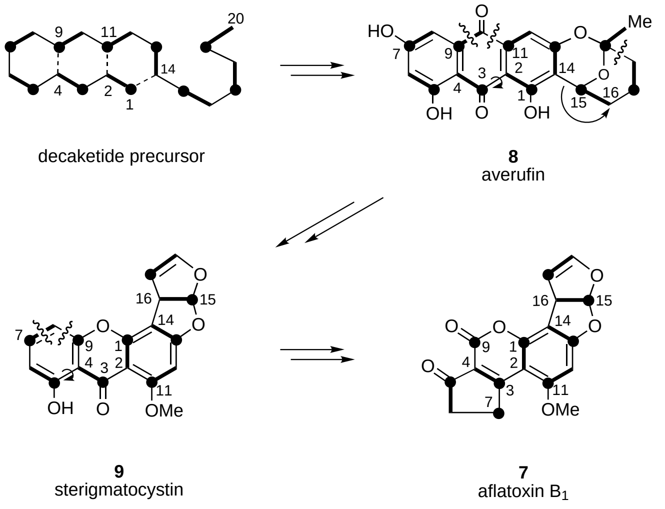
The rare tricyclic phenalenone fused ring system of the *Penicillium herquei* heptaketide deoxyherqueinone (**6**),[16] could in theory form by three different modes of folding of a linear intermediate. However, only one of these accommodates the couplings observed by Simpson in [$^{13}C_2$]acetate-derived **6** (Scheme 3).[17]



6
deoxyherqueinone
Penicillium herquei

Scheme 3. *The heptaketide folding pattern of fungal phenalenones, as exemplified by deoxyherqueinone (**6**).*

Structurally complex *Aspergillus* fused-ring polyketides studied in this manner include the potent carcinogen aflatoxin B_1 (**7**) and its precursors averufin (**8**) and sterigmatocystin (**9**) (Scheme 4).[18, 19] The resulting $^{13}C-{}^{13}C$ couplings were consistent with the proposed formation of the heterocyclic skeletons of **7** and **9** through oxidative ring cleavages of an anthraquinone intermediate[20] and derivation of the bisfuranoid moiety through oxidative rearrangement of the linear C_6 substituent of averufin (**8**).[21] Subsequent labelling studies and genetic investigations have clarified many details of the overall pathway, including the key finding that the C_6 substituent of averufin (**8**) is derived from an *n*-hexanoyl starter unit.[22]



Scheme 4. *Incorporation of [$^{13}C_2$]acetate into aflatoxin-related metabolites.*

3. Fungi and streptomycetes utilise different polyketide-folding strategies

Streptomycetes similarly form a wide variety of fused-ring aromatic polyketides, including some with carbon skeletons that closely parallel those produced by fungi. However, with very few exceptions, such as the melanin spore pigment precursor 1,3,6,8-tetrahydroxynaphthalene (THN) produced, for example, by the fungus *Alternaria alternata*[23] and a recombinant strain of *Streptomyces griseus*,[24] fungi and streptomycetes do not appear to form identical fused-ring structures.[25]

This contrasts with the production by both classes of microorganisms of simple monocyclic compounds, such as the fungal tetraketide orsellinic acid (**1**)[26] and its deoxy product 6-methylsalicylic acid (6-MSA).[27] Derivatives of both of these products are constituents of the streptomycete metabolites everninomycin B[28] and polyketomycin.[29] 6-MSA was first isolated from *Penicillium griseofulvum* by Anslow and Raistrick in 1931,[27] while the 6-MSA synthase of *P. patulum* was the first PKS to be investigated in a series of studies initiated in the 1960s by Lynen and co-workers.[30]

The *Streptomyces rimosus* antibiotic oxytetracycline (**10**) (Figure 1) is a tetracyclic nonaketide[31, 32] which has been shown to incorporate an intact malonate starter unit and eight malonate-derived C_2 extender units.[33] Although the timing of the introduction of the amide nitrogen atom is not known, the candidate primer malonamyl acyl carrier protein (ACP) could be formed by amidation of malonyl-CoA or malonyl-ACP.

Acetyldecarboxamidooxytetracycline (ADCOTC, **11**)[34] is a decaketide co-metabolite of **10**, which requires an acetyl starter unit (possibly generated through decarboxylation of malonyl-ACP[35]) and is presumably assembled via acetoacetyl and triacetyl-ACP intermediates. Since the diketide acetoacetyl-ACP is a carbon isostere of malonamyl-ACP, the similarity in chain lengths of the respective linear nonaketide and decaketide precursors of **10** and **11** may allow their assembly and cyclisation in *S. rimosus* by common PKS chain-length-determining and cyclising enzymes.

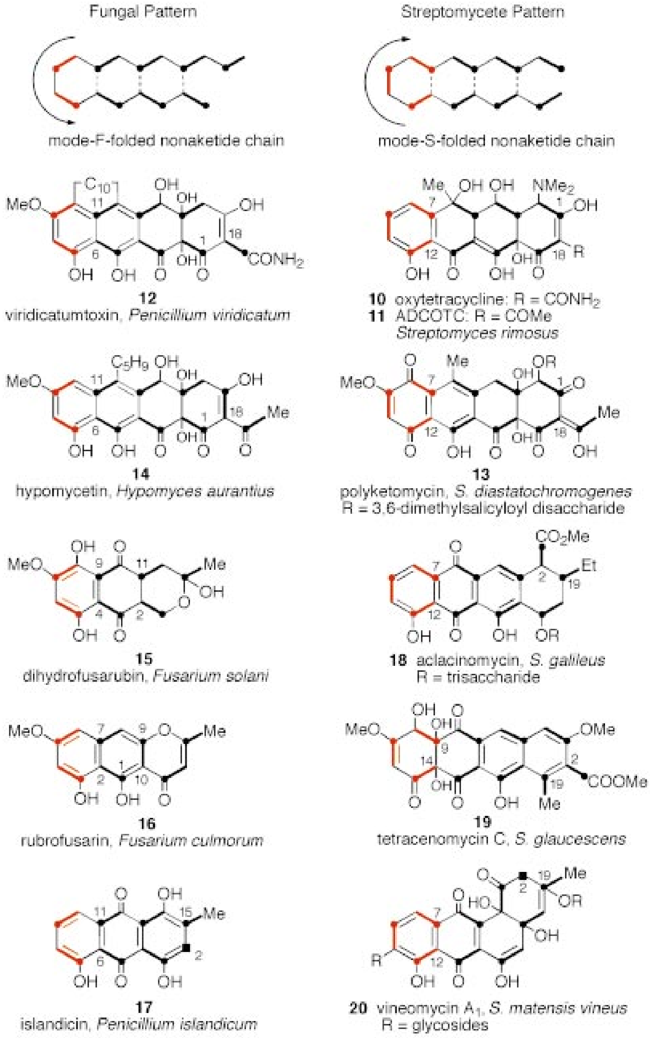


Figure 1. *Distribution of intact C_2 units in microbial fused-ring aromatic polyketides (red bonds denote intact C_2 units in the initial rings of these and subsequent structures).*

Investigations of the incorporation of [$^{13}C_2$]acetate into oxytetracycline (**11**)[32] and the structurally related *Penicillium viridicatum* tetracyclic amide viridicatumtoxin (**12**)[36] unexpectedly revealed two different folding strategies (Figure 1). Similarly contrasting folding patterns are also apparent in the corresponding decaketides polyketomycin (**13**),[29] a product of *Streptomyces diastatochromogenes*, and hypomycetin (**14**),[37] a metabolite of the fungus *Hypomyces aurantius*.

Particularly noteworthy is the derivation of the initial cyclohexanoid rings of both of the bacterial polyketides **10** and **13** from three intact C_2 units (hereafter designated polyketide-folding mode S). This contrasts with the incorporation of only two intact C_2 units in the initial rings of the corresponding fungal tetracyclines **12** and **14** (folding mode F).

Other fungal metabolites shown in Figure 1 include the naphthalene derivatives dihydrofusarubin (**15**),[38] rubrofusarin (**16**)[39] and the anthraquinone islandicin (**17**),[40] all of which conform to mode F folding. Additional tetracyclic streptomycete polyketides shown in this figure are the decaketides aclacinomycin (**18**),[41] tetracenomycin C (**19**)[42] and the angucycline vineomycin A_1 (**20**),[43] which are mode-S-folded.

