Abstract

The α-pyrene moiety is a structural feature found in a huge variety of biologically active metabolites. In recent times new insights into additional biosynthetic mechanisms, yielding in such six-membered unsaturated ester ring residues have been obtained. The purpose of this mini-review is to give a brief overview of α-pyrone and the mechanisms forming the basis of their natural synthesis. Especially the chain interconnecting enzymes, showing homology to ketosynthases which catalyze Claisen-like condensation reactions, will be presented.

Introduction

α-Pyrone (1, also 2-pyrone) represent a moiety widespread in nature (Figure 1). The motif of a six-membered cyclic unsaturated ester is present in a large number of natural products, and molecules containing α-pyrones can be found in all three kingdoms of life. Additionally α-pyrone, especially the structurally simple ones, i.e., triacetic acid lactone (2) and tetraacetic acid lactone (3) (Figure 1), represent widely exploited building blocks in synthetic chemistry. Examples are the syntheses of compounds like α-chymotrypsin, coumarins, pheromones, and solanopyrones [1]. Known biological functions reach from intermediates and end products in primary metabolism to signaling molecules and molecules which are applied for defense against competitors and predators. The biological activities these compounds exhibit is immense, including antimicrobial [2], antitumor [3,4], and cytotoxic activities [5]. Aflatoxins, produced by several Aspergillus species, are known to cause food poisoning due to their cytotoxic activity. They can regularly be found in improperly stored food, hence, entering the food supply chain [6]. Further coumarin derivatives, e.g., umbelliferone (4), esculetin (5), and scopoletin (6), are subject of investigation due to their pharmacological properties, i.e., anticancer effects (Figure 1) [7]. α-Pyrone has also been shown to be HIV protease [8-10] and selective COX-2 inhibitors [11,12], and further, signaling functions were attributed to them. Already in the 1990s an unusual dialkyl-substituted α-pyrone (supellapyrone, 7) was detected to be the cockroach sex pheromone [13], and recently it was reported that so called photopyrones (8–15) act as signaling molecules in the cell–cell communication system of the bacterium Photorhabdus luminescens (Figure 1) [14].
Figure 1: Selected monocyclic and monobenzo α-pyrene structures.

Since the biological activities of α-pyrones are very diverse, these compounds are in the focus of synthetic chemists [15]. Hence, the phenomenal abundance of natural products and of chemically synthesized derivatives therefrom justifies several reviews, and comprehensive articles exist [1,16]. However, in the present review the diverse biosynthesis of α-pyrones will be the focus. Different mechanisms for the biosynthesis of these mostly polyketide-derived structures exist, thus it is assumed that the route towards α-pyrones has been developed several times in evolution. They can be built up by the catalytic activities of the different types of polyketide synthase (PKS) systems, and especially the final ring formation yielding in the α-pyrone moiety can be accomplished in different ways. The different biosynthetic routes towards an α-pyrene ring will be presented.

The biosynthetic mechanisms to yield saturated lactones, like the statin drug lovastatin, which is in application for lowering cholesterol, will not be discussed.

Review

1 Occurrence and activities

In this chapter special sub-types of α-pyrones will be described. The compounds are grouped into three categories depending on their structural features: (i) dibenzo-α-pyrones, (ii) monocyclic α-pyrones, and (iii) monobenzo-α-pyrones.

1.1 Dibenzo-α-pyrones

Dibenzo-α-pyrones (16) harbor the α-pyrone moiety in the middle part and consist of three ring structures (Figure 2). Aromatic rings are fused to edge c and e of the central 2-pyrone, yielding the basic structure of 16.

Many dibenzo-α-pyrene-producing fungi have been described. However, it seems that they are mainly distributed in the Alternaria species and mycobionts. Especially endophytic fungi can be regarded as source organisms. Alternariol (17), altenuene (18), and alternariol 9-methyl ether (19) have been described from Alternaria sp. [17], botrallin (20) from Hyalodendriella sp. [18], and graphis lactone A (21) from Cephalosporium acremonium IFB-E007 (Figure 3) [19]. These compounds show toxic effects in plants and animals. In addition, Alternaria spp. have been involved in the contamination of food, even in refrigerated stocks, since the fungi is able to grow also at low temperature. Alternaria spp. had also been linked to a poultry disease outbreak called poultry hemorrhagic syndrome. However, the main toxic effects seem to be linked to other toxins produced, e.g., the non pyrone metabolite tenuazonic acid [20]. Nevertheless, alternariol (17) and altenuene (18) were studied for their toxicity using different assays. Toxicity to Artemia salina larvae was examined by measuring the optical motility and resulted in IC₅₀ values of 150 µg/mL [21]. A comparable result was obtained using the disk method of inoculation, whereby the IC₅₀ values were 100 µg/mL for 17 and 375 µg/mL for 18 [22].
Further, alternariol (17) and derivatives were tested against L5178Y mouse lymphoma cells. Here 17 was the most active compound with an EC$_{50}$ value of 1.7 μg/mL [23]. In another in vitro assay, this time a biochemical assay using protein kinase, the IC$_{50}$ values were determined, and 17 inhibited 10 out of the 24 kinases tested. The results of the MTT and the kinase assay showed a similar pattern, and hence it was concluded that protein kinase inhibition should be one mechanism leading to the cytotoxicity of 17. In a study using human colon carcinoma cells to elucidate the cell death mode and the pathways triggered by 17, the induction of an apoptotic process was revealed. Further investigations showed that cell death was mediated through a mitochondria-dependent pathway [24]. In murine hepatoma cells it was shown that 17 and its methyl ether 19 interfere with the transcription factor and by inducing the so-called aryl hydrocarbon receptor, apoptosis is mediated by inducing cytochrome P450 1A1 [25]. For alternariol 9-methyl ether (19) and the graphislactone A (21) cytotoxic effects against the human cancer cell line SW1116 with IC$_{50}$ values between 8.5 and 21 μg/mL were reported [26].

These toxic fungi-derived metabolites are often pathogenic to plants, and are therefore called phytotoxins. Phytotoxins are divided into host-specific and host non-specific toxins, whereby the here named *Alternaria*-derived dibenzo-α-pyrones 17, 18, and 19 represent host-specific phytotoxins [26].

