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Oxygen-independent enzymatic dearomatization of homocyclic aromatic compounds

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Covering: 1995 up to the end of 2025

Homocyclic aromatic compounds (HAC) represent the second most abundant class of natural and anthropogenic products. Their biodegradation is central to the global carbon cycle and the bioremediation of aromatic pollutants. A key step in this process is enzymatic dearomatization, historically attributed exclusively to oxygen-dependent mono- or dioxygenases. However, over the past three decades, a growing diversity of oxygen-independent dearomatizing reductases has been identified. These enzymes act either on partially activated di- or trihydroxybenzenes and trihydroxynaphthalenes with *meta*-oriented hydroxyl groups, or on coenzyme A (CoA) thioesters of carboxylated HAC, converting aromatic substrates into cyclic dienes. Class I and II benzoyl-CoA reductases catalyze Birch-like reductions *via* radical intermediates at metal cofactors, with low-potential electrons supplied either through ATP-dependent electron transfer (class I) or flavin-based electron bifurcation (class II), whereas 2-naphthoyl-CoA reductase appears independent of an electron-activation system. An alternative, non-redox dearomatization mechanism has been identified in *S*-adenosyl-L-methionine-dependent methyltransferases involved in the anaerobic bacterial estrogen-to-androgen conversion, as well as in polyketide tailoring. Together, these findings reveal a broad enzymatic repertoire for overcoming arene resonance stabilization under anoxic conditions. Beyond their ecological significance, these pathways provide mechanistically diverse routes and opportunities for biocatalysis and the sustainable synthesis of valuable chemical building blocks.

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1. Introduction

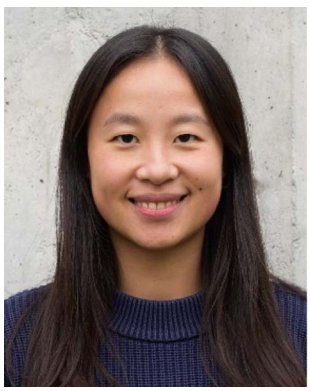
Homocyclic aromatic compounds (HAC) constitute, after carbohydrates, the second most abundant class of biomolecules on Earth, with lignin as the predominant natural source, accounting for approximately 30% of terrestrial organic carbon.[1] Indeed, plants are the primary global producers of aromatic ring structures, generating phenylpropanoids *via* the shikimate pathway, which serve as precursors for lignin and a wide range of other aromatic secondary metabolites. Following the colonization of terrestrial environments by plants approximately 450 million years ago, lignin-derived aromatic structures progressively accumulated, severely impacting the global carbon cycle. A second major reservoir of HAC is biogenic crude oil derived from plant material, which contains 5–30% aromatic constituents, depending on its geological origin.[2] Petroleum-derived aromatics,

including benzene, toluene, ethylbenzene, and xylenes (BTEX), as well as polycyclic aromatic hydrocarbons, are widely used as industrial solvents and as a key feedstock for synthesizing a vast range of materials and chemicals, such as polymers, plasticizers, resins, foams, insecticides, herbicides, pharmaceuticals, explosives, food additives and fragrances. However, many of these compounds and their derivatives are associated with significant environmental and health concerns, including toxicity, carcinogenicity and endocrine-disrupting activity, and can cause severe ecological damage when released through industrial processes during extraction, storage, transport or accidental spills.[3,4] In addition to lignin and crude oil, polyketides constitute a distinct source of HAC with an alternative biosynthetic origin and are produced by bacteria, fungi, plants, and some marine animals.[5]

Although plants are the largest producers of HAC worldwide, only microorganisms are capable of utilizing these compounds as carbon and energy source and mineralizing them completely to CO_2. Given the widespread abundance of HAC and their numerous adverse effects on the environment and human health, microbial HAC degradation is of considerable ecological and biotechnological importance. Lignin depolymerization is mediated by aerobic white-rot fungi, such as *Phanerochaete chrysosporium*, which employ a suite of nonspecific oxidative extracellular enzymes, including lignin peroxidases, manganese peroxidases, and laccases.[6,7] These enzymes cleave the complex polymeric structure of lignin into smaller aromatic units. Brown-rot fungi and certain bacteria also contribute to lignin breakdown, albeit to a lesser extent.[8] The complete microbial degradation of HAC derived from lignin, crude oil, or other sources requires enzymatic dearomatization of the aromatic ring. This step is particularly challenging due to the high resonance stability of homocyclic aromatic systems (approximately 150 kJ mol^{-1}). Depending on oxygen availability, fundamentally different metabolic strategies have evolved in aerobic bacteria and fungi *versus* anaerobic bacteria to accomplish this transformation. In contrast, the capacity of archaea to completely degrade HAC, whether under aerobic or anaerobic conditions, appears to be comparatively rare and is likely the result of lateral gene transfer events.[9] Collectively, these examples highlight the pivotal role of dearomatization reactions in the degradation of aromatic compounds. Beyond this catabolic function, such reactions also contribute to biosynthetic polyketide pathways.[10]

2. Dearomatization of