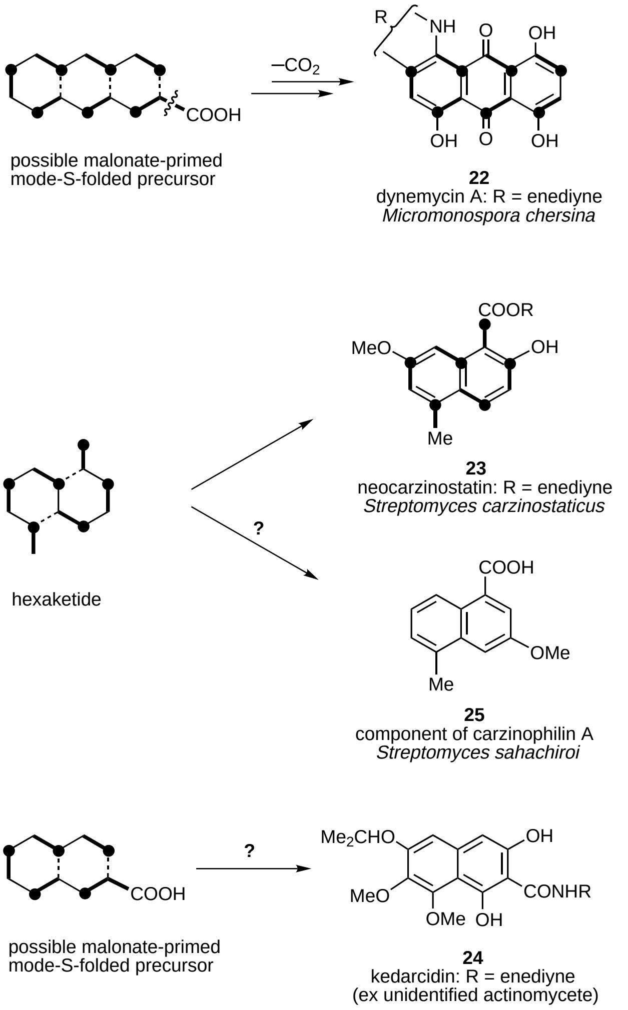
The incorporation of $[^{13}C_2]$acetate into tetracenomycin C (**19**) does not appear to have been studied per se. However, the proposed derivation of the initial ring of tetracenomycin C from three intact C_2 units is in accord with extensive genetic and enzymatic evidence.[42]

The distribution of intact C_2 units in these representative metabolites is consistent with the selective utilisation of mode F and mode S cyclisation pathways in the formation of the respective fused-ring polyketides of fungi and streptomycetes. A possible exception to this generalisation is apparent in the reported labelling assignments of the naphthopyrone fonsecin, a metabolite of the fungus *Aspergillus fonsecaeus*.

The interpretation of the ^{13}C NMR spectrum of $[^{13}C_2]$acetate-derived fonsecin leading to the isotopic carbon distribution **21S** proposed by Bloomer, Smith and Caggiano[44] is based on the assignments summarised in Table 1. This necessitates mode S folding and thereby differs from that of its anhydro derivative rubrofusarin (**16**) (Figure 1).[39] However, the reported values do not appear to exclude the alternative labelling pattern of **21F**, in which the distribution of seven intact C_2 units parallels that observed by Leeper and Staunton in their NMR spectroscopic study of the methyl ethers of $[^{13}C_2]$acetate-derived rubrofusarin.

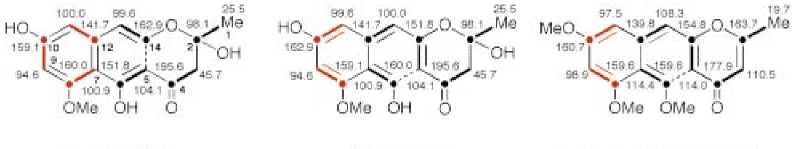
4. Aromatic fused-ring constituents of enediyne antibiotics

Some bacterial enediyne antibiotics also contain aromatic fused-ring components. These include the anthraquinone **22**, the 5-methyl-1-naphthoic acid ester **23** and the 2-naphthoic acid amide **24**, which are, respectively, constituents of dynemicin A, neocarzinostatin and kedarcidin (Scheme 5).[45, 46] $[^{13}C_2]$Acetate



Scheme 5. *Aromatic fused-ring constituents of enediyne antibiotics and related structures.*

Table 1. *^{13}C NMR spectroscopic assignments of $[^{13}C_2]$acetate-derived fonsecin folding options **21S** and **21F** and rubrofusarin dimethyl ether.*[a, b]



fonsecin **21S**
(mode S folding)

fonsecin **21F**
(mode F folding)

rubrofusarin dimethyl ether
(mode F folding)

*Fonsecin folding mode **21S** assignments*[44]														
coupled carbons	1	2	3	4	5	6	7	8	9	10	11	12	13	14
δ [ppm]	25.5	98.1	45.7	195.6	104.1	151.8	100.9	160.0	94.6	159.1	100.0	141.7	99.6	162.9
J [Hz]	–	46	40	40	59	59	70	70	72	71	56	56	66	66
*Fonsecin folding mode **21F** assignments*														
coupled carbons	1	2	3	4	5	14	7	6	9	8	13	12	11	10
δ [ppm]	25.5	98.1	45.7	195.6	104.1	151.8	100.9	160.0	94.6	159.1	100.0	141.7	99.6	162.9
J [Hz]	–	46	40	40	59	59	70	70	72	71	56	56	66	66
Rubrofusarin dimethyl ether assignments[34]														
coupled carbons	1	2	3	4	5	14	7	6	9	8	13	12	11	10
δ [ppm]	19.7	163.7	110.5	177.9	114.0	154.8	114.4	159.6	98.9	159.6	108.3	139.8	97.5	160.7
J [Hz]	52	52	57	57	61	61	70	70	75	75	59	59	59	61

[a] Carbon atoms are numbered as in ref. [44]. [b] Broken bonds show cross-links forming the second carbocyclic ring.

labelling studies have established the polyketide origins of the anthraquinone **22**,[47] the naphthalene **23**[48] and their enediyne substituents.

The pathways leading to the enediyne moieties of this group of antibiotics remain uncertain despite extensive labelling, gene-cloning and blocked-mutant studies. Thus, it has not yet proved possible to distinguish between the derivation of enediynes via fatty acid intermediates, de novo polyketide biosynthesis involving type I or type II PKSs, or other mechanisms such as the retro-Bergman reaction of an aromatic intermediate.[46, 49]

In **22**, the only carbon substituent of the anthraquinone is the enediyne moiety, to which it is linked through a bond derived from two [1-^{13}C]acetate-derived carbons, consequently the mode of folding of this anthraquinone is not readily discernible. It has been suggested that the anthraquinone constituent of dynemicin may result from cyclisation of an enediyne rather than a poly-β-ketoacyl heptaketide.[45, 47] Alternatively, **22** could be formed by decarboxylation of a mode-S-folded 2-carboxyanthraquinone intermediate which retains an intact malonate starter unit, as does oxytetracycline (**10**).[33]

If the unusual 1,5-disubstituted bicyclic ring system of **23** is formed through intramolecular aldol condensations, then the observed mode of incorporation of two intact C_2 units in each ring would differ from the general mode S pattern exhibited by the bacterial polyketides shown in Figure 1. In this context, it is interesting to compare the structurally related disubstituted naphthalene constituent **25** of the *Streptomyces sahachiroi* antibiotic carzinophilin A,[50] which is identical to the *Streptomyces griseofuscus* metabolite azinomycin B.[51, 52] Although this has the same carbon skeleton as **23**, its naphthalene rings have only one oxygen substituent which, if **25** is a hexaketide, would be attached to a [2-^{13}C]malonate-derived carbon atom and therefore probably introduced by oxidation. The absence of oxygen substituents on the [1-^{13}C]malonate-derived carbon atoms of either ring favours the derivation of **25** by cyclisation of a reduced polyketide through a non-aldol mechanism, which could similarly give rise to **23**. Conceivably, the naphthalene rings of **23** and **25** could result from cyclisation of an enediyne, as has been suggested for the derivation of the anthraquinone moiety of the dynemicins.[45, 47] As previously pointed out, under these circumstances the mode F and mode S classification criteria would not apply.

While the origin of the kedarcidin 2-naphthoic acid component of **24** remains to be determined, it has an amido substituent analogous to that of oxytetracycline (**10**) and accordingly may be formed by mode S folding of a pentaketide which retains an intact malonate starter unit. It is noteworthy that this naphthoic acid amide is a derivative of 1,3,6,8-tetrahydroxynaphthalene (THN). As noted in Section 3, THN is a rare example of a fused-ring polyketide produced by streptomycete and fungal PKSs; furthermore, both synthases have been shown to utilise a malonate starter unit.[24, 53] Since the malonate carboxyl group is eliminated during the formation of THN, the resulting symmetrical structure obscures the geometry of folding, thus leaving open the possibility that it may be biosynthesised in fungi and streptomycetes through different cyclisation pathways (as discussed later in Section 5).

5. A general rule for the biosynthetic classification of fungal and streptomycete fused-ring aromatic polyketides

The distribution of intact C_2 units in the [$^{13}C_2$]acetate-derived carbon skeletons of the fungal polyketides dihydrofusarubin (**26**), rubrofusarin (**27**), averufin (**28**), islandicin (**29**) and hypomycetin (**30**), and the streptomycete products aclacinomycin (**31**), tetracenomycin C (**32**) and polyketomycin (**33**) is illustrated in Figure 2. The full structures of these compounds are shown in Figure 1, other than that of averufin (**8**), which is shown in Scheme 4. Carbon skeletons of other products in Figure 2 for which the [$^{13}C_2$]acetate incorporation patterns have been determined include the fungal metabolite bikaverin (**34**)[54] and the streptomycete products actinorhodin (**35**),[55] spectomycin A (**36**)[56] and RM18[57, 58] (**37**; RM18 is formed by a streptomycete recombinant strain).