Several dibenzo-α-pyrones have been isolated from plant parts. Purified from roots, bulbi, heartwood, or whole plant material, the origin of some plant-derived pyrones is not finally clarified, since the production by endophytic fungi cannot be excluded. Djalonensone was isolated from *Anthocleista djalonensis* (Loganiaceae) roots, but is identical to alternariol 9-methyl ether (the corresponding bioactivities are described above.) The latter was isolated from a series of fungi including endophytic species. Thus, the possibility that a fungus is the real producer cannot be ruled out. In addition, production by a fungus and modification of the metabolites by plant enzymes is also possible. Further α-pyrene plant secondary metabolites are ellagitannins and ellagic acid (22) [27] (Figure 4). These metabolites are important constituents of different foods, e.g., berries, nuts, medicinal plants and tisanes, as well as of grapes and oak-aged wines. These natural products are not absorbed in the intestinal tract; rather they are metabolized by intestinal bacteria, yielding so called urolithins (23–27, Figure 4). Therefore, it can be assumed that the urolithins are responsible for the biological activities related to the intake of ellagitannins by higher organisms. Such urolithins show different phenolic hydroxylation patterns and have been isolated from animal feces.

Concerning the activity urolithin A (23), urolithin B (24), and isourolithin A (27), all isolated from fruits of *Trapa natans* (water chestnut) showed antioxidative activity [28]. Testing urolithins A, B, C, D (23–26) in an assay using myelomonocytic HL-60 cells showed antioxidative activities for 23, 25 and 26. These three derivatives inhibited the reactive oxygen species (ROS)-dependent oxygenation of the non-fluorescent 2',7'-dichlorodihydrofluorescein (DCFH) to the fluorescent 2',7'-dichlorofluorescein (DCF) [29]. This antioxidant activity

![Figure 3: Selected dibenzo-α-pyrones.](image)

![Figure 4: Structure of ellagic acid and of the urolithins, the latter metabolized from ellagic acid by intestinal bacteria.](image)
was also linked to anti-inflammatory effects by testing the in vivo effects of 23 in a carrageenan-induced paw edema assay. Oral administration of 23 to mice prior to carrageenan injection resulted in a significant decrease in paw edema, compared to the control group [30]. Further, weak antiallergic activity in the mM range was indicated for urolithin A (23), urolithin B (24), and isourolithin A (27), by testing the influences of these compounds on the activity of the enzyme hyaluronidase. The latter is involved in inflammation reactions. The authors isolated 23, 24 and 27 from the feces of Trogopterus xanthipes (flying squirrel) by bioactivity-guided fractionation, and determined IC50 values for the pure compounds to be in the low mM range (1.33, 1.07 and 2.33 mM, respectively) [31]. Also, estrogenic and antiestrogenic activities in a dose-dependent manner were shown for 23 and 24. Thus, the authors suggested further research to evaluate the possible role of ellagitannins and ellagic acid as dietary “pro-phytoestrogens” [32].

Even though many α-pyrones have been isolated from bacteria, only one dibenzo variant was described, i.e., murayalactone (28) isolated from Streptomyces murayamaensis (Figure 5) [33].

![Figure 5: Structure of murayalactone, the only dibenzo-α-pyrone des-cribed from bacteria.](image)

### 1.2 Monocyclic α-pyrones

In addition to the aforementioned examples also the simplest α-pyrones show remarkable biological effects. Isolated from several fungi, e.g., Trichoderma viride, 6-pentyl-α-pyrone (29) showed antifungal activity against Rhizoctonia cerealis, Gaemannomyces graminis and Botrytis cinerea (Figure 6) [34]. The structural related trichopyrone (30) instead showed no antimicrobial activity [35]. For compound 29 it was further revealed that it represents the prominent headspace volatile of Trichoderma asperellum IsmT5 [36]. Deeper investigation of the volatiles released by Trichoderma species revealed the complexity of the volatile mixture consisting of many derivatives [37]. Several alkylated and alkenylated α-pyrones with length variations in the side chain and different positions of olefinic double bonds were isolated in the headspace extracts and unambiguously assigned by comparison to authentic standards [37]. Co-cultivation experiments of T. asperellum and Arabidopsis thaliana without physical contact resulted in smaller but vital and robust plants. Therefore, 29 was applied to A. thaliana, and the growth and defense reactions were verified. A. thaliana pre-exposed to 29 showed significantly reduced symptoms when challenged with B. cinerea and Alternaria brassicicola [36].

![Figure 6: Structures of the 6-pentyl-2-pyron (29) and of trichopyrone (30). Only 29 showed antifungal activity.](image)

Beside the examples of simple substituted α-pyron derivatives, such as triacetic acid lactone (2), tetracetic acid lactone (3), and 6-pentyl-2-pyron (29) also more complex systems, e.g., bufalin (31) [38], fusapyrones (32,33) [39], or the α-pyron antibiotics corallopyronins (34,35) [40] and myxopyronins (36,37) [41], exist in the group of monocyclic α-pyrones (Figure 7).

The bufadienolides are an important group of steroids containing an α-pyron moiety. The α-pyron ring is here connected to a steroid nucleus, as exemplified in bufalin (31, Figure 7). These α-pyrones were detected in several plants, as well as in animals. The vast amount of derivatives shows also very diverse biological activities. The bufadienolides from succulent plants of the family Crassulaceae cause the symptoms of cardiac poisoning in animals. Animal sources are the name giving toad genus Bufo and others, e.g., Photinus (fireflies) and Rhabadophis (snake). The abundance of bufadienolides in some Bufo species is extremely high, and all together, over eighty derivatives have already been isolated, e.g., the epoxide-containing resibufogenin (38, Figure 7) was isolated from the Chinese toad skin extract drug Ch’an Su. It showed growth inhibition effects on human oral epidermoid carcinoma KB cells and murine leukemia MH-60 cells [42].

