Acetogenic anthraquinones: biosynthetic convergence and chemical evidence of enzymatic cooperation in nature

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Abstract Phenylanthraquinones belong to the quite rare class of fully unsymmetric biaryls, consisting of two different molecular portions, an anthraquinone part, chrysophanol, and a phenyl part, 2,4-dihydroxy-6-methoxyacetophenone, linked together by phenol-oxidative coupling. The biosynthesis of these two moieties, from eight and four acetate units, respectively, bears some unique features: Chrysophanol is the first example of an acetogenic natural product that is, in an organism-specific manner, formed via more than one folding mode: In eukaryotes, like, e.g., in fungi, in higher plants, and in insects, it is formed via folding mode F, while in prokaryotes it originates through mode S. It has, more recently, even been found to be synthesized by a third pathway, which we have named mode S′. It is thus the first example of biosynthetic convergence in polyketide biosynthesis. The monocyclic "southern" portion of the molecule, which is much simpler (arising from only four acetate units and without decarboxylation), unexpectedly does not show the anticipated randomization of the C_2-labeling in the aromatic ring, but has clearly localized C_2 units, excluding any symmetric intermediate like, e.g., 2,4,6-trihydroxyacetophenone. This is confirmed by competitive feeding experiments with specifically $^{13}C_2$-labeled acetophenones, showing the O-methylation to be the decisive symmetry-preventing step, which hints at a close collaboration of the participating enzymes. The results make knipholone an instructive example of structure, function, and evolution of polyketide synthases and O-methyltransferases, and their cooperation.

Abbreviations

CD	Circular dichroism
HPLC	High performance liquid chromatography
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
NMR	Nuclear magnetic resonance
OMT	O-Methyltransferase
PKS	Polyketide synthase

Introduction

Nature provides a huge variety of structurally diverse natural products. Many of them originate from simple acetate-malonate units, catalyzed by polyketide synthases (PKSs), often in combination with other enzymes (Rix et al. 2002). For the directed search for novel acetogenic metabolites right from crude extracts, we have composed the analytical triad LC-MS/MS-NMR-CD (HPLC coupled to tandem mass

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spectrometry, nuclear magnetic resonance, and circular dichroism) (Bringmann et al. 2002b, 2003) in combination with quantum chemical CD calculations (Bringmann and Busemann 1998; Bringmann et al. 2008).

Among the novel-type structures thus discovered recently by our group, are, i.a., unique acetogenic alkaloids (Bringmann and Pokorny 1995; Bringmann et al. 1998a, 2005, 2006a) but also nitrogen-free compounds like, e.g., mono- (Abegaz et al. 2002; Bringmann et al. 2002a) and dimeric (Bringmann et al. 2007c) phenylanthraquinones, with knipholone (1) (Fig. 1) as the longest-known representative (Dagne and Steglich 1984). These secondary metabolites from tropical plants of the Asphodelaceae family show remarkable properties:

- Structurally, due to the presence of a biaryl axis, which links together two very different aromatic systems. This is quite rare because most biaryls in nature are constitutionally more or less symmetric (Bringmann et al. 2001). Furthermore, this axis is rotationally hindered, leading to stable atropo-enantiomers, (M)-1 and (P)-1, whose absolute configurations have been revised recently (Bringmann et al. 2007b). But, interestingly, these occur mostly scalemic in nature (Mutanyatta et al. 2005), i.e., not enantiomerically pure, but not fully racemic either, as analyzed by our LC-CD hyphenation: HPLC on a chiral phase with online circular dichroism monitoring (Bringmann et al. 2008).

- Natural phenylanthraquinones show a broad structural variety: Besides knipholone itself, the plants produce the—oxygen-poorer—respective anthrones like 2 (Dagne and Yenesew 1993), the corresponding O-sulfates like 3 (Mutanyatta et al. 2005), O-glucosides like 4 (Kuroda et al. 2003; Qhotsokoane-Lusunzi and Karuso 2001a, b; Abegaz et al. 2002), and side chain-oxygenated analogs, like gaboroquinone B (5) (Abegaz et al. 2002), and we have recently even discovered mixed 'dimers' like joziknipholone A (6)—consisting of a knipholone moiety and a knipholone anthrone part, with even three such axes (Bringmann et al. 2007c).

- Furthermore, phenylanthraquinones are interesting pharmacologically, exhibiting antiplasmodial (Bringmann et al. 1999; Bringmann et al. 2002a; Wube et al. 2005) and antitumoral (Qhotsokoane-Lusunzi and Karuso 2001a; Bringmann et al. 2007c) activities, different for the two respective rotational isomers (Kuroda et al. 2003), showing that the phenomenon of atropisomerism is of concrete relevance and not just an academic issue.

- And, in particular, these compounds are formed via a most remarkable biosynthetic pathway!

But can there be anything interesting about the biosynthesis of knipholone (1)? Its anticipated biosynthesis looks so clear, predictable, and straightforward, without any surprises to be expected (Fig. 2). It seems evident that 1 is built up from the known (Thomson 1997) anthraquinone chrysophanol (7) and from the
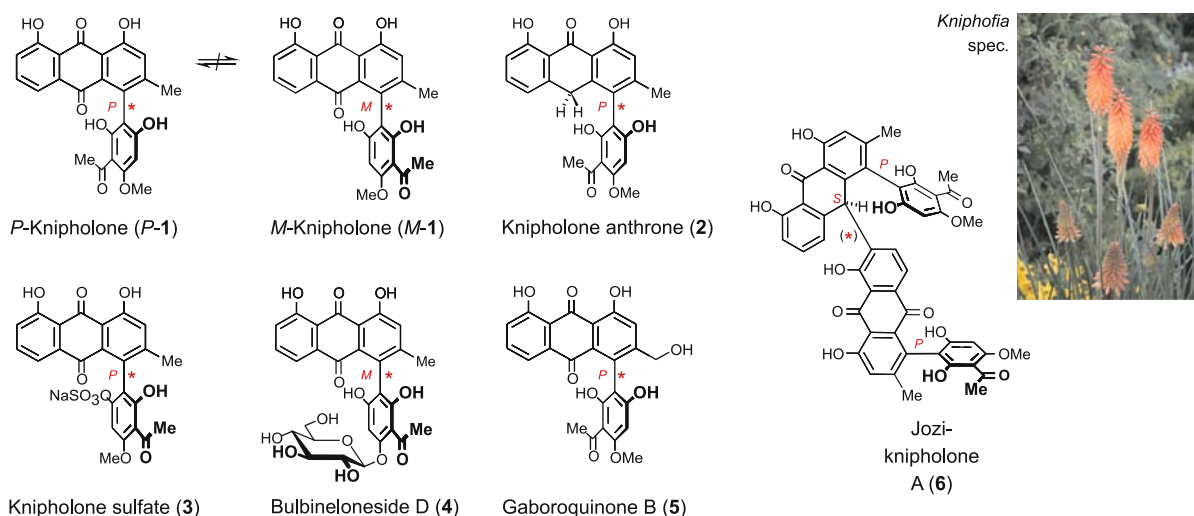