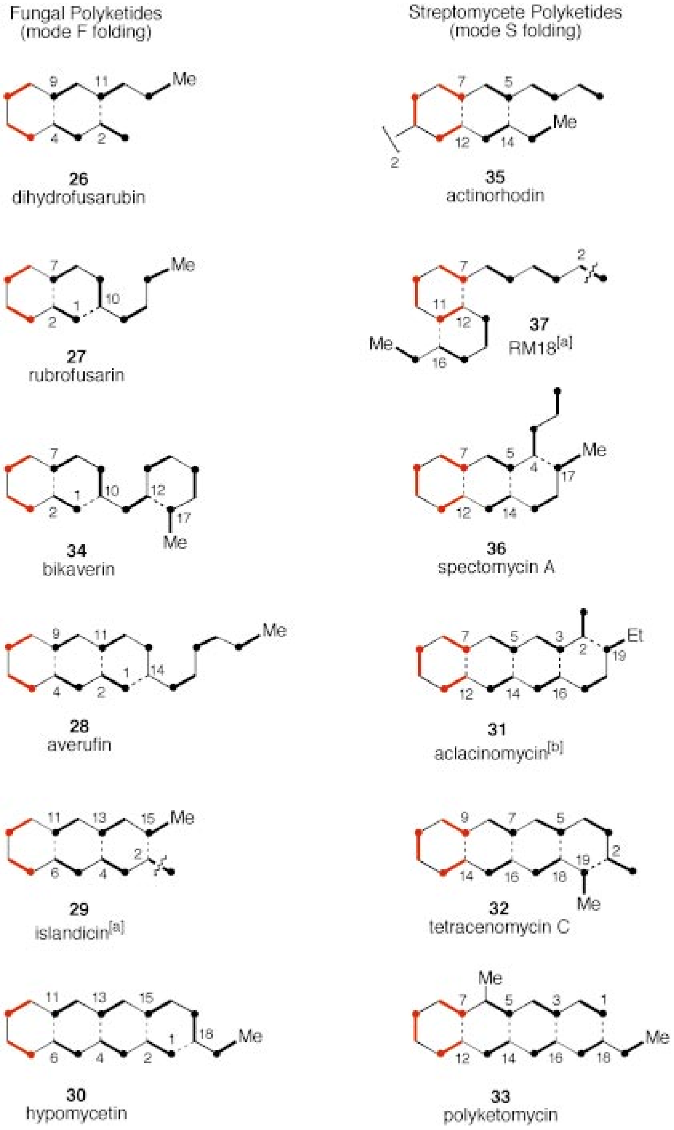


Figure 2. *Distribution of C_2 units in the carbon skeletons of aromatic polyketides. The complete structures of the majority of these compounds are shown in Figure 1 and Scheme 4. [a] Decarboxylated polyketides. [b] The starter unit in aclacinomycin (**31**) is propionate.*

The above [$^{13}C_2$]acetate incorporation results are collectively representative of a broad range of carbocyclic fused-ring products of microbial origin, in which cross-linking takes place through aldol or Claisen condensations. It is evident from the observed labelling patterns that, with the possible exception of the naphthopyrone fonsecin (**21**) (Table 1), all of these fungal metabolites exhibit mode F folding. On the other hand, the streptomycete metabolites are all mode-S-folded. Only the atypical 1,5-disubstituted naphthalene moiety of the enediyne neocarzinostatin (**23**) appears to necessitate mode F folding, irrespective of which ring is initially formed. However, as noted earlier, its cyclisation through an aldol condensation is not the only option, in which event the polyketide mode F and mode S folding criteria would not apply.

These observations are consistent with a general rule for the biosynthetic classification of fused-ring aromatic polyketides of fungi and filamentous bacteria, based on the regiospecificity of aldol cross-linking leading to the initial carbocyclic ring. The archetypal mode-F- and mode-S-derived carbon skeletons shown in Figure 3 illustrate the incorporation patterns of malonate-derived C_2 units in the initial rings and their two *ortho*-oriented carbon side chain substituents. The classification criteria underlying these incorporation patterns are outlined in Table 2.

Thus, in fungal metabolites, mode F folding of a linear polyketide chain yields an initial cyclohexane ring containing *two* intact C_2 units with two adjacent side chains, both of which have an *odd* number of carbon atoms. The corresponding streptomycete polyketides exhibit mode S folding, leading to an initial ring derived from *three* intact C_2 units and also having two adjacent side chains, but with *even* numbers of carbon atoms in each side chain. These characteristics of fungal and streptomycete fused-ring polyketides are exemplified in Figure 3 by the respective carbon skeletons of averufin (**8**) and polyketomycin (**13**). Streptomycete polyketides frequently utilise propionate starter units, which consequently give rise to an initial ring with a primer side chain containing an odd number of carbon atoms, as in aclacinomycin (**18**). Here, the propionate unit is the source of the ethyl substituent of the terminal ring.

Table 2. *Classification of fused-ring polyketides of fungi and streptomycetes based on the mode of cyclisation of the initial ring.*[a]

Fungal polyketides: C_2 unit distribution (mode F folding)
(i) The initial benzene ring contains 2 malonate-derived intact C_2 units
(ii) Primer side chain $C_p = p + n_p$ intact C_2 units + 1 [2-^{13}C]malonate-derived benzylic carbon atom $= p + 2n_p + 1$ carbon atoms
(iii) Terminal side chain $C_t = n_t$ intact C_2 units + 1 [1-^{13}C]malonate-derived benzylic carbon atom $= 2n_t + 1$ carbon atoms
Where the primer unit is acetate ($p = 2$), both side chains have an *odd* number of carbon atoms

Streptomycete polyketides: C_2 unit distribution (mode S folding)
(i) The initial benzene ring contains 3 malonate-derived intact C_2 units
(ii) Primer side chain $C_p = p + n_p$ intact C_2 units $= p + 2n_p$ carbon atoms
(iii) Terminal side chain $C_t = n_t$ intact C_2 units $= 2n_t$ carbon atoms
Where the primer unit is acetate ($p = 2$), both side chains have an *even* number of carbon atoms

[a] The initial rings of fungal and streptomycete polyketides, represented by the structures in Figures 3 a and b, respectively, have two vicinal side chains derived from a primer unit (e.g. acetate or propionate) and malonate-derived extender units. The following abbreviations also apply to the structures shown in Figure 3: C_P = primer unit side chain, C_t = terminal carboxyl side chain, p = primer unit carbon atoms, n_p and n_t = number of intact extender C_2 units in the C_p and C_t side chains, respectively.

On inspection of the carbon skeletons in Figure 2, it can be seen that the first ring of each of the fungal products involves

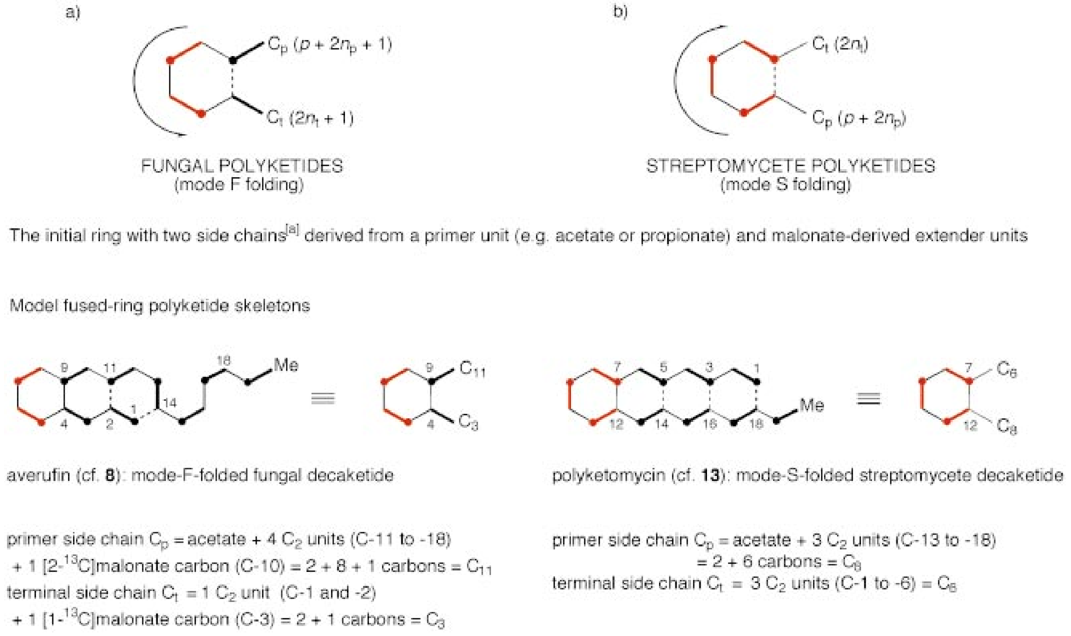


Figure 3. *Archetypal carbon skeletons of monocyclic precursors of mode-F- and mode-S-folded fused-ring polyketides and their corresponding acetate incorporation patterns (as detailed in Table 2). [a] The following side chain abbreviations are also used in Table 2: C_p = primer unit side chain, C_t = terminal carboxyl side chain, p = primer unit carbon atoms, n_p and n_t = number of intact extender C_2 units in the C_p and C_t side chains, respectively.*

cross-linking of mode-F-folded polyketide chains between C-2/C-7, C-4/C-9 or C-6/C-11. This differs from the mode S folding of the fused-ring metabolites of wild-type strains of streptomycetes, in which initial cross-linking takes place between C-7/C-12 or C-9/C-14. Examples of these cyclisation patterns are listed in Table 3, together with other cross-linking patterns observed in so-called "unnatural" metabolites of recombinant streptomycetes. These include the mode-S-folded naphthalene RM18b[57] (an octaketide analogue of RM18 (**37**)), TW94b[58] and TW93h.[59] The initial C-5/C-10 aldol condensation of RM18b and TW94b is infrequently observed, while the C-13/C-18 cross-link of the complex 2,4-dioxoadamantane dodecaketide TW93h is without precedent. The atypical cross-linking of the fungal polyketide fonsecin (**21**) has already been noted and those of the hybrid streptomycete structures TW93f and TW93g are discussed later.