Testing the inhibitory effect of corallopyronin A (34) against various microorganisms revealed promising activity against Gram-positive bacteria, but no relevant effect on Gram-negative bacteria (only at concentrations >100 µg/mL activity was observed). Against Staphylococcus aureus a MIC of 0.097 µg/mL and against Bacillus megaterium of 0.39 µg/mL was obtained [40]. Myxopyronin B (37), the most active derivative of the myxopyronins, showed comparable activities, e.g., MIC of 0.3 and 0.8 µg/mL against S. aureus and B. megaterium, respectively [43]. In addition corallopyronin A was also...
tested successfully using an in vivo mouse model for the treatment of infections with filarial nematodes [44]. Such antibiotics produced by heterotroph bacteria, e.g., marine and terrestrial myxobacteria which can feed on other bacteria, are suggested as predatory weapons to paralyze and kill their prey [45,46].

Fusapyrone (32) and the derivative deoxyfusapyrone (33) had been isolated from *Fusarium semitectum* [39]. These compounds show considerable antifungal activity, e.g., a minimum inhibitory concentration against *Botrytis cinerea*, *Aspergillus parasiticus*, and *Penicillium brevi-compactum* in the range of 0.78–6.25 µg/mL [47]. Testing the zootoxicity of 32 and 33, using brine shrimp assays, revealed that only approximately 50-fold higher concentrations had a negative effect. Therefore, it was concluded that these compounds might be used together with biocontrol yeasts to control crop diseases which can occur while storing the crops [47]. From another strain of this fungal genus, i.e., *Fusarium fujikuroi*, the gibepyrones A–F (39–44) were isolated (Figure 8) [48]. The activity of these compounds was tested against bacterial and fungal strains. However, the activities were extremely low, e.g. gibepyrone A inhibited *B. subtilis* and *S. cerevisiae* at 100 µg/mL.

The diastereomeric pair of phomenin A (45) and phomenin B (46) was isolated from the phytopathogenic fungus *Phoma tracheiphila*,[49] and from *Alternaria infectoria* (Figure 9) [50]. Further, the same compound 45 was isolated from *Leptosphaeria maculans* and named phomapyrone A, as well as from the mediterranean ascoglossan mollusc *Ercolania fune-
Figure 9: Structures of the phomenins A and B.

Real, described as cyercene [51]. Phomenin A displayed phytotoxicity at a concentration of 100 µg/mL. Chemical synthesis approaches enabled then to investigate many more α-pyron derivatives for their antimicrobial and cytotoxic properties [2].

The volatile α-pyrene 5-(2,4-dimethylheptyl)-3-methyl-2H-pyran-2-one (7, Figure 1), also named supellapyrone) is used by female brownbanded cockroaches to attract males [13]. It is known that cockroaches use pheromones in many aspects of influencing interacting behavior between individuals. Hence, such volatiles are used in courtship behavior to find mating partners. Also another α-pyrene fulfilling pheromone function in insects is known, i.e., the queen recognition pheromone of the red imported fire ant, 6-(1-pentenyl)-2H-pyran-2-one (47, Figure 10) [52].

Also antitumor activities of α-pyrones had been shown. Thus, pironetin (47, Figure 10) induced apoptosis in a dose- and time-dependent manner, and tubulin assembly was inhibited in vitro [53]. The natural product was isolated from Streptomyces sp. NK10958 [54], and its biosynthesis was investigated using various 13C-labeled precursors [55]. Hence, it was concluded that beside four acetate units also two propionate units and one butyrate unit form the backbone, while the O-methylation is S-adenosyl-methionine dependent.

Several derivatives of 6-alkyl (alkoxy or alkylthio)-4-aryl-3-(4-methanesulfonylphenyl)pyrones 49 had been synthesized to get insights into structure activity relationships, whereby 6-methyl-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-one (50) showed the best combination of inhibitory concentration and selectivity (IC50 = 0.68 µM, SI = 904; Figure 11) [56].

Figure 10: Structures of monocyclic α-pyrones showing pheromone (47) and antitumor activity (48), respectively.

A further group of compounds are the kavalactones 51 (Figure 12), e.g., yangonin (52, Figure 12), which have been isolated from Piper methysticum [57]. At various regions of the Pacific Ocean the roots of the plant have been used for a long time to produce a drink with sedative and anesthetic properties. The α-pyrones responsible for the influence on the nervous system have a wide variety of effects including amnestic, analgesic, anticonvulsant, anxiolytic, nootropic, and sedative/hypnotic activities [58].

Figure 11: Structures of 6-alkyl (alkoxy or alkylthio)-4-aryl-3-(4-methanesulfonylphenyl)pyrones.

Highly active α-pyrones, i.e., germicidins (53, 54, Figure 13), were isolated from Streptomyces viridochromogenes NRRL B-1551, whereby the compounds had been detected in the supernatant of germinated spores, as well as in the supernatant of the submerged culture [59]. The excretion of these compounds prevents the germination of the spores too close to the
parent culture. Germination of *S. viridochromogenes* NRRL B-1551 spores is inhibited at pM concentrations, i.e., 200 pM (40 pg/mL). A comparable effect was also observed by applying 53 and 54 to seeds, however, only at much higher concentrations. Germination of *Lepidium sativum* (garden cress) seeds was clearly retarded. An additional in vitro effect was inhibition of porcine Na\(^+\)/K\(^+\)-activated ATPase. Germicidin was the first known autoregulative inhibitor of spore germination in the genus *Streptomyces* [59]. Influence on plant germination was also shown for further lactones. An inhibiting effect was proven for 3,4-dimethylpentan-4-olid from the plant pathogenic fungus *Hymenoscyphus pseudoalbidus*, which inhibited germination of *Fraxinus excelsior* (European ash) seeds [60]. In contrast, 3-methyl-2\(H\)-furo[2,3-\(c\)]pyran-2-one, a component of smoke derived from burning plant material, promotes seed germination [61].

Recently, a further regulatory function for \(\alpha\)-pyrones within bacteria was discovered. The so called photopyrones (8–15, Figure 1) represent extracellular signals involved in cell–cell communication [14]. *Photorhabdus luminescens*, an entomopathogenic bacterium species, excretes these molecules, and binding of the latter to the respective receptor, i.e., the PluR protein, leads to the activation of the *Photorhabdus* clumping factor (PCF) operon (pcfABCDEF). The phenotypic change observed due to PCF expression was cell clumping, which in turn contributed to insect toxicity [14]. Structurally related are the pseudopyronines A (55), B (56), and C (57, Figure 14), which have been isolated from different *Pseudomonas* strains [62,63]. Compounds 55 and 56 had been initially tested positive for antimycobacterial and antiparasitic activities and both inhibited fatty acid biosynthesis [62]. The new derivative 57, possessing a longer eastern acyl moiety, was identified in *Pseudomonas* sp. GM30, and it was subsequently proven by heterologous expression experiments with ketosynthase which is responsible for the biosynthesis of these derivatives [63].