Fig. 1 Knipholone and related phenylanthraquinones: Secondary metabolites from *Kniphofia* and *Bulbine* (Asphodelaceae) species

likewise known (Nagarajan and Parmar 1977) 2,4,6-trihydroxyacetophenone (**8**), then linked together by a mixed phenol-oxidative coupling (Pal and Pal 1996)—nothing extraordinary, except for the fact that such truly unsymmetric biaryls are quite rare in nature (Bringmann et al. 2001), most of them are more or less symmetric dimers. And that these two building blocks in turn originate from polyketide precursors, seems to be evident, too, the acetophenone **8** from four acetate units, via a tetraketide intermediate **10**, chrysophanol (**7**) from eight acetate units, via an octaketide chain **11** (with decarboxylation), possibly via a bicyclic diketo precursor, as postulated earlier by Harris (Harris et al. 1976) and Franck (Franck and Stange 1981), although never found in nature.

Biosynthesis of chrysophanol (7): many roads lead to Rome!

But even if this "Harris–Franck ketone" (**9**) is a true intermediate en route to chrysophanol (**7**) (which is not

sure!), its octaketide precursor **11** might be folded like in **11a** or like in **11b**, since the diketone **9** might have had its carboxyl at any of its two methyl groups, before-hand, at C-2″, but possibly also at C-3′, instead. The array as in **11b** would be in agreement with the structure of the known (Asahina and Fujikawa 1935) lichen metabolite endocrocin (**12**), which still possesses its carboxylate group. But of course this does not exclude, a priori, an alternative polyketide folding as in **11a** for chrysophanol (**7**), moreover since the producers are different—plants versus lichens.

Already by this uncertainty, chrysophanol (**7**) becomes a promising candidate. It might be the very first polyketide-derived product that can be formed via more than one folding mode, which is depicted in more detail in Fig. 3. As mentioned, the diketone **9** as a postulated intermediate might have had its carboxyl group at C-2″ or at C-3′, corresponding to **13a** or to **13b** as possible bicyclic precursors—independent of when exactly the decarboxylation takes place and, hence, whether **9** is an authentic precursor or not. In any case, although always starting from
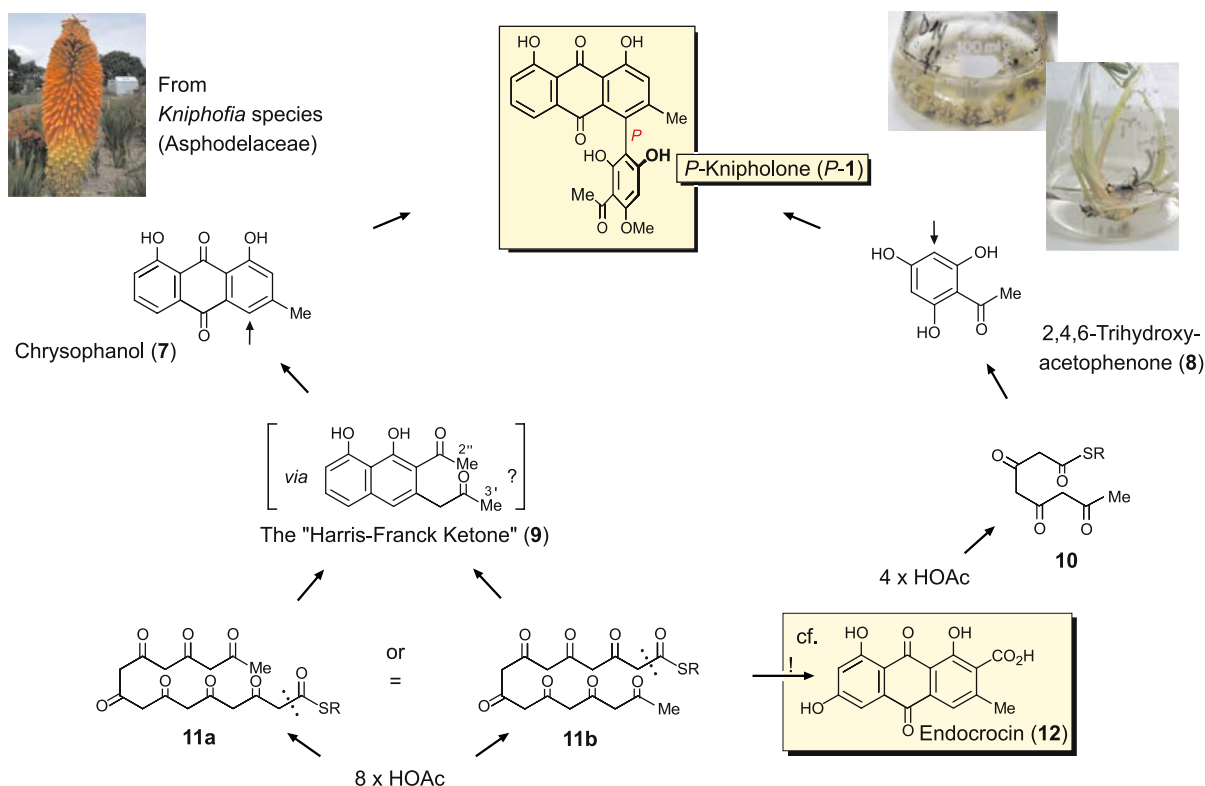