Table 3. *Initial cyclisations of representative fungal and streptomycete aromatic fused-ring polyketides.*[a]

Cross-link	Compound	Source	Ref.
Typical mode F cross-linking			
C-2/C-7	rubrofusarin (**16**)	ex fungus	[39]
C-4/C-9	averufin (**8**)	ex fungus	[19]
C-6/C-11	islandicin (**17**)	ex fungus	[40]
Typical mode S cross-linking			
C-5/C-10	RM18b, TW94b	ex streptomycete (hybrid)	[57, 58]
C-7/C-12	aclacinomycin (**18**)	ex streptomycete	[41]
C-9/C-14	tetracenomycin C (**19**)	ex streptomycete	[42]
C-13/C-18	TW93h	ex streptomycete (hybrid)	[59]
Atypical cross-linking			
C-3/C-8	fonsecin (**21S**)[b]	ex fungus	[44]
C-8/C-13	TW93f (**67**)	ex streptomycete (hybrid)	[59]
C-10/C-15	TW93g (**68**)	ex streptomycete (hybrid)	[59]

[a] Carbon atoms numbered as in the parent polyketide. [b] See the alternative C-2/C-7 cross-linked mode-F-folded structure **21F** (Table 1).

In fungal products, the U-turn fold leading to the first ring is rarely initiated beyond carbon atom 6 of the nascent chain, whereas the corresponding cross-link in naturally occurring streptomycete products seldom commences before carbon atom 7. Either F- or S-folding mode could arise from comparatively minor differences in the location of PKS binding pockets. Thus, regiospecific folding of adjacent segments of a linear octaketide could generate conformations favourable to F or S cyclisation (Figure 4). Further cross-linking could subsequently give rise to the respective polycyclic carbon skeletons of the fungal anthraquinone islandicin (**29**) or the streptomycete naphthoquinone actinorhodin (**35**).

A simple ring-forming sequence may, for example, involve growth of a polyketide on its synthase with partial folding until further migration of the starter unit is blocked. Two additional extender units could then be accommodated with mode F or S folding in strategically located midchain cyclisation pockets, yielding fully grown chains with conformations conducive to aldol cross-linking.

The above cyclisation characteristics suggest that the binding pocket of a fungal fused-ring PKS may be located closer to the polyketide carboxyl terminus than that of a typical bacterial synthase. Such differences would not be surprising in view of the contrasting size and shape of iterative fungal type I aromatic PKSs, which are large multifunctional polypeptides, compared to streptomycete type II PKSs, which are non-covalently bound dissociable aggregates of individual monofunctional enzymes.

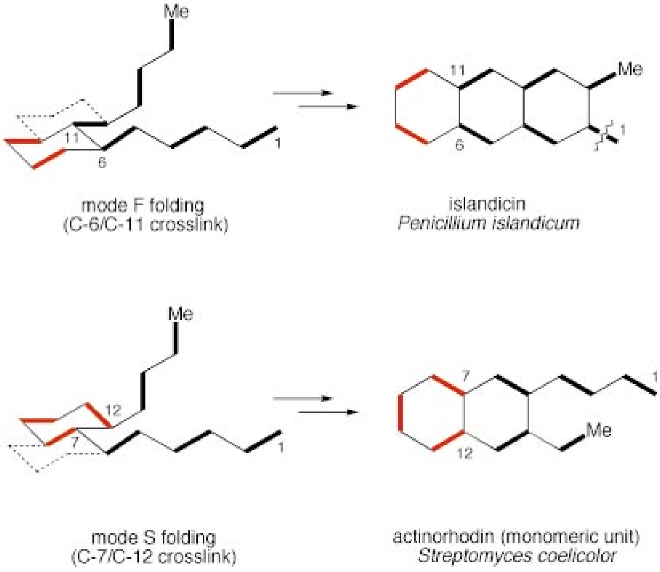


Figure 4. *Model conformations of mode-F- and mode-S-folded octaketide skeletons leading to initial ring formation. Broken bonds in the mode-F-folded chain show the relative orientation of the alternative mode-S-cyclised skeleton and vice versa.*

The proposed classification only applies to fused-ring polyketides, since fungi and bacteria are known to form aromatic monocyclic products with benzene rings derived from either two or three intact C_2 units. For example, both cyclisation modes are utilised in the formation of the fungal pentaketides α-acetylorsellinic acid (**38**)[60] and curvulin (**39**),[61] which are, respectively, metabolites of *Penicillium brevi-compactum* and *Curvularia siddiqui* (Figure 5). Analogous examples among bacterial polyketides include the dichloroorsellinic acid ester constituent of the *Micromonospora carbonaceae* antibiotic everninomicin B (**40**)[28] and diacetylphloroglucinol (**41**),[62] which is formed by various *Pseudomonas* species. More complex monobenzenoid bacterial metabolites include the 3,5-dihyroxyphenylacetate-derived tetraketide moiety **42** of the *Amycolatopsis orientalis* antibiotic vancomycin[63] and the unusual carbocyclic *ansa*-bridged quinone methide kendomycin (**43**), a constituent of *Streptomyces violaceoruber*.[64]

Some single rings could conceivably form by spontaneous cyclisation of linear tetraketides, particularly where these are close to chain termini and consequently may not be subject to the regiospecific mode F or S folding constraints of midchain cyclases. Comparatively few bacterial monocyclic polyketides have been isolated from bacteria other than derivatives of orsellinic acid (**1**) and 6-methyl salicylic acid (6-MSA) and the examples shown in Figure 5. In this regard, it may be significant that the formation of **41**, **42** and **43** appears to involve chalcone-type synthases.[62–64] The latter are well-known plant enzymes, which differ from microbial type I and II PKSs in that the primer

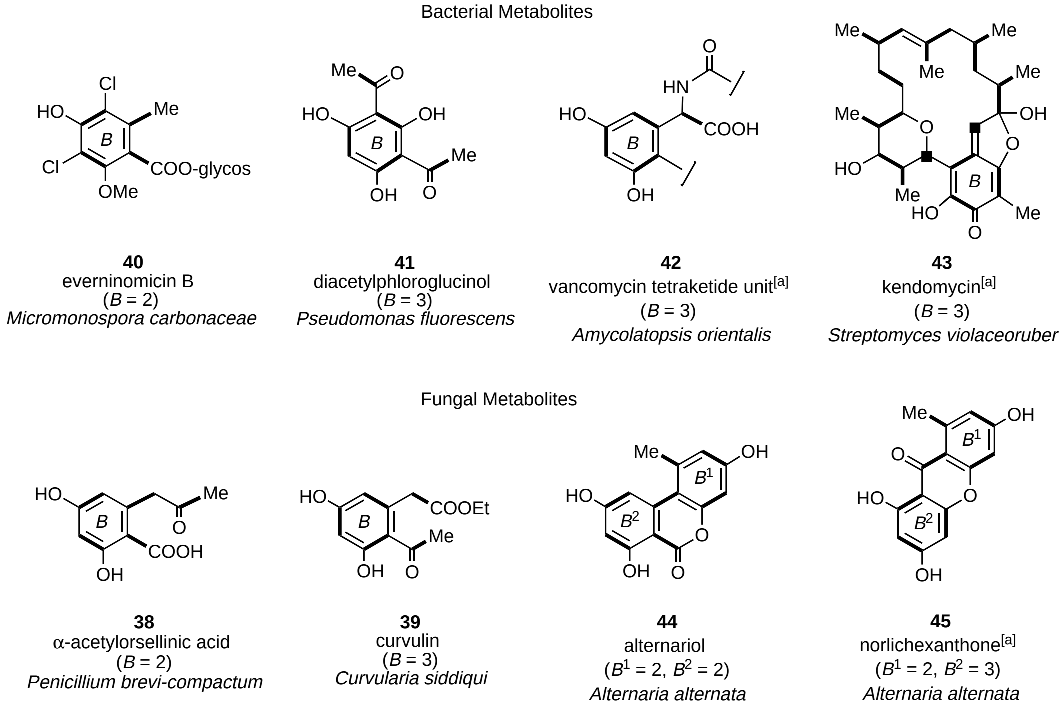


Figure 5. *Fungal and bacterial monocyclic aromatic polyketides.* B = *number of intact* C_2 *units in each benzene ring. [a] Rings with 3 intact* C_2 *units shown to be derived via symmetrical intermediates.*

and the malonate extender units are utilised as thioesters of coenzyme A rather than acyl carrier proteins. On the other hand, fungi are an abundant source of monocyclic phenols which, in addition to orsellinic acid (**1**) and 6-MSA, include many derivatives with *S*-adenosylmethionine-derived C_1 substituents. While C_1-substituted fused rings are extremely rare among fungal metabolites, these are occasionally observed in streptomycete products such as oxytetracycline (**10**) and polyketomycin (**13**).

Fungi also form biscarbocyclic non-fused-ring aromatic compounds derived from a single polyketide chain. These include the dibenzo-α-pyrone alternariol (**44**), one of the earliest biosynthetically investigated polyketides,[65] and norlichexanthone (**45**),[66] which are heptaketide congeners of *Alternaria alternata*. Whereas both carbocyclic rings of **44** and one ring of **45** incorporate two intact C_2 units, the second benzene ring of **45** is formed from three intact C_2 units. The suggested precursor–product relationship of **44** and **45**[66] was excluded by the results of a specifically targeted ^{13}C-labelling study.[67] As noted later, the structural interrelationship of the second rings of alternariol (**44**) and norlichexanthone (**45**) parallels that of plant chalcone and stilbene tetraketide co-metabolites.