Aflatoxins are poisonous and cancer-causing monobenzo-\(\alpha\)-pyrones [6]. Several derivatives exist, whereby aflatoxin B\(_1\) (61, Figure 16) represents the most poisonous compound. Usually these toxins are ingested, but 61 can also permeate through the skin. The aflatoxins are PKS-derived molecules which undergo an extreme rearrangement [66]. The cytotoxic effects of the coumarin derivatives umbelliferone (4, Figure 1), esculetin (5, Figure 1), and scopoletin (6, Figure 1) are subject of anticancer research [67]. Marmesin (62) was first isolated from the fruits of *Ammi majus* [67], and is currently under investigation as an agent for the treatment of angiogenesis-related diseases, e.g., cancer [68]. A structurally related compound, i.e., isopim-
pinellin (63), was also first isolated from fruits of Ammi majus [69]. It was shown that 63 blocks DNA adduct formation and skin tumor initiation in mice [70]. Psoralen (64), isolated from plants, e.g., Ficus carica, had been used against skin diseases due to its mutagenic effect [71].

Bacterial monobenzo-α-pyriones were isolated from the myxobacterium Stigmatella aurantiaca MYX-030. Myxocoumarins A (65) and B (66) were identified, and 65 was tested for antifungal activity [72]. It showed a promising activity against agronomically important pathogens, e.g., complete inhibition of Magnaporthe grisea and Phaeosphaeria nodorum at 67 µg/mL, and Botrytis cinerea was inhibited at 200 µg/mL.

2 Biosynthesis

Even though the α-pyriones possessing interesting activities were in the focus of chemical synthesis approaches for a long time, for most of them the clarification of the biosynthesis remained unknown for many years.

An early example for a biosynthetic hypothesis is the biosynthesis of the simple 6-pentyl-α-pyrone (29), which was hypothesized to start with the C-18 linoleic acid. This acid is then shortened by β-oxidation reactions to a C-10 intermediate, i.e., 5-hydroxy-2,4-decenoic acid (72), which undergoes lactonization to yield 29 (Figure 17) [34]. This hypothesis is based on the fact that feeding studies with Trichoderma harzianum and T. viride using [U-14C]linoleic acid or [5-14C]sodium mevalonate revealed the incorporation of these labelled compounds into 6-pentyl-α-pyrone (29). Labelled sodium mevalonate was used to test for the possible link between the isoprenic pathway and biosynthesis of 29. The experiments revealed that the incorporation of labelled linoleic acid reached within the first 24 hours 18-fold higher ratios than labelled sodium mevalonate. Therefore, the authors suggested that β-oxidation of linoleic acid is a probable main step in the biosynthetic pathway of 29 in Trichoderma species [34]. The incorporation of labelled sodium mevalonate is hypothesized to be due to degradation to acetate with following polymerization to fatty acids [34].

Now, it is generally accepted that most α-pyriones are synthesized via the polyketide pathway. Solely for plant-derived ellagitannins another biosynthetic origin was described. Via the shikimate pathway gallic acid is generated, which represents the precursor in ellagitannin biosynthesis [73]. The ellagitannins...
Figure 18: Proposed biosynthetic pathway of alternariol (modified from [77]). Malonyl-CoA building blocks are applied to build up the enzyme-bound polypeptide chain. Cyclization between C-2, C-7 and C-8, C-13, as well as lactonization takes place, resulting in alternariol (17). Subsequently, a methylation and a hydroxylation reaction occur, catalyzed by the respective enzymes.
terminal module, which results in a cis-configured double bond. Through the formation of the cis double bond the sterical arrangement of the nascent chain favors the lactone ring closure which results in the α-pyrene moiety. Hence, the polyketide is released from the assembly line, whereby the thioesterase (TE) domain catalyzes the ring-closure and therewith also the off-loading from the PKS_I system [79]. A comparable mechanism, in which a TE is involved in off-loading the nascent chain from the PKS_I assembly line by lactonization, was described for other natural products, e.g., the isochromanone ring formation for the ajudazols A and B in Chondromyces crocatus Cm c5 [80].

2.2 Biosynthesis by PKS_{II} systems

In the type II PKS-catalyzed biosynthesis, the subunit type of such megaenzyme systems, the starter molecule and the extender units, mostly malonate molecules, are assembled at the same ACP. A lactonization at the ACP-bound terminus yields the pyrone ring. As an example the enterocin (76, Figure 19) biosynthesis will be regarded (Figure 20). In the marine bacterium Streptomyces maritimus a gene cluster corresponding to enterocin (ene) biosynthesis was identified [81]. The minimal ene PKS, EncABC, is encoded by a set of genes architecturally similar to most other type II PKS clusters. EncA represents the KS{alpha}, EncB the KS{beta}, and EncC the ACP domain. First, an uncommon benzoate starter unit gets elongated by EncB the KS{alpha}, and EncC the ACP domain. α-represents the KS{alpha} structurally similar to most other type II PKS clusters. EncA minimal PKS, EncABC, is encoded by a set of genes archi-
cering to enterocin (76) biosynthesis was identified [81]. The biosynthesis of

2.4 Biosynthesis by free-standing ketosynthases

In contrast to the α-pyrene formation by intramolecular cyclization reactions, also the condensation of two polyketide chains can result in a pyrone ring. Such a mechanism was indicated by feeding experiments for the antibiotically active compounds 36 [83] and 34 [84]. The resulting labeling pattern clearly showed that the central α-pyrene ring of the molecule was not the result of a usual intramolecular reaction. Rather, an interconnection of two independent chains should form the central ring structure. In addition further molecules, e.g., photopyrones (8-15) from Photorhabdus luminescens are synthesized by such a head-to-head condensation of two acyl moieties [60]. Also the csypyrones (79-81, Figure 21), first reported from Aspergillus oryzae, are composed of two independent chains which are interconnected thereafter [85]. Recently, the biosynthetic origin of the pseudopyronines A (55) and B (56) in Pseudomonas putida BW11M1 was clarified — and again two chains are fused to yield the final products [86]. Thus, it can be assumed that this mechanism is exemplified quite often in natural products. Therefore, in the next paragraph the chain interconnecting mechanism will be described.