Fig. 2 Expected biosynthetic origin of knipholone [(*P*)-**1**]
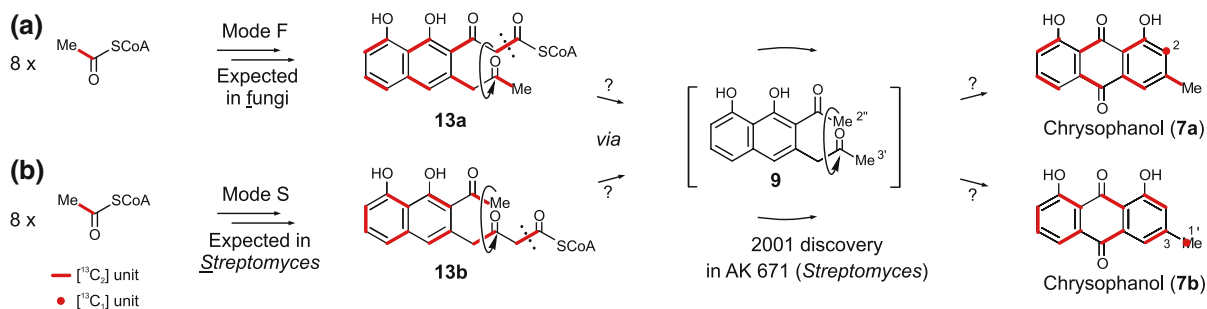
Fig. 3 Two possible folding modes that might lead to chrysophanol (**7**), with or without the Harris-Franck ketone (**9**) as a true intermediate—an imaginable first example of convergence in polyketide biosynthesis

eight acetate units, this would mean two different metabolic pathways and, thus, two entirely different sets of intermediates—still, in the end, convergently leading to the very same product, chrysophanol (**7**).

Experimentally, it should be possible to distinguish between these two imaginable pathways by feeding twofold ^{13}C-labeled acetate, which would lead to chrysophanol with two different isotopic patterns, **7a** or **7b**, and these could be distinguished by NMR: The residual C_1-'single' (in red), left behind after the decarboxylation, would be located at C-2 in **7a**, or, in the case of **7b**, on the methyl group, C-1'. And, in particular, all of the $^{13}C_2$ units in **7a** and **7b** (again drawn in red) would be complementary to each other.

In principle, one can classify such folding types by just counting the number of intact C_2 units—two or three—in the first ring (i.e., in the left one). According to this simple, but useful empirical criterion, as introduced by Thomas (2001), one would call the type that leads to **7a** (i.e., with two such C_2 units in the first ring) folding mode F, because that would be typical of fungal metabolites, and the other one, leading to **7b** (which has three C_2 units in the first ring) folding mode S, as expected for _Streptomyces_. The rule has, however, been deduced from results on other compounds, while chrysophanol (**7**) itself has never been investigated for its folding mode and, in particular, there is as yet not a single example of a natural product that is formed via both, the F and the S modes.

Thus, already from its structure, the long-known anthraquinone chrysophanol (**7**) is a rewarding candidate to become the first example of such a convergence of polyketide pathways in nature. It is

even more promising by the fact that it is found in most different classes of organisms: It has been found to occur in fungi (Thomson 1997), in higher plants (Hammouda et al. 1974), in lichens (Mishchenko et al. 1980), and even in insects (Howard et al. 1982; Hilker and Schulz 1991)—but always only in eukaryotes, never in streptomycetes or other prokaryotes.

But then, the authors' group has, quite unexpectedly, discovered the diketone **9** in a _Streptomyces_ strain, in a nice cooperation with H.-P. Fiedler and M. Goodfellow. It is exactly the Harris–Franck ketone (**9**), which had so far only been postulated as a precursor (Harris et al. 1976; Franck and Stange 1981), but had never been found in nature. Its discovery now made it rewarding to look for chrysophanol (**7**) in this diketone producer and in other prokaryotes, which was, again, done together with H.-P. Fiedler. Although **7** could not be traced up in that particular organism, we did find it in a _Nocardia_ strain, where it even occurs as a main component![1] This discovery opened the way for the first comparative study on the organism-specific formation of chrysophanol (**7**) in most different classes of organisms, now even including prokaryotes.

Among the fungal chrysophanol producers, _Drechslera catenaria_ (aka _Helminthosporium catenarium_) proved to be the most reliable one. The feeding experiments with $^{13}C_2$-labeled acetate (Fig. 4) clearly show the expected fungal folding type F: the decarboxylation site at C-2, in the 'northeast' of the molecule, and, in particular, two intact C_2 units in the first ring, i.e., mode F, as expected for a fungal producer.

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[1] For the parallel discovery of 7 in another prokaryote, though as a minor component, see Fotso et al. (2003).

The same result—formation of chrysophanol (**7**) via mode F (i.e., with the labeling pattern as in **7a**)—was obtained with higher plants (not shown), exemplarily with a torch lily species, *Kniphofia uvaria*, by feeding the labeled acetate to callus cultures grown in liquid medium.

But how about prokaryotic organisms? Feeding experiments on the above-mentioned *Nocardia* strain, again using $^{13}C_2$-acetate, gave an unexpected result. On the one hand, one does find hints at the now expected second folding type, S, as seen in **7b** (Fig. 5); but the $^{13}C_2$ interactions, in particular that of

C-4 to C-3, are very weak and not really stronger than, e.g., the respective interaction from C-4 to C-4a (but that would, formally, correspond to mode F, as in **7a**, which would not fit here at all). Much stronger, and dominating everything else, is yet another, third isotope pattern as seen in **7c**. This specific labeling pattern again possesses three intact C_2 units in the first ring, thus also to be addressed as an S type, but now with the decarboxylation site at C-4, and this is even, by far, the main folding type, much stronger than the faint hints at the initially expected type S. So, besides the pleasure to have found yet another folding
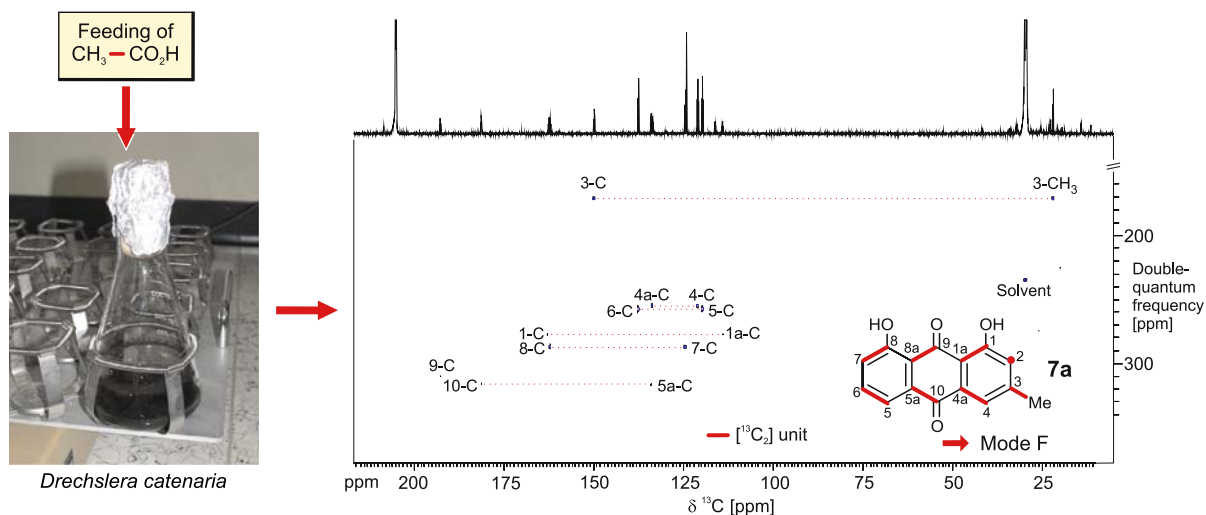