Fujii and co-workers have recently reported the first preparation of a cell-free fungal fused-ring type I PKS.[53] This was isolated from *Colletotrichum lagenarium* and appears to utilise malonyl-CoA as the source of both the starter and extender units in the formation of the pentaketide 1,3,6,8-tetrahydroxynaphthalene (THN) and its by-product α-acetylorsellinic acid (**38**). As proposed by the authors, these two metabolites could be formed in fungi through decarboxylation of a common monocyclic intermediate such as **46**, which requires prior mode F folding (Figure 6). Decarboxylation leading to the second ring of THN may take place in either a concerted or a stepwise manner.

A hybrid *Streptomyces griseus* chalcone-related synthase is responsible for the biosynthesis of THN, which is the source of the red-brown pigment of *Streptomyces* species and *Escherichia coli*.[24] The 372 amino acid sequence of this enzyme, which is encoded by the *S. griseus rppa* gene, shares 30% identity with

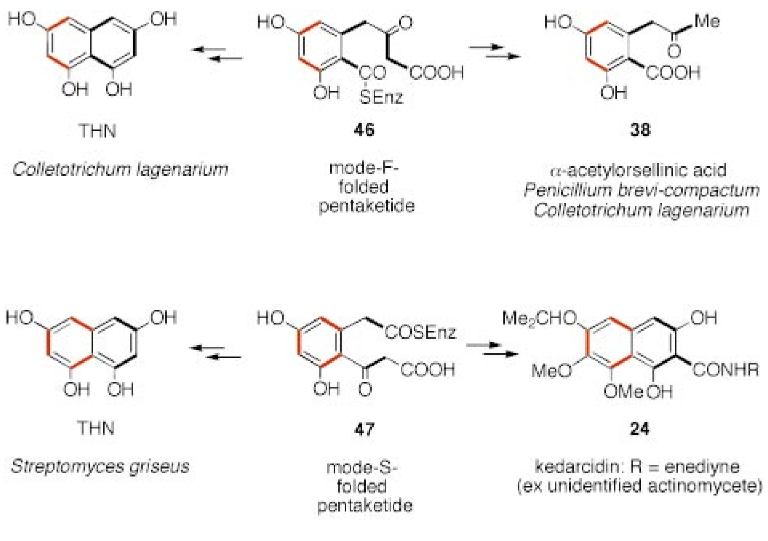
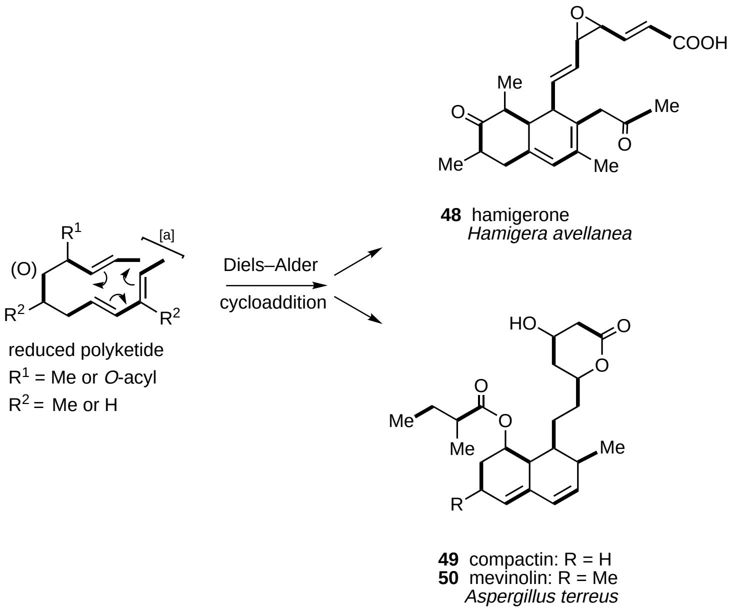


Figure 6. *Hypothetical alternative mode F or S folding options for the formation of 1,3,6,8-tetrahydroxynaphthalene in fungi and streptomycetes.*

known plant chalcone synthases. This contrasts with the formation of THN in fungi by type I PKSs where it serves as a precursor of melanins following reduction to 1,8-dihyroxynaphthalene (DHN). Recent feeding and mutant studies by Bode and Zeeck have demonstrated the key role of DHN in a general fungal pathway leading to cladospirone[68] and a family of related spirobisnaphthalenes.[69]

As noted earlier (Section 4), the symmetrical structure of THN allows the possibility that it may be formed in fungi and streptomycetes by different cyclisation pathways. Thus, in addition to the above *Colletotrichum langenarium* pathway involving decarboxylation of the mode-F-folded monocyclic intermediate **46**, decarboxylation of its mode-S-folded isomer **47** would also yield THN. Interestingly, the structure of the hypothetical intermediate **47** derived from five intact malonate units corresponds to that of the suggested precursor of the 2-naphthoic acid amide constituent of the streptomycete enediyne antibiotic kedarcidin (**24**) (Scheme 5).

The general rule for the selective modes of biosynthesis of fused-ring polyketides by fungi and bacteria is only applicable to polycyclic compounds in which the initial ring is formed through an intramolecular aldol condensation. It does not apply to the products of olefinic cycloaddition or other non-aldol pathways. For example, the initial ring of the bicyclic fungal decaketide hamigerone (**48**) has been shown to incorporate three intact C_2 units,[70] which a priori might appear to indicate its formation through mode S folding and aldol cyclisation. However, this ring could alternatively form in concert with the second ring through Diels–Alder cyclisation of a partially reduced polyketide, as may also account for the origin of the fungal products compactin (**49**) and mevinolin (**50**)[6, 71] (Scheme 6).



Scheme 6. *Putative Diels–Alder-cyclised fused-ring polyketides. [a] Compound **48** requires one more malonate extender unit than **49** and **50**.*

The proposed biosynthetic classification facilitates the structure elucidation of microbial fused-ring polyketides by restricting the selection of candidate structures to those that satisfy predictable F or S modes of cyclisation. It is also of value in identifying structures which do not conform to expected cyclisation patterns and consequently may warrant further examination. For example, the fungal polyketide viomellein was originally assigned the dimeric bisnaphthalene structure **51**,[72] in which one of the monomeric components was an unprecedented naphthoquinone requiring mode S cyclisation, whereas the second naphthalene unit conforms to the predicted F mode of folding. This structure was subsequently revised to **52** following synthetic,[73] X-ray crystallographic[74] and ^{13}C NMR spectroscopic studies[75] of the closely related fungal metabolite xanthomegnin (**53**), in which both rings are products of mode F cyclisation (Figure 7).

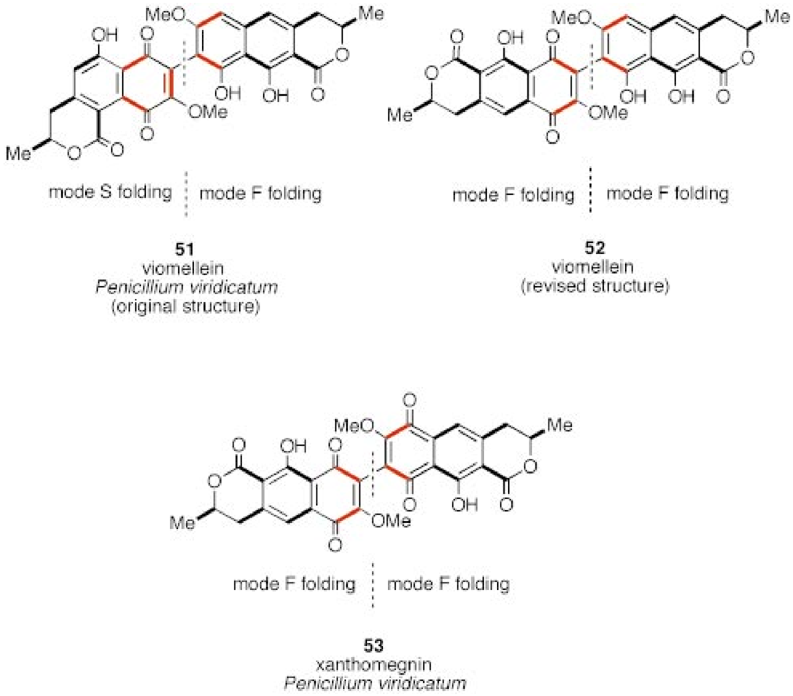
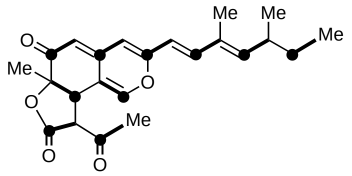


Figure 7. *Fungal dimeric naphthalene polyketides.*

6. Rearrangement of linear precursors leading to fused-ring polyketides

Although it is likely that some polyketides such as ochrephilone (**54**)[76] are derived from more than one chain, the majority of known fungal and streptomycete fused-ring polyketides are formed from single-chain intermediates.