For α-pyrene antibiotics, the corallopyronin and myxopyronin derivatives, free-standing KSs encoded in the respective cluster, i.e., CorB and MxnB, were suggested as the chain-interconnecting enzymes [84,87]. These enzymes have now been investigated in detail.

In vitro assays using NAC thioesters of the western and eastern chains in the biosynthesis of 36 [88], as well as simplified sub-
Figure 20: Pyrone ring formation. Examples for the three types of PKS systems are shown in A–C. In D the mechanism catalyzed by a free-standing ketosynthase is depicted. Herein the keto–enol tautomeration is shown. A) Polyketide synthase (PKS) type I: The end part of the phenylannolone A biosynthesis is given. The ACP-tethered nascent chain gets elongated by the incorporation of acetate units. The corresponding reductive domains (ketoreductase, KR; and dehydratase, DH) reduce the β-keto group to a cis double bond. The chain is then released from the assembly line through pyrone ring formation catalyzed by the thioesterase (TE) domain, resulting in 73. B) PKS type II: The precursor of the enterocin biosynthesis, comprising the uncommon benzoate starter unit, is shown attached to the ACP domain, which forms a complex with the KSα and the KSβ domain. Modification, rearrangement and lactonization of this bound precursor yield enterocin (76). C) PKS type III: The starter molecule, e.g., a CoA-activated fatty acid, gets loaded to the PKS III enzyme. Two rounds of chain elongation via malonyl-CoA take place before the molecule is released by pyrone ring formation, resulting in 77. D) The two ACP-tethered chains are interconnected by the catalytic activity of a free-standing KS. In the second step the lactonization takes place, facilitated by the keto–enol tautomeration. Thereby the α-pyrene 78 is formed.

strate mimics of both antibiotics [88,89] provided experimental evidence that the free-standing ketosynthases are responsible for the α-pyrene ring formation. In both publications non-enzymatic condensation was ruled out, since in the absence of the respective protein no product formation was detectable. For MxnB it was further shown that in vitro conditions can be optimized by applying carrier-protein-bound substrates instead of the SNAC-coupled substrates, i.e., this resulted in a 12-fold increase of product formation. This is an additional hint that protein–protein interactions represent an important factor in PKS systems. Further, it seemed that the carrier proteins conferred specificity for α-pyrene ring formation, since once the carrier proteins were primed in each case with the other substrate (mimic), the production rate decreased significantly.
However, a certain degree of flexibility in α-pyrene ring formation was proven by the in vitro experiments using the ketosynthases CorB and MxnB. In addition, the substrate specificity was analyzed in vivo in a mutasynthesis study employing a Myxococcus fulvus mutant unable to biosynthesize the western chain. This study revealed that MxnB is capable of condensing a wide variety of activated synthetic western chains with the carrier protein bound native eastern chain [90].

The two proposed mechanisms for CorB and MxnB closely resemble each other, but certain differences have also been proposed, as will be discussed here. First, one chain is transferred and covalently linked to the active-site cysteine. This results in an activation of the cysteine-tethered chain. In the second step, the other chain is placed into the proximal cavity, orienting the α-carbon in a position suitable for the nucleophilic attack by the cysteine-tethered, activated chain. Thereby, the second chain is still attached to the ACP, the phosphopantetheine residue reaching into the T-shaped catalytic cavity, enabling the placement of the two chains in opposite directions (Figure 22 and Figure 23). In that way a nucleophilic attack of the enzyme-bound chain onto the carbonyl carbon of the ACP-tethered chain is facilitated. Hence, a diketothioester is formed, which results in chain interconnection and the release of the catalytic cysteine. Subsequently, lactonization can take place. It is assumed that an enolate exists as an intermediate in the formation of the C–O bond [88]. Even though for both enzymes no experimental evidences for the chronological order of the two condensation reactions exist, it can be expected that the C–C

Figure 21: Structures of csypyrones.
bond is formed prior to lactonization [88]. For the following lactonization process a spontaneous reaction can be anticipated, which takes place once the two chains are interconnected, since thereby the atoms needed for lactonization are positioned in close proximity to each other. The sterical requirements within the catalytic cavity of CorB and MxnB do not favor the ring closure, thus the second step might take place in solution [90].

It has to be mentioned that the results between CorB and MxnB differ slightly. The in vitro results obtained for MxnB imply that the western chain gets covalently attached, prior to condensation with the second chain. The transfer of the western chain from the corresponding ACP to MxnB occurred much faster than the transfer of the eastern chain [88]. However, concerning CorB it was possible to observe a substantial positive electron density at the catalytic cysteine as a result of substrate incubation prior to crystallization. This was only possible with a very short substrate mimic which renders more similarity to the eastern chain. Using the longer western chain mimic no suitable crystals for structure determination could be produced (neither for CorB, nor for MxnB). Thus, in the CorB model the eastern chain was covalently attached. These inconsistent results indicate that the use of different chains could alter the binding preference.