Fig. 4 Incorporation experiments on the biosynthesis of chrysophanol with a filamentous fungus: mode F!
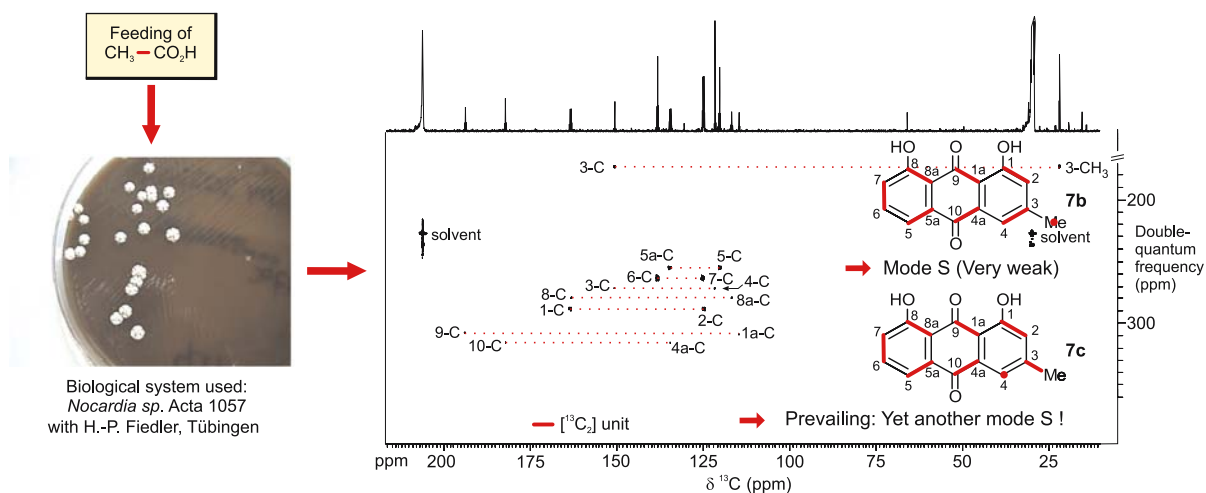

Fig. 5 Incorporation experiments with bacteria: Discovery of an as yet unexpected further S-type mode—how many more folding modes may lead to chrysophanol?

mode, the question arises: how many more folding modes may there be?

A complete set of all imaginable folding modes that could provide chrysophanol (7) can be seen in Fig. 6. The labeling patterns of 7a (lane a) and 7b (lane b) represent the two folding modes that we initially expected; of these we had, at this point, already identified mode F in fungi and in plants and had now found slight, still preliminary hints at an isotope pattern 7b corresponding to mode S—and have now discovered that additional, significantly different, but again S-related, folding type leading to 7c (lane c), so that it is unambiguously sure now: chrysophanol (7) *is* the first proven polyketide-derived natural product that is formed through more than one folding type.

But why had this second S type as yet been overlooked and where does it come from? Of course, the last ring closure to give chrysophanol (7) might not only have taken place between C-2 and C-3 (see green lines in 7a and 7b), but also between C-3 and C-4 (see green line as in 7c) and then one may expect the alternative diketone 14, i.e., an isomer to the Harris-Franck ketone—and, hence, the corresponding thioester 13c—to be probable biosynthetic precursors.

Because of the structure of the isolated diketone 9, the second possible S folding mode (leading to 7c)

had previously not been taken into consideration, but now, due to its prevalence in *Nocardia*, we have named the one leading to 7b, via 13b, which is indeed chemically less favorable, mode S′ and the new, predominant one via 13c, mode S. There should, theoretically, be one further pathway if one again assumes that (like already in the case of 9) the imaginable alternative intermediate diketone 14 might have been carboxylated at any of its methyl groups, at C-1" or possibly also at C-3′, and this would lead to 7d and thus to yet another folding mode (Fig. 6, lane d), now again F-related, with *two* C_2 units in the first ring.

From a chemical point of view, this fourth possible type, now called mode F′, should be less probable than the other three folding types, and we had not found this mode F′ in fungi, nor in plants—but how about other eukaryotes, like, e.g., insects? Feeding experiments with $^{13}C_2$-labeled acetate to more than 800 larvae of *Galeruca tanaceti*, a chrysophanol-producing tansy beetle, in cooperation with M. Hilker, Berlin, again revealed mode F, which thus seems typical of eukaryotic producers. Again, there were no hints at mode F′.

Chrysophanol is thus the first polyketide that can—and does—originate via more than one folding type in nature: although always starting from eight acetate
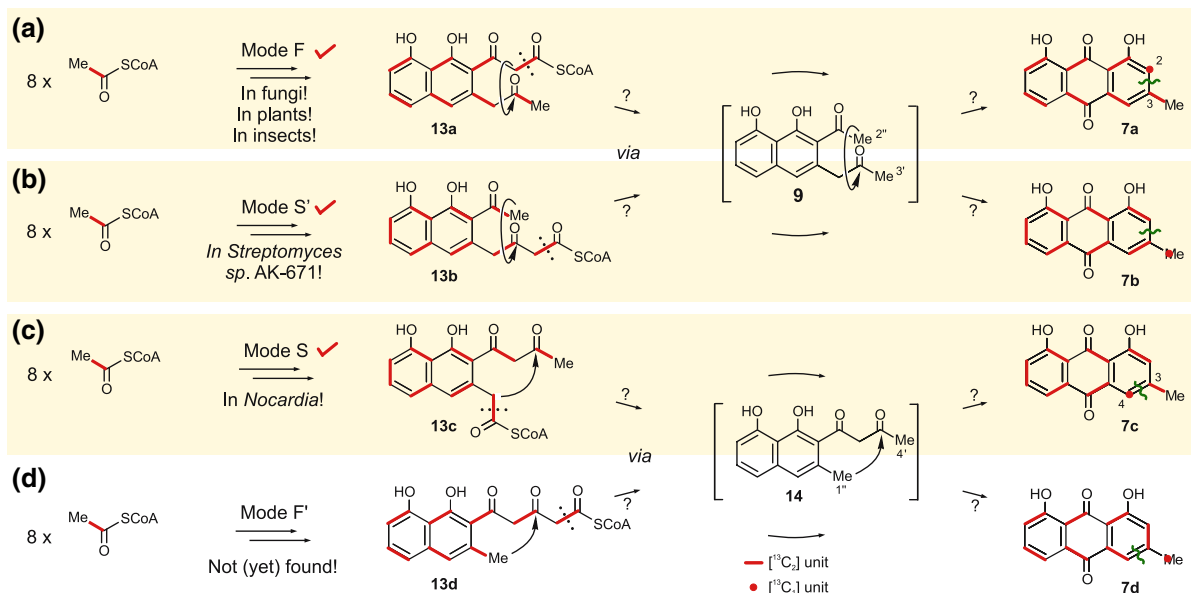