This is normally apparent on inspection of the carbon skeletons of cyclic polyketides, unless their linear origin is



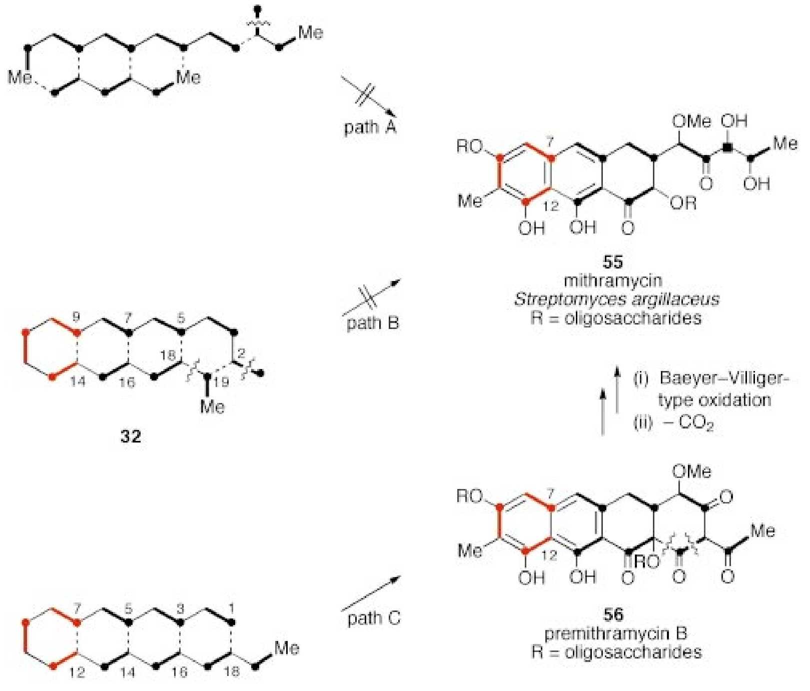
54

ochrephilone
Penicillium multicolor

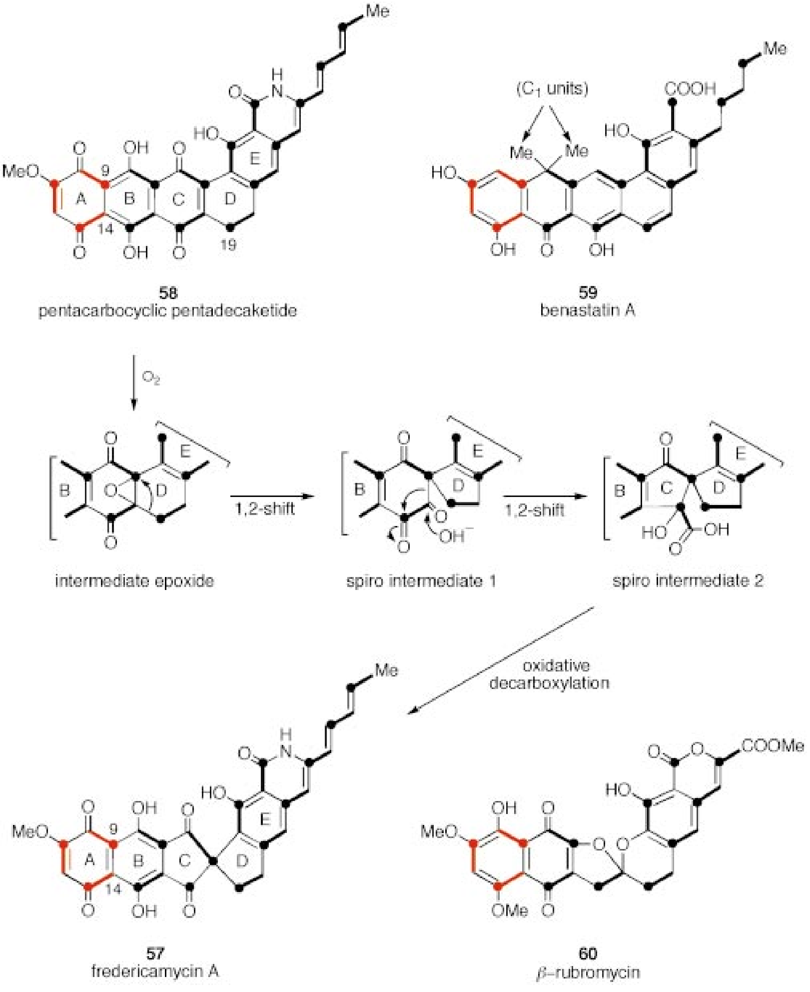
obscured by ring fission or intermediate rearrangements. Examples of fungal polyketides derived from a linear chain by a combination of ring fissions and rearrangements include the aflatoxins[18–22] as shown in Scheme 4. An interesting example among streptomycete metabolites occurs in the biosynthesis of the decaketide-derived tricyclic nucleus of mithramycin (aureolic acid (**55**)). Rohr and co-workers[77] have recently shown that **55** probably arises from a Baeyer–Villiger-type oxidative cleavage of the mode-S-folded tetracylic precursor premithramycin B (**56**) (Scheme 7, path C), the aglycone of which has a similar carbon skeleton to that of ADCOTC (**11**) and polyketomycin (**13**). This precluded earlier feasible schemes based either on multiple polyketide chains (path A in Scheme 7) or cleavage of the tetracenomycin skeleton **32** (path B in Scheme 7).

The unusual labelling of the spirocyclopentane rings of the [$^{13}C_2$]acetate-derived streptomycete metabolite fredericamycin A (**57**) appears to favour its formation from two polyketide chains.[78] However, a single-chain pathway may be envisaged involving an oxidative rearrangement with two sequential 1,2-shifts of the mode-S-folded pentacarbocyclic pentadecaketide intermediate **58** related to benastatin A (**59**),[79] as shown in Scheme 8.[80]

The recently revised structure of the streptomycete polyketide β-rubromycin (**60**)[81] and the mode of incorporation of [U-$^{13}C_3$]malonate is also accommodated by this scheme. The authors concluded that **60** is derived from two chains (a decaketide and a diketide, or a heptaketide and a pentaketide), or an unusually folded single dodecaketide chain which undergoes oxidative C–C bond cleavage. An alternative single-chain pathway via an angular fused-ring polyketide similar to the suggested precursor **58** of fredericamycin A (**57**), would require the oxidative removal of an intermediate C_2 unit from ring C and possibly one or more C_2 units from the starter side chain. Analogous mechanisms could account for the origin of structurally related metabolites such as heliquinomycin.[82]



*Scheme 7. Biosynthesis of mithramycin (**55**): hypothetical polyketide pathways A – C.*



*Scheme 8. Formation of fredericamycin A (**57**) via rearrangement of a mode-S-folded linear polyketide.*

Other examples of the apparent incorporation of [$^{13}C_2$]acetate through oxidative rearrangements of fused-ring intermediates were reported by Gould and co-workers, following extensive biosynthetic investigations of the streptomycete angucycline polyketide PD 116198 (**61**)[83] and the phenanthrene murayaquinone (**62**)[84] (Scheme 9). These authors proposed mechanistically feasible routes to **61** via the anthracycline **63** (path A1) and to **62** via the phenanthrene **64** (path B1).

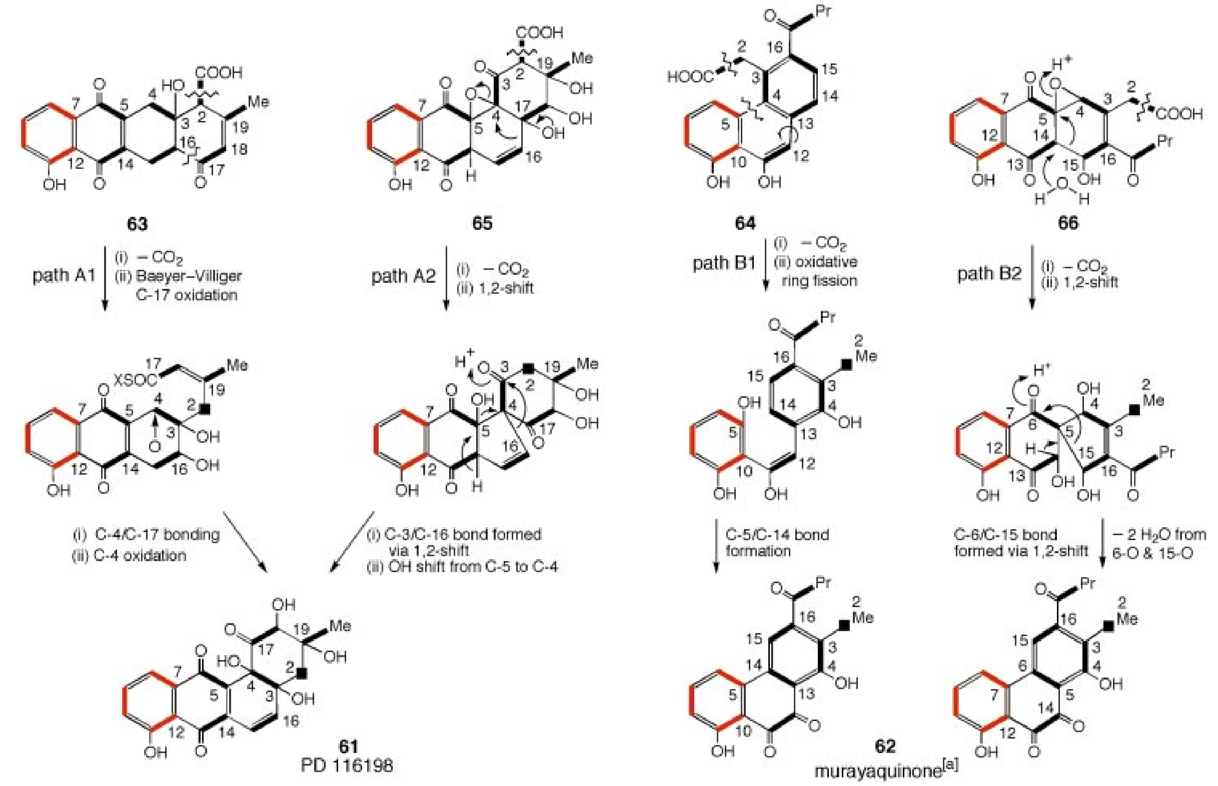
Alternative epoxide-driven rearrangements allied to the hypothetical fredericamycin A pathway could utilise mode-S-folded angucycline and anthraquinone precursors such as **65** and **66**. These could form PD 116198 (**61**) and murayaquinone (**62**) via spiro intermediates, as outlined in paths A2 and B2, respectively. In paths B1 and B2 to murayaquinone (**62**), the bridgehead carbon atoms are derived from different polyketide precursor carbon atoms.