Also CsyB from Aspergillus oryzae catalyzes the condensation of two β-ketoacyl-CoAs [85]. However, this mechanism to form 3-acetyl-4-hydroxy-6-alkyl-α-pyrones (79–81) significantly differs from the one catalyzed by the myxobacterial ketosynthases described before [89]. CsyB is indeed an up to now unexemplified case of a type III PKS with dual function. First, CsyB catalyzes chain elongation – as many other PKS III enzymes. Secondly, it catalyzes the condensation of two β-ketoacyl units – a mechanism comparable to the enzymes described in the previous paragraph. It possesses two β-ketoacyl-CoA coupling activities to synthesize acylalkylpyrone. The initially proposed mechanism for the formation of 3-acetyl-4-hydroxy-6-alkyl-α-pyrone by CysB was the coupling of a β-keto fatty acid acyl intermediate with acetoacetyl-CoA, followed by pyrone ring formation (Figure 24 A) [85]. Then, as the crystal structure was solved the authors proposed the detailed mechanism as follows [91]: First, acetoacetyl-CoA is loaded onto the catalytic cysteine residue. Subsequently, the thioester bond is cleaved by the nucleophilic water molecule, which itself is activated through hydrogen bonding to the catalytic cysteine and a histidine residue. Thereby, the β-keto acid intermediate is generated. This intermediate is proposed to be placed within the novel pocket, a cavity accessible from the conventional elongation/cyclization pocket. After the replacement of the first β-keto acid, the second
β-ketoacyl unit is produced. The catalytic cavity of CysB is loaded with a fatty acyl-CoA which is elongated with one molecule of malonyl-CoA, yielding the second β-ketoacyl chain. Condensation of the two chains generates the final product, whereby first the two chains are interconnected due to a nucleophilic attack, and subsequently an intramolecular lactonization takes place. In that way the ring closure results in the elimination of a water molecule, yielding the csypyrones harboring four O-atoms. The first step of the proposed mechanism was delineated from a set of in vitro assays, which indicated that the $^{18}$O atom of the H$_2^{18}$O molecule – which should be activated by hydrogen bonds networks with a histidine and the catalytic cysteine residue – is enzymatically incorporated into the final product (Figure 24 B). However, this mechanism is hard to prove, because $^{18}$O incorporation into the molecule can occur due to spontaneous exchange. Anyway, CysB clearly differs from CorB and MxnB. The latter condense two β-ketoacyl chains in a Claisen-like reaction to form the α-pyrone, while CysB should first generate a β-keto acid intermediate by hydrolysis of the thioester bond. Then the starter of the second chain is loaded onto the free catalytic cysteine, gets elongated by a malonyl-CoA before the nucleophilic attack of the first chain. In that way the thioester bond is cleaved and subsequently lactonization takes place, yielding in the final product (Figure 24 B).

In *Photorhabdus luminescens* it was shown that α-pyrones act as bacterial signaling molecules at low nanomolar concentrations [14]. A similar mechanism for the biosynthesis of these photopyrones as for the above mentioned α-pyrole antibiotics myxo- and corallopyronin was expected. To identify the gene corresponding to the biosynthesis of these so-called photopyrones, all ketosynthases which are not part of the usual fatty acid biosynthesis had been identified in the genome of *P. luminescens*. Thereby the ketosynthases neighbored by genes related to fatty acid synthesis had not been considered. Insertion mutants were generated and the influence on photopyrone production was analyzed. Thus, the gene *ppys* (for photopyrone synthase) was identified, since all other disruption mutants did not yield in a photopyrone negative strain. Heterologous expres-

![Figure 24: Proposed mechanism for the CysB enzymatic reaction. A) Coupling reaction of the β-keto fatty acyl intermediate with acetoacetyl-CoA followed by pyrone ring formation (modified from [85]). B) Detailed mechanism; the two chains are color coded (orange and violet), as well as the water molecule (red) whose oxygen atom is incorporated into the α-pyrene (modified from [91]).](image-url)
sion of ppyS in E. coli, together with the bkdABC operon (encoding the branched chain α-ketoacid dehydrogenase (Bkd) complex) and ngrA (encoding a phosphopantetheinyl-transferase which is essential to generate the holo-acyl carrier protein BkdB) for the biosynthesis of branched-chain iso-fatty acid, resulted in the production of photopyrone derivatives. This was a functional proof that PpyS catalyzes the formation of α-pyrones, as indicated before by feeding experiments with stable isotope-labeled precursors. PpyS should connect 5-methyl-3-oxohexanoyl thioester and different thioesters of straight-chain and iso-branched chain fatty acids [14]. The mechanism proposal also includes the catalytic cysteine. The first chain, i.e., thioester-activated 9-methyldecanoic acid, gets covalently tethered to that important residue within the active site. This reflects the same mechanism as for the other KS-like enzymes described. Also for PpyS the proposal postulates that the α-carbon of the enzyme-bound chain acts as a nucleophile. Thus, this activated carbon executes a nucleophilic attack on the carbonyl carbon of chain two, i.e., 5-methyl-3-oxohexanoyl thioester, which is itself synthesized by the Bkd complex. In that way a C–C bond is formed, and both chains are still attached to the catalytic cysteine residue. This bound intermediate undergoes a further deprotonation, which enables the formation of the α-pyrone ring. Through the ring closure the α-pyrene is released from PpyS. This second deprotonation can occur spontaneously, or enzyme catalyzed. In contrast to the cases of myxopyronins 36 and 37 and corallopyronins 34 and 35, no PKSI system provides the ACP-bound chains. Therefore, the substrates for the chain interconnection might be either ACP or CoA bound. This would be depending on their origin in the cell, either fatty acid biosynthesis or degradation. The flexibility of the system in regard to the first chain to be bound to PpyS was already shown by the photopyrones A–H, which differ in the chain length and in the either branched or unbranched starting unit.

No crystal structure for PpyS exists. Therefore, the structure was modeled using OleA from Xanthomonas campestris, which is showing the highest sequence identity (27%) of all available PDB-deposited crystal structures as template. Using the generated homodimeric model of PpyS, docking studies of the substrates onto the catalytic cysteine were performed. The resulting model suggested that a glutamate residue, which reaches into the catalytic cavity of the respectively other homodimer, acts as a base by forming a hydrogen bond with the α-carbon of the covalently bound substrate (Figure 25). Indeed, the exchange of this glutamate against an alanine residue resulted in an inactive version of the protein. Further an arginine residue, which could be involved in dimerization, was mutated to an aspartate. Also this mutant lost its catalytic activity, indicating that dimerization is essential [63].

The pseudopyronine synthase PyrS represents a homologue of PpyS. Using PpyS from Pseudomonas sp. GM30, it was analyzed if this KS is also involved in the formation of α-pyrones. The two pseudopyronines A (55) and B (56) have been up to now isolated from different Pseudomonas strains. Recently, in an independent publication 55 and 56 have been rediscovered from the banana rhizobacterium Pseudomonas putida BW11M1 [86]. Feeding studies with isotopically labelled precursors supported the biosynthesis from two chains. Subsequent analysis of the draft genome of the strain revealed a ppyS homologue. However, instead of the syntenic genomic region where pseudomonads usually harbor the ppyS homologue, it appeared that the gene has inserted between genes belonging to carbohydrate metabolism in P. putida BW11M1. An in-frame deletion mutant of the ppyS homologue was constructed and yielded in a strain which lost the opportunity for pseudopyronine biosynthesis [86]. Despite the similar mechanism for α-pyrene formation by PpyS homologues in the different Pseudomonas strains, a phylogenetic analysis revealed that different clades of PpyS exist. These different clades reflect also different locations in the genome sequences of the differ-
dent *Pseudomonas* species: On a taxonomic level closely related strains harbor the *ppyS* homologue in the same region of their genome. Therefore, it can be assumed that the genetic information coding for the enzyme needed to synthesize pseudopyrones was acquired several times. Hence, *Pseudomonas* species from different habitats, e.g., rhizosphere, soil, water, acquired the gene set independently [86].