Fig. 6 Chrysophanol (7) as the first proven example of convergence in polyketide biosynthesis: four possible folding modes, of which three (F, S, and S′) have been recently identified

units, it then follows, divergently, at least two different pathways, F and S, i.e., passing through at least two series of completely different intermediates, and then, in the end, convergently always forms the same metabolite, chrysophanol (**7**) (Bringmann et al. 2006b).

The only issue that remained unsettled, was the fact that there were hints at the likewise imaginable, though chemically less probable, S′ folding mode, but no solid evidence, which, however, rather stimulated us to look for this third possible folding mode in nature. And the concept was to screen for prokaryotic—and thus S-type—micro-organisms that produce chrysophanol (**7**) *and*, simultaneously, the Harris-Franck ketone (**9**), because the idea was: This diketone can originate only from mode F (which, however, would be extremely improbable in a prokaryote!)—or from mode S′ (which we were looking for), but in no case from S, since that would require a different diketo intermediate, viz. **14**. Again together with H.-P. Fiedler, we finally found such a double producer: A *Streptomyces* strain from Tübingen, registered as AK 671. And thus—in expectation of a new record—again the labeled acetate was fed, now clearly giving mode S′ for the diketone (see **9a**, Fig. 7)! But this does not necessarily mean that chrysophanol (**7**) is formed through S′ as well: Maybe **9** is not a precursor to **7**, but an end product after a premature decarboxylation, thus losing its chemical reactivity, and chrysophanol would be formed

independently, in parallel, again via mode S as in *Nocardia*. But this is not the case: the INADEQUATE measurements now unambiguously evidence mode S′ for chrysophanol (Bringmann G, Gulder TAM, Hamm A, Dieter A, Goodfellow M, Fiedler H-P, unpublished results)!

Thus not only two, but even three different pathways lead to chrysophanol (**7**): F, S, and S′. So this is a most remarkable biosynthesis, always starting from eight acetate units, presumably even always via the same octaketide chain **11** (see Fig. 2), which then, however, gets diversely folded and further reacts in a divergent way, via three different series of intermediates, all of them, eventually, providing chrysophanol—thus, many roads lead to Rome!

This may give interesting comparative insight into the structure, function, and evolution of polyketide synthases in phylogenetically very different organisms. In bacteria, usually PKS systems of type II are responsible for the formation of aromatic polyketides (Staunton and Weissmann 2001; Hertweck et al. 2007)—and probably also for the biosynthesis of chrysophanol (**7**). In fungi, however, all known aromatic polyketide synthases belong to type I (Shen 2000). In plants, a third type of (aromatic) polyketide forming enzymes seems to be responsible, viz. chalcone synthase-like PKS, whose function is probably not only restricted to *p*-coumaryl-CoA, but also to other starter units (Austin and Noel 2003). From *Aloe*
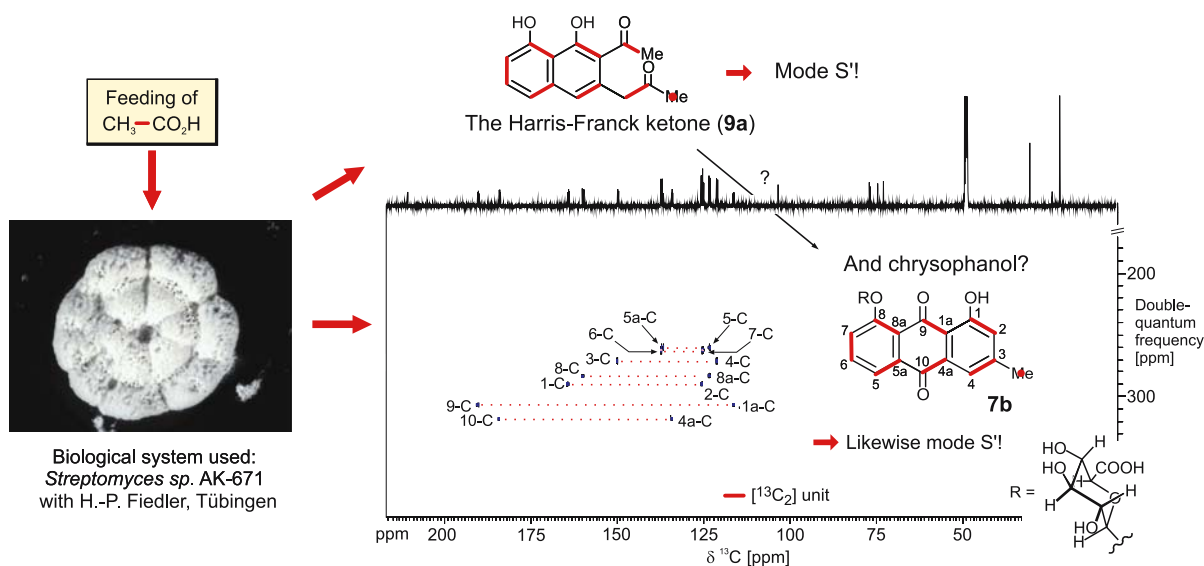

Fig. 7 Incorporation experiments with bacteria (*Streptomyces* sp. AK 671): recent discovery of mode S′, the third pathway to chrysophanol!

arborescens, Abe et al. (2005) have recently identified a gene encoding a chalcone-synthase related enzyme catalyzing octaketide synthesis. The knowledge about polyketide synthases from animals, by contrast, is as yet rather marginal, thus making it impossible to predict the structure of the respective PKS, which might constitute another member of the novel group of animal-derived type I polyketide synthases (Calestani et al. 2003), whose origin is, however, as yet unclear (Castoe et al. 2007). Given the above described diversity of PKS, it seems well possible that chrysophanol (**7**) is not only built up via different folding modes, but also from three (or even more) different *types* of enzymes, hinting at a truly convergent development of different pathways towards one target molecule, in the course of evolution. This makes an investigation of both, the enzymes of the biosynthesis of chrysophanol and their genetic background a rewarding task, which is now under investigation together with our cooperation partners.