7. Exceptions to mode S cyclisation in the formation of hybrid streptomycete polyketides

Among the above examples of wild-strain streptomycete fused-ring products, only the unusual 5-methyl-1-naphthoic acid constituent **23** of the enediyne antibiotic neocarzinostatin provides a potential exception to the predominant S mode of cyclisation. However, as already indicated, this may be formed by a non-aldol cyclisation pathway, in which case the mode F and S classification criteria would not apply. On the other hand, the streptomycete polyketide structures TW93f (**67**) and TW93g (**68**)[59] are clearly formed by aldol condensations, but neither is in accord with the classification outlined in Figure 3 and Table 2.

These two compounds are among an impressive array of more than thirty so-called "unnatural" products of a *Streptomyces coelicolor* recombinant strain. In all of the co-metabolites other than **67** and **68**, the initial rings are formed from three C_2 units and require cross-linking of carbon atoms C-5/C-10, C-7/C-12, C-9/C-14 or C-13/C-18, consistent with ring formation by the expected mode S cyclisation pathway (Table 3). The corresponding initial rings of TW93f (**67**) and TW93g (**68**), however, incorporate only two intact C_2 units and require C-8/C-13 and C-10/C-15 cross-linking, respectively, characteristic of mode F folding (Figure 8).

The remarkable variety of recombinant *S. coelicolor* structures led to the conclusion that genetically engineered streptomycetes produce modified PKSs with relaxed control of the regiospecificity of cross-linking and that some of the observed ring systems may arise through spontaneous rather than enzyme-catalysed cyclisations. Such flexibility could conceivably account for the formation of the two structurally anomalous mode-F-folded hybrid polyketides **67** and **68**. Given such enzymatic versatility, it would be interesting to know if this prolific recombinant strain also produces the corresponding C-7/C-12 and C-9/C-14 cross-linked polyketides **69** and **70**, which are, respectively, mode-S-folded isomers of TW93f (**67**) and TW93g (**68**).



*Scheme 9. Possible rearrangements of mode-S-folded polyketide skeletons leading to PD 116198 (**61**) and murayaquinone (**62**). [a] The bridgehead carbon atoms of **62** are derived from different polyketide precursor carbon atoms in paths B1 and B2.*

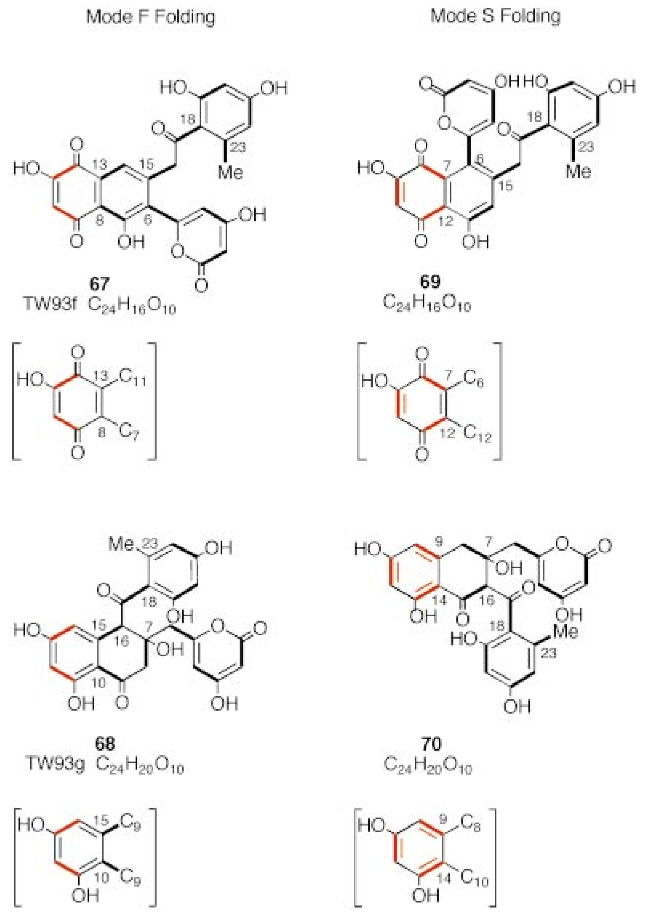


Figure 8. Exceptions to mode S folding in two polyketide products of a hybrid streptomycete.

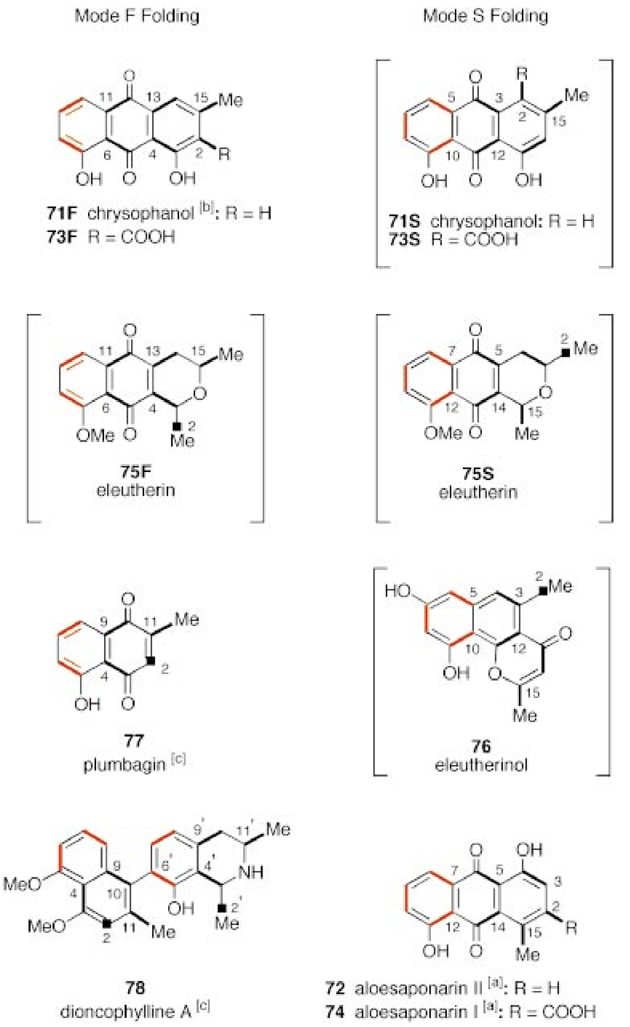


Figure 9. Acetate-labelling patterns of fused-ring polyketides of bacteria ([a]), fungi ([b]) and higher plants ([c]). Alternative possible mode F and S folding patterns of some plant products are shown in square brackets.

8. Folding patterns of plant aromatic polyketides

In addition to anthraquinone constituents of higher plants such as alizarin, which is derived from shikimate, glutamate and mevalonate precursors, other plant anthraquinones are structurally identical to those of microbial origin. Of particular interest in the context of the proposed biosynthetic classification of fused-ring polyketides is the co-occurrence in root extracts of *Aloe* species of the 2-methylanthraquinone chrysophanol (**71**) and the isomeric 1-methylanthraquinone aloesaponarin II (**72**) (Figure 9),[85] which are also produced by microorganisms. Both chrysophanol and aloesaponarin II lack the 1-carboxy group and one of the oxygen substituents of the probable parent linear octaketide.

In microorganisms, chrysophanol is a product of the fungus *Penicillium islandicum* and would be expected to exhibit the same acetate-labelling pattern **71F** as its co-metabolite islandicin (**17**).[40] These are probably formed by decarboxylation of a mode-F-folded tricyclic octaketide with the carbon skeleton **73F**. Aloesaponarin II (**72**), on the other hand, is produced in a recombinant streptomycete through decarboxylation of aloesaponarin I (**74**), which is a product of mode S cyclisation.[86] Consequently, the formation of chrysophanol and aloesaponarin II (**72**) by similar pathways in microorganims and plants would a priori appear to necessitate the simultaneous operation in plants of both F and S folding modes. In an alternative scenario, chrysophanol labelled as in **71S** could form in plants by decarboxylation of the mode-S-folded intermediate **73S**. However, this pathway would then require fungi and plants to produce chrysophanol with the differing acetate-labelling patterns **71F** and **71S**.

Eleutherin (**75**) and eleutherinol (**76**) are naphthalene cometabolites of the plant *Eleutherine bulbosa*,[87] the possible origins of which present a similar anomaly. Thus, both structures could in theory form by mode F or S folding of an octaketide, as illustrated by the alternative eleutherin labelling patterns **75F** and **75S**. The structure of eleutherinol, which was revised by Birch and Donovan in the course of their original polyketide studies,[88] is depicted in **76** as the mode-S-folded option, but this remains to be verified.