In summary different types of chain-interconnecting KSs which catalyze α-pyrene ring formation were identified in the last years. One mechanism is to fuse two ketoacyl moieties, as exemplified by CorB and MxnB. Another mechanism is the fusion of one ketoacyl moiety with one acyl moiety, as shown for *PpyS*-like KSs. All evolved from FabH-type KSs, but form different clades in phylogenetic analyses. *PpyS*-like enzymes show the conserved glutamate residue – indicating a mechanism distinct from the ketoacyl–ketoacyl-connecting KSs – and were identified in different bacterial genera, i.e., *Burkholderia*, *Legionella*, *Nocardia*, *Microcystis* and *Streptomyces*, therewith also in clinically relevant pathogens [63]. Future work will reveal which natural products are biosynthesized by such KSs, and which relevance these products have.

**Conclusion**

The α-pyrones show an extraordinary wide variation in biological activities, independently if structurally simple or complex, naturally or non-naturally synthesized. Therefore, α-pyrones represent a rich source for isolation studies and lead discovery. Now, new insights into the biosynthesis of these molecules through chain interconnecting ketosynthases were obtained. This opens up the possibility to use these enzymes as tools; both, in bio- as well as in semi-synthetic approaches. The potential of these enzymes in combinatorial biosynthesis has to be further evaluated in the future.