Biosynthesis of the entire phenylanthraquinone knipholone

But coming back to the intact plant metabolite knipholone (**1**) from the torch lily, *Kniphofia*: For the chrysophanol part one would now clearly anticipate the mode F, since *Kniphofia* is a higher plant, thus expectedly leading to the isotope labeling pattern as in **7a**, with the 'decarboxylation single' at C-2.

In the case of the trihydroxyacetophenone portion **8**, the situation looked even more predictable (Fig. 8): **10** is a short tetraketide that does not lose CO_2, so there should be only one single folding type: as in chain **10a**—or like in **10b**, but that is of course entirely identical. For the final isotope labeling in **1**, however, this means that the acetate units in the aromatic ring would be located as in **10a** *plus*, complementarily, as in **10b**, i.e., 'blue' plus 'red', hence expectedly leading to a C_2-wise randomization in **8** (i.e., **8a/b**) and thus in *P*-**1** (i.e., *P*-**1a/b**). Or, in other words: 2,4,6-trihydroxyacetophenone (**8**)[2] as the expected intermediate is symmetric and will thus
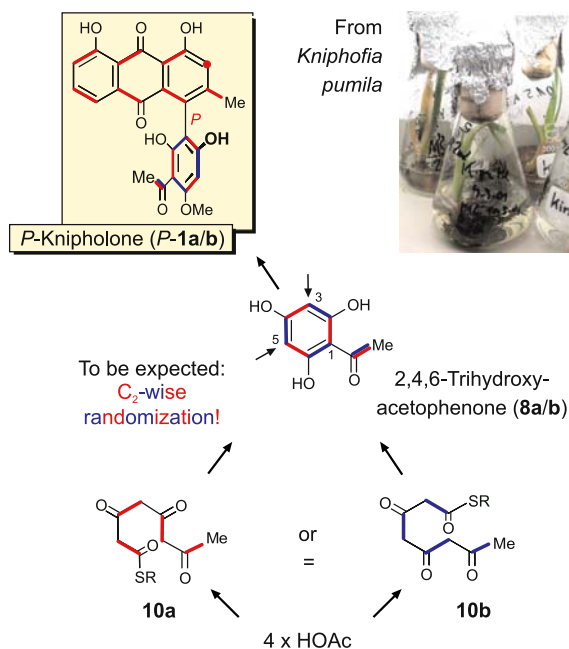
Fig. 8 Anticipated biosynthesis of the 'southern' portion of knipholone: with C_2-wise isotope scrambling in the aromatic ring

be coupled equally at C-3 and C-5—these are, after all, homotopic coupling positions. Only in the side chain, the C_2 unit would be expected to be localized, since both options, 'blue' and 'red', should lead to the same isotope pattern.

The acetate feeding experiments (Fig. 9) were performed on plants of *Kniphofia pumila*, grown under sterile conditions. As expected, the chrysophanol portion is formed via the anticipated F type folding mode; there is, however, *no* C_2-wise randomization in the phenyl ring, but, discrete, localized C_2 units. By SELINQUATE (Berger 1988) experiments, any randomization can be excluded down to less than 10 % (Bringmann et al. 2007d).

This result in the phenyl portion of knipholone is absolutely surprising: Of the two possible isotopomers, *P*-**1a** and *P*-**1b** (i.e., 'blue and red', see also Fig. 11), only the red one is formed from the $^{13}C_2$ labeled acetate, which clearly means that this molecular portion is not formed via any symmetric intermediate at any time, hence 2,4,6-trihydroxyacetophenone (**8**) cannot be a precursor, its monomethyl ether **15**, i.e., the authentic "southern" portion of **1**, by contrast, might have a chance, since that is unsymmetric per se.
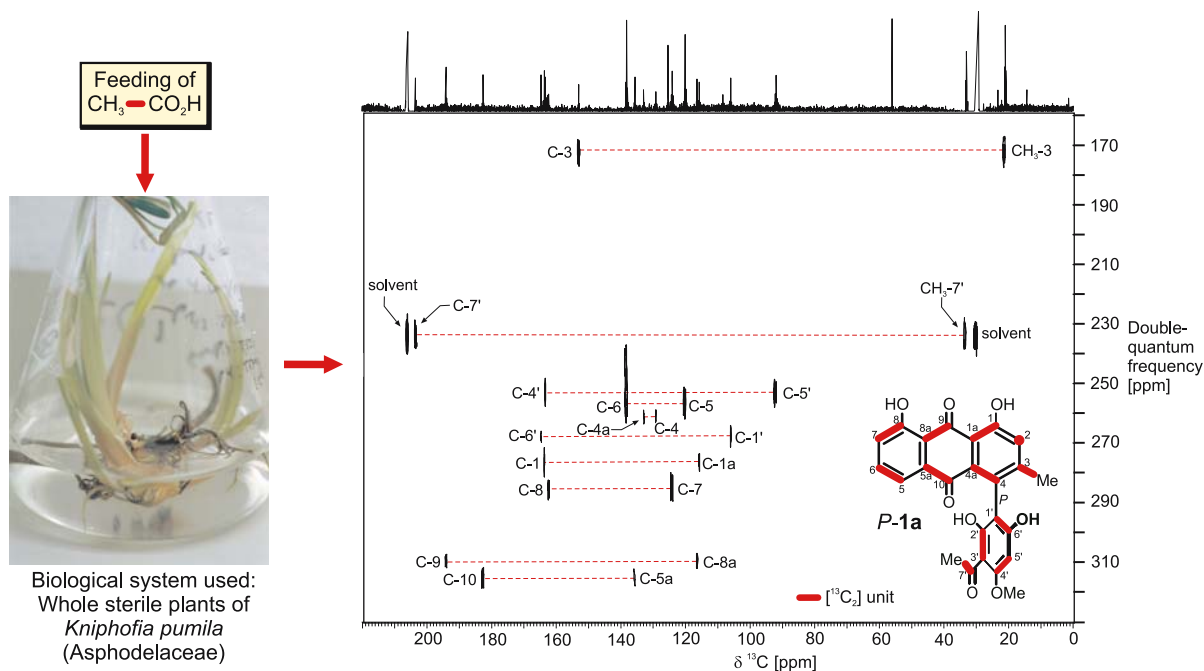
Fig. 9 Incorporation experiments on knipholone using aseptic whole plants: folding mode F in the chrysophanol part and, unexpectedly, *no* C$_2$-wise randomization in the phenyl portion
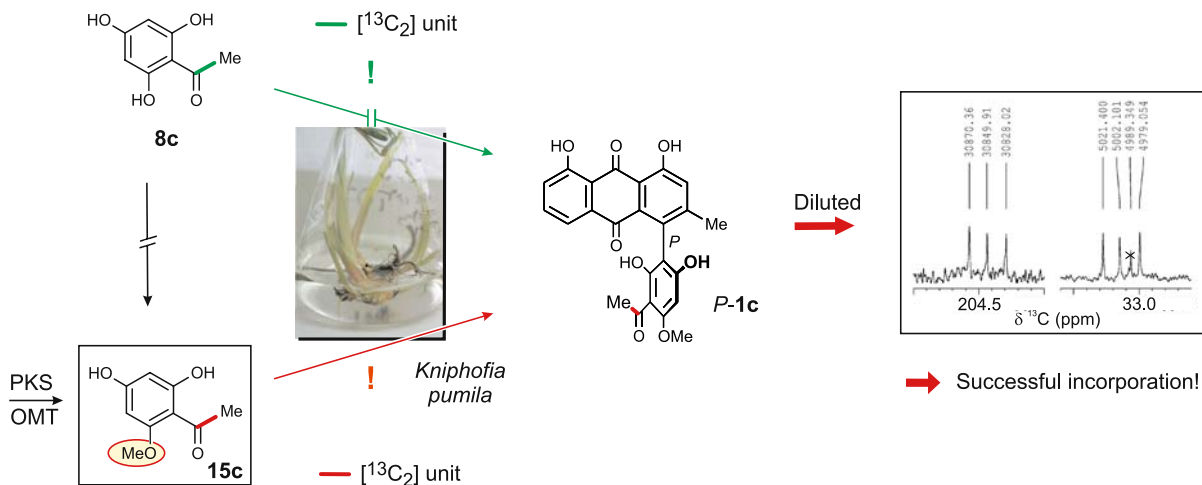