The paucity of plant fused-ring polyketide labelling data currently precludes any firm conclusions regarding the possible derivation of such co-metabolites by different cyclisation pathways, or alternatively by rearrangements of common precursors. The incorporation of [$^{13}C_2$]acetate into the naphthoquinone plumbagin (**77**)[89] by cell cultures of *Ancistocladus heyneanus* and by *Triphophylum peltatum* into the naphthalene alkaloid dioncophylline A (**78**)[90] have clearly established that these fused-ring plant polyketides are formed by mode F folding. However, no instances of the utilisation by plants of the corresponding mode S cyclisation pathway have yet been demonstrated.

A major group of higher plant PKSs which have been extensively investigated are the synthases responsible for the formation of the tetraketide chalcone precursors of flavonoids such as naringenin (**79**) and the stilbene resveratrol (**80**). Chalcone synthase (CHS) and stilbene synthase (STS) assemble polyketides directly from acylthioesters of coenzyme A, in contrast to fungal and bacterial PKSs, which mostly utilise acyl carrier proteins (ACPs). These enzymes are thought to be members of a superfamily of polyketide synthases capable of producing di-, tri- and tetraketides from a variety of starter units, as described in a recent review by Schröder.[91]

Structurally diverse products of this superfamily of plant polyketide synthases may include acridones and the rare phenylphenalenones, such as the aglycone haemocorin (**81**), a constituent of the monocotyledon *Haemodorum corymbosum*. It was proposed that **81** could be formed from one acetyl-CoA and two cinnamoyl-CoA units via a diarylheptanoid related to curcumin (**82**) (Figure 10).[16] This pathway was consistent with early ^{14}C- and ^{13}C-labelled precursor feeding experiments[92–94] and has recently been confirmed by Schneider and co-workers, who demonstrated the incorporation of ^{13}C-labelled advanced intermediates into the phenylphenalenone hydroxyanigorufone (**83**) from *Anigozanthos preissii*.[95, 96]

As previously noted (Section 5), CHS-related enzymes are also found in bacteria and appear to catalyse the formation of phenolic polyketides such as THN, diacetylphloroglucinol (**41**) and the aromatic constituents of vancomycin (**42**) and kendomycin (**43**). While flavonoids are widely distributed in plants, interestingly, chlorflavonin (**84**) has been isolated from the fungus *Aspergillus candicus*,[97] but the nature of the associated synthase has not been investigated.

CHSs and STSs are relatively small homodimeric proteins (subunits 40 – 45 kDa). The recently reported crystal structure of recombinant alfalfa CHS2, together with the structures of its complexes with substrate and product analogues,[98] reveals its active-site architecture. CHSs show little homology with bacterial and fungal PKSs and probably evolved via an independent pathway. However, there may be some general similarities in their assembly strategies that can serve as a conceptual basis for the characteristic mode F and mode S cyclisation pathways utilised by fungi and streptomycetes. Of potential relevance in this regard is the presence of polyketide-binding and -cyclisation pockets, the spatial and hydrogen-bonding geometry of which is thought to determine the mode of folding leading to the formation of either a chalcone or a stilbene tetraketide. The combined crystal structures indicate that the growing carbon skeleton undergoes progressive conformational changes in the course of sequential addition of three malonate-derived C_2 units.

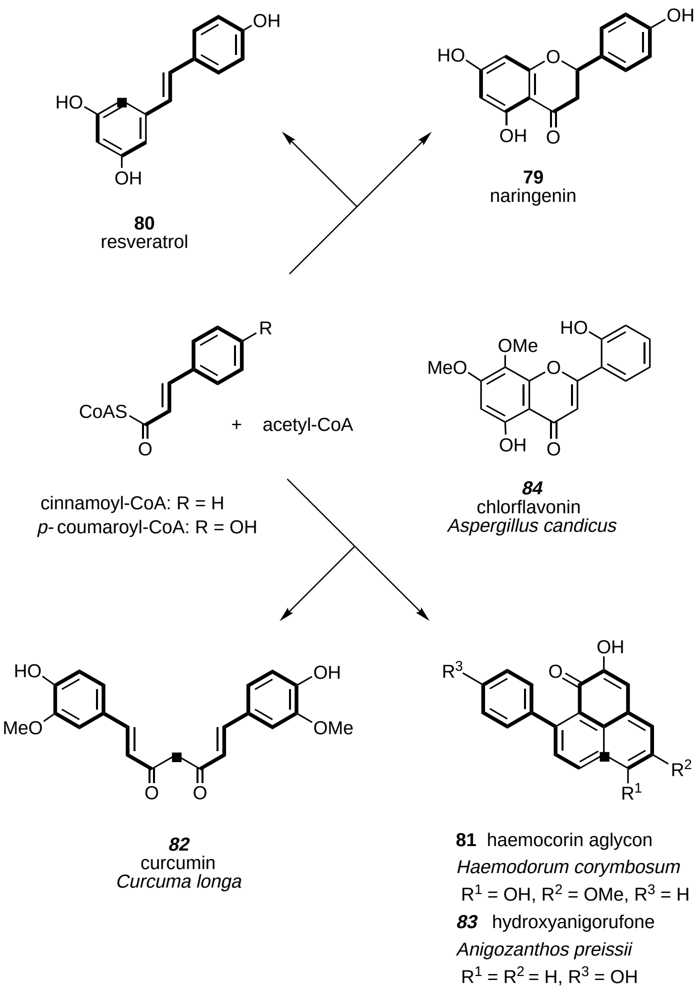


Figure 10. *Tetraketides and possible decarboxylated diketides formed by the chalcone and stilbene superfamily of plant synthases.*

It was shown by site-directed mutagenesis of an inactive hybrid of CHS and STS that the exchange of only three amino acid residues in the CHS component of the hybrid protein was sufficient to restore STS activity.[91, 99] Analogous minor modifications of fungal aromatic PKSs might similarly lead to the different modes of folding of the corresponding rings of the *Alternaria alternata* heptaketides alternariol (**44**) and norlichexanthone (**45**). While these co-metabolites share common methyl-substituted resorcinol rings incorporating two intact C_2 units and the second resorcinol ring of **44** is similarly derived (Figure 5), in **45** the second ring is formed from three C_2 units. The contrasting modes of formation of the latter rings of **44** and **45** parallel the formation of the corresponding rings of the stilbene resveratrol (**80**) and the flavonoid naringenin (**79**) (Figure 10).

Although the fused-ring PKSs of plants have received little attention relative to those of microbial origin, the fatty acid synthases (FASs) of a wide range of plant, animal and microbial sources have been subjected to intensive study[100] and shown to differ in their molecular structure and complexity. Thus, type I FASs produced by animals, fungi and yeasts are high molecular weight multifunctional complexes, in which the individual active sites may be covalently linked components of one or two polypeptide chains. In the type II FASs of bacteria and plants, the constituent enzymes are freely dissociable proteins, which may be organised in vivo as loose aggregates.

As previously mentioned (Section 5), microbial PKSs also exist as type I and type II enzymes.[5, 101, 102] Both synthases produce aromatic polyketides through the iterative operation of single sets of active sites in successive rounds of chain assembly, involving the sequential addition of malonate units and intramolecular cyclisation. While far less is known of the properties of the aromatic PKSs of fungi relative to those of streptomycetes, it would appear that the former are generally type I and the latter are type II synthases. Few fused-ring PKSs have been purified to homogeneity and little information is currently available regarding their three-dimensional structures. However, the solution structure of the actinorhodin ACP from the type II PKS of *Streptomyces coelicolor* has been determined by two-dimensional ^{1}H NMR spectroscopy and shown to contain pockets which may harbour growing polyketide chains.[103, 104]

9. Summary and outlook

A general rule is proposed for the biosynthetic classification of fused-ring aromatic polyketides formed by fungi and filamentous bacteria through intramolecular aldol and Claisen condensations. This is based on the observation of two characteristic modes of folding of linear intermediates leading to the initial cyclohexane ring. In fungal polyketides, two intact malonate-derived C_2 units are incorporated in the initial ring (designated folding mode F), whereas three intact units are incorporated in the corresponding ring of streptomycete polyketides (mode S).

This classification has a number of potential applications (see above). Thus, it: i) facilitates the structural elucidation of new microbial fused-ring polyketides by favouring the selection of candidate structures which satisfy predictable F or S modes of cyclisation; ii) identifies biosynthetically atypical structures such as that initially proposed for the fungal metabolite viomellein and the anomalous structures of two "unnatural" hybrid streptomycete polyketides, TW93f and TW93g; iii) recognises aberrant [$^{13}C_2$]acetate incorporation patterns such as that assigned to the fungal polyketide fonsecin; iv) facilitates the taxonomic classification of unidentified fungal and bacterial sources of fused-ring polyketides, including PKS genes cloned from soil DNA without prior isolation of the parent microorganisms.[105]

Higher plants also produce a number of fused-ring polyketides. Some of these are structurally identical to those isolated from fungi and bacteria, such as the respective mode-F- and mode-S-folded isomeric octaketide anthraquinones chrysophanol and aloesaponarin II. Hence, the folding specificities of plant PKSs may be less rigid than those of microbial origin. Although the biosynthesis of plant fused-ring polyketides have received far less attention, recent $^{13}C_2$-labelling studies have established the derivation of the naphthalene skeletons of plumbagin and also the alkaloid dioncophylline A by mode F cyclisation.

The specific catalysis of mode F or S folding may be inherent properties of the respective type I and type II aromatic PKSs of fungi and bacteria. The preparation of viable hybrids of equivalent PKS component genes from both microbial sources may facilitate the biosynthesis of novel aromatic fused-ring products with various combinations of the distinctive structural characteristics of fungal and bacterial polyketides.

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