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**References**

1. McGlacken, G. P.; Fairlamb, I. J. S. Nat. Prod. Rep. 2005, 22, 369–385. doi:10.1039/b416865p
2. Fairlamb, I. J. S.; Marrison, L. R.; Dickinson, J. M.; Lu, F.-J.; Schmidt, J. P. Bioorg. Med. Chem. 2004, 12, 4285–4299. doi:10.1016/j.bmc.2004.01.051
3. Suzuki, K.; Kuwahara, A.; Yoshiida, H.; Fujita, S.; Nishikiori, T.; Nakagawa, T. J. Antibiot. 1997, 50, 314–317. doi:10.7164/antibiotics.50.314
4. Kondoh, M.; Usui, T.; Kobayashi, S.; Tsuchiya, K.; Nishikiori, T.; Mayumi, T.; Osada, H. Cancer Lett. 1998, 126, 29–32. doi:10.1016/S0304-3835(97)00028-4
5. Calderón-Montaño, J. M.; Burgos-Morón, E.; Orta, M. L.; Pastor, N.; Austin, C. A.; Mateos, S.; López-Lázaro. M. Toxicol. Lett. 2013, 222, 64–71. doi:10.1016/j.toxlet.2013.07.007
6. Villers, P. Front. Microbiol. 2014, 5, No. 158. doi:10.3389/fmicb.2014.00158
7. Musa, M. A.; Cooperwood, J. S.; Khan, M. O. F. Curr. Med. Chem. 2008, 15, 2664–2679. doi:10.2174/092986708786248277
8. Thaisrivongs, S.; Romero, D. L.; Tommasci, R. A.; Janakiraman, M. N.; Strohbach, J. W.; Turner, S. R.; Biles, C.; Morge, R. R.; Johnson, P. D.; Aristoff, P. A.; Tomich, P. K.; Lynn, J. C.; Hong, M.-M.; Chong, K.-T.; Hinshaw, R. R.; Howe, W. J.; Finzel, B. C.; Watenpaugh, K. D. J. Med. Chem. 1996, 39, 4630–4642. doi:10.1021/jm960228q
9. Poppe, S. M.; Slade, D. E.; Chong, K. T.; Hinshaw, R. R.; Pagano, P. J.; Markowitz, M.; Ho, D. D.; Mo, H.; Gorman, R. R., III; Dueweke, T. J.; Thaisrivongs, S.; Tarpley, W. G. Antimicrob. Agents Chemother. 1997, 41, 1058–1063.
10. Turner, S. R.; Strohbach, J. W.; Tommasci, R. A.; Aristoff, P. A.; Johnson, P. D.; Skučnik, H. I.; Dolak, L. A.; Seest, E. P.; Tomich, P. K.; Bohanon, M. J.; Hong, M.-M.; Lynn, J. C.; Chong, K.-T.; Hinshaw, R. R.; Watenpaugh, K. D.; Janakiraman, M. N.; Thaisrivongs, S. J. Med. Chem. 1998, 41, 3467–3476. doi:10.1021/jm9802158
11. Yeh, P.-P.; Daniels, D. S. B.; Cordes, D. B.; Slawn, A. M. Z.; Smith, A. D. Org. Lett. 2014, 16, 964–967. doi:10.1021/ol403697h
12. Liaw, C.-C.; Yang, Y.-L.; Lin, C.-K.; Lee, J.-C.; Liao, W.-Y.; Shen, C.-N.; Sheu, J.-H.; Wu, S.-H. Org. Lett. 2015, 17, 2330–2333. doi:10.1021/acs.orglett.5b00739
13. Charlton, R. E.; Webster, F. X.; Zhang, A.; Schal, C.; Liang, D.; Sreng, I.; Roelofs, W. L. Proc. Natl. Acad. Sci. U. S. A. 1993, 90, 10202–10205. doi:10.1073/pnas.90.21.10202
14. Brachmann, A. O.; Brameyer, S.; Kresovic, D.; Hiltkova, I.; Kopp, Y.; Manske, C.; Schubert, K.; Bode, H. B.; Heermann, R. Nat. Chem. Biol. 2013, 9, 573–578. doi:10.1038/nchembio.1295
15. Lee, J. S. Mar. Drugs 2015, 13, 1581–1620. doi:10.3390/md13031581
16. Dickinson, J. M. Nat. Prod. Rep. 1993, 10, 71–98. doi:10.1039/np9931000071
17. Harvan, D. J.; Pero, R. W. Adv. Chem. Ser. 1976, 149, 344–355. doi:10.1021/ba-1976-0149.ch015
18. Luo, H.; Liu, H.; Cao, Y.; Xu, D.; Mao, Z.; Mou, Y.; Meng, J.; Lai, D.; Liu, Y.; Zhou, L. Molecules 2014, 19, 14221–14234. doi:10.3390/molecules190914221
19. Song, Y. C.; Huang, W. Y.; Sun, C.; Wang, F. W.; Tan, R. X. Biol. Pharm. Bull. 2005, 28, 506–509. doi:10.1248/bpb.28.506
20. Griffin, G. F.; Chu, F. S. Appl. Environ. Microbiol. 1983, 46, 1420–1422.
21. Zajkowski, P.; Grabarkiewicz-Szaconska, J.; Schmidt, R. Mycotoxin Res. 1991, 7, 11–15. doi:10.1007/BF03192168
22. Panigrahi, S.; Dallin, S. J. Sci. Food Agric. 1994, 66, 493–496. doi:10.1002/jsfa.2740660411
23. Ath, A. H.; Edrada-Ebel, R.; Indriani, I. D.; Wray, V.; Müller, W. E. G.; Totzek, F.; Zirrgebiel, U.; Schächtele, C.; Kubbubat, M. H. G.; Lin, W. H.; Proksch, P.; Ebel, R. J. Nat. Prod. 2008, 71, 972–980. doi:10.1021/np070447m
24. Bensassi, F.; Galline, C.; Sharaf El Dein, O.; Hjalafou, M. R.; Bacha, H.; Lemaire, C. Toxicol. In Vitro 2012, 26, 915–923. doi:10.1016/j.tiv.2012.04.014
25. Schreck, I.; Deigendesch, U.; Burkhardt, B.; Marko, D.; Weiss, C. Arch. Toxicol. 2012, 86, 625–632. doi:10.1007/s00204-011-0781-3
26. Mao, Z.; Sun, W.; Fu, L.; Luo, H.; Lai, D.; Zhou, L. Molecules 2014, 19, 5088–5108. doi:10.3390/molecules19045088
75. Saha, D.; Fetzner, R.; Burkhardt, B.; Podlech, J.; Metzler, M.; Dang, H.; Lawrence, C.; Fischer, R. *PloS One* **2012**, *7*, e40564. doi:10.1371/journal.pone.0040564
76. Dasenbrock, J.; Simpson, T. J. *J. Chem. Soc., Chem. Commun.* **1987**, *1235–1236*. doi:10.1039/C98700001235
77. Sun, J.; Awakawa, T.; Noguchi, H.; Abe, I. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 6397–6400. doi:10.1016/j.bmcl.2012.08.063
78. Throckmorton, K.; Wiemann, P.; Keller, N. P. *Toxins* **2015**, *7*, 3572–3607. doi:10.3390/toxins7093572
79. Bouhired, S. M.; Crüsemann, M.; Almeida, C.; Weber, T.; Piel, J.; Schäberle, T. F.; König, G. M. *ChemBioChem* **2014**, *15*, 757–765. doi:10.1002/cbic.201300676
80. Buntin, K.; Weissman, K. J.; Müller, R. *ChemBioChem* **2010**, *11*, 1137–1146. doi:10.1002/cbic.200900712
81. Piel, J.; Hertweck, C.; Shipley, P. R.; Hunt, D. M.; Newman, M. S.; Moore, B. S. *Chem. Biol.* **2000**, *7*, 943–955. doi:10.1016/S1074-5521(00)00044-2
82. Nakano, C.; Otsuka, H.; Akanuma, G.; Funai, N.; Horinouchi, S. *J. Bacteriol.* **2009**, *191*, 4916–4923. doi:10.1128/JB.00407-09
83. Kohl, W.; Irschik, H.; Reichenbach, H.; Höflé, G. *Liebigs Ann. Chem.* **1984**, *1088–1093*. doi:10.1002/jlac.198419840605
84. Erol, Ö.; Schäberle, T. F.; Schmitz, A.; Rachid, S.; Gurgui, C.; El Omari, M.; Lohr, F.; Kehraus, S.; Piel, J.; Müller, R.; König, G. M. *ChemBioChem* **2010**, *11*, 1253–1265. doi:10.1002/cbic.2010000085
85. Hashimoto, M.; Koen, T.; Takahashi, H.; Suda, C.; Kitamoto, K.; Fujii, I. *J. Biol. Chem.* **2014**, *289*, 19976–19984. doi:10.1074/jbc.M114.569095
86. Bauer, J. S.; Ghequire, M. G. K.; Nett, M.; Josten, M.; Sahl, H.-G.; De Mot, R.; Gross, H. *ChemBioChem* **2015**, *16*, 2491–2497. doi:10.1002/cbic.201500413
87. Sucipto, H.; Wenzel, S. C.; Müller, R. *ChemBioChem* **2013**, *14*, 1581–1589. doi:10.1002/cbic.201300289
88. Sucipto, H.; Sahner, J. H.; Prusov, E.; Wenzel, S. C.; Hartmann, R. W.; Koehnke, J.; Müller, R. *Chem. Sol.* **2015**, *6*, 5076–5085. doi:10.1039/C5CSS01013F
89. Zocher, G.; Vilsstrup, J.; Heine, D.; Hallab, A.; Goralski, E.; Hertweck, C.; Stahl, M.; Schäberle, T. F.; Stehle, T. *Chem. Sci.* **2015**, *6*, 6525–6536. doi:10.1039/C5SC02488A
90. Sahner, J. H.; Sucipto, H.; Wenzel, S. C.; Groh, M.; Hartmann, R. W.; Müller, R. *ChemBioChem* **2015**, *16*, 946–953. doi:10.1002/cbic.201402666
91. Mori, T.; Yang, D.; Matsui, T.; Hashimoto, M.; Morita, H.; Fujii, I.; Abe, I. *J. Biol. Chem.* **2015**, *290*, 5214–5225. doi:10.1074/jbc.M114.626416

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