Fig. 10 Incorporation experiments with specifically $^{13}C_2$-labeled acetophenones, **8c** and **15c**

We have thus synthesized both acetophenones, **8** and **15**, in a specifically $^{13}C_2$-labeled form (viz. **8c** and **15c**) and fed them to aseptic *Kniphofia* plants (Fig. 10). Unfortunately, the two compounds are quite toxic to the plants and drive them into necrosis. Still, if one sufficiently dilutes the precursors, the feeding experiments give a conclusive result: the unambiguous *non*-incorporation of the symmetric trihydroxy compound **8c** and the likewise clear specific incorporation of the monomethyl ether **15c**. This also confirms that **15** itself does not originate from a free trihydroxy compound **8**, but somehow
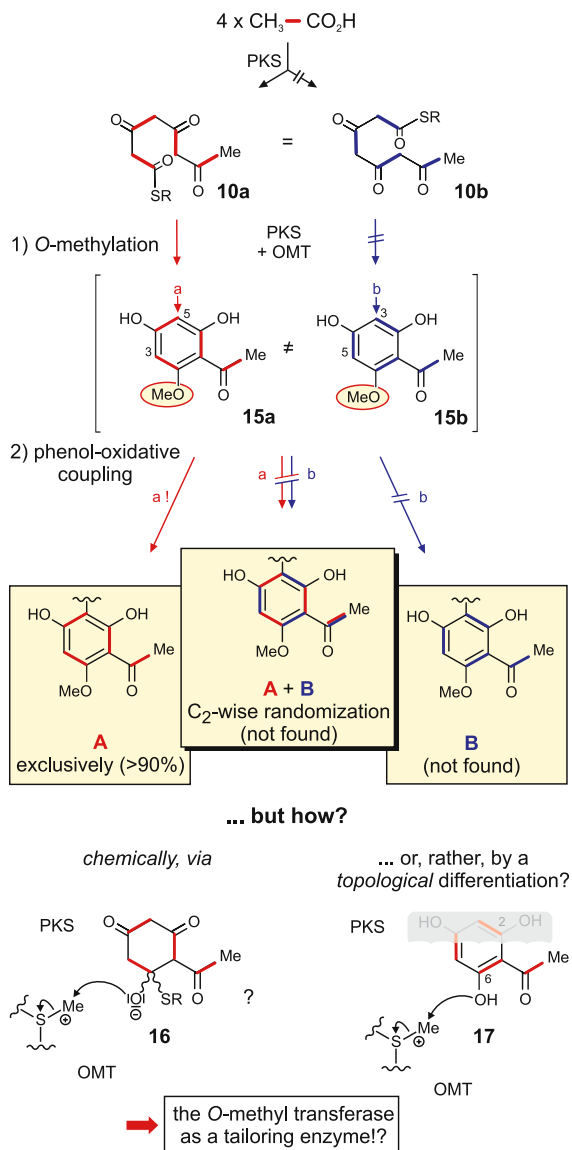
Fig. 11 The exclusive formation of only one 'isotopomer A' of knipholone (1) from [1,2-^{13}C$_2$]-acetate: as a consequence of a close cooperation of the PKS with the *O*-methylation enzyme, OMT!

'directly' from the PKS and the *O*-methyltransferase (OMT).

Thus, apparently, the methyl ether **15** is the immediate coupling substrate and, hence, the *previous O*-methylation reaction is the deciding desymmetrizing (or, rather, symmetry-preventing) step (Fig. 11, top)—and not the biaryl coupling, which must occur later!

Still, how can that be? The unsymmetry of the acetophenone **15** provenly results from the *O*-methylation, but before the *O*-methylation, it must have been symmetric, i.e., **8**—or not? Alternatively this decisive desymmetrizing step occurs so early and in such a close cooperation with the polyketide synthase that the resulting cyclization product is enzymatically 'trapped' before becoming a symmetric acetophenone.

This would be imaginable *chemically*, by an *O*-methylation before the aromatization, possibly at the level of a still 'pre-aromatic' intermediate **16** (Fig. 11, bottom left), which is not yet symmetric.

More probably, however, the differentiation may take place *topologically*, in a way that the trihydroxyacetophenone **8** is indeed formed, but is still in the PKS enzyme pocket as in **8a** and thus is unsymmetrically shielded and hence "protected" in the dimmed area. It could thus only be *O*-methylated at the exposed OH group at C-6, but not at the shielded (and hence not equivalent) one at C-2. In both cases, the OMT must be very close to the PKS—spatially and organizationally—acting as a 'tailoring enzyme' (Rix et al. 2002).

Conclusion

In summary, the expected C$_2$-wise scrambling of the acetate-derived labeling in the acetophone portion of knipholone does *not* occur, there is consequently *no* free symmetric intermediate like **8**, because the coupling substrate is the monomethyl ether **15**, and the coupling position is at C-4 in the phenyl portion and C-4 in the chrysophanol part (Fig. 12).

Chrysophanol (**7**) in turn is, as we have demonstrated, the first compound that is formed through more than one polyketidic pathway—here even three roads lead to Rome: F, S, and S′! In the case of knipholone from *Kniphofia*, the chrysophanol portion is formed according to the F type folding mode, as expected for a higher plant.

Other examples of secondary metabolites of *identical* structures, yet originating through different pathways, mainly concern the MEV/MEP dualism (Dewick 2002); for the recently established first secondary metabolite that is, simultaneously and by the same organism, built up via the MEP (20%) and the MEV (80%) route, furanonaphthoquinone I (FNQ I), see Bringmann et al. (2007a).
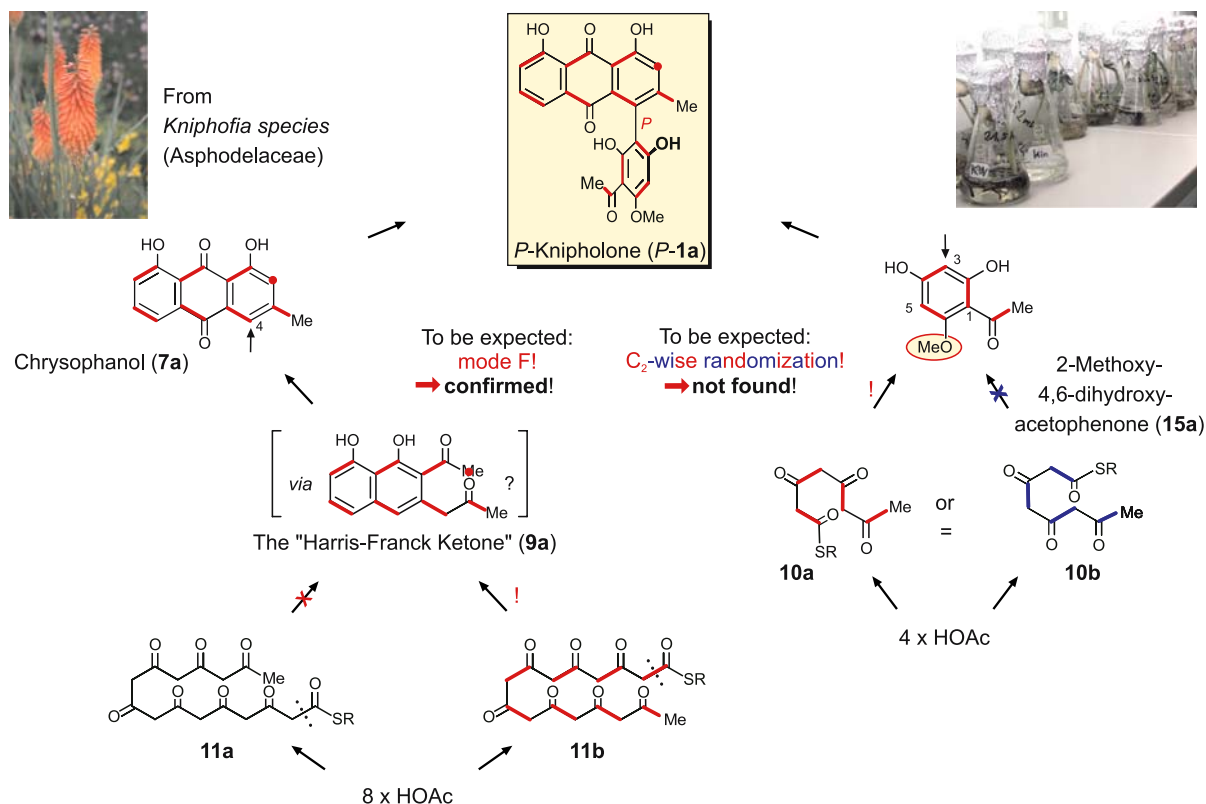
Fig. 12 The biosynthesis of knipholone (1)—an instructive example of structure, function, and evolution of PKSs and OMTs, and their cooperation

All other cases[3] of biosynthetic convergence known to date concern *similar* compounds, just from identical *classes* of metabolites produced via different pathways (Dewick 2002; Thomson 1997), like, e.g., amino acid derived versus acetogenic isoquinoline and piperidine alkaloids (Leete 1971; Stadler et al. 1988; Herbert 1995; Bringmann et al. 2000b), shikimic acid versus acetate derived versus amino acid and prenyl derived naphthoquinones (Bolkart and Zenk 1968; Cox and Gibson 1964; Durand and Zenk 1971; Inouye et al. 1979; Bringmann et al. 1998b, 2000a), and microbial versus plant-derived carbazol alkaloids (Kaneda et al. 1990; Chakraborty 1991).

Chrysophanol (7), by contrast, is the first—moreover multiple (three pathways!)—case of one identical compound originating through a divergent–convergent biosynthesis starting from the same precursors (eight molecules of acetate, one octaketide chain), divergently forming three sets of intermediate, and then converging to chrysophanol (7).

This biosynthetic convergence of chrysophanol (7) and the enzymatic cooperation in the enzymes producing the acetophenone portion make knipholone (1) an interesting model system to get insight into the structure, function, and evolution of PKSs and their interaction with other enzymes, here with the *O*-methyltransferase.

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[3] The biosynthesis of gibberellins (Hedden et al. 2002) may be considered as a different case. The pathways in fungi and plants only differ in the sequence of—otherwise identical—reactions, viz. in the order in which the hydroxyl groups are introduced, not in divergent folding types as for the biosynthesis of chrysophanol (7).

Apparently not a case of biosynthetic convergence is the virtually identical biosynthesis of β-lactam antibiotics like cephalosporin C in fungi and bacteria, since it does not only follow the same sequence of reaction types, but is, mostly, even assisted by the same sorts of enzymes—probably due to horizontal gene transfer between bacteria and fungi (Brakhage et al. 2005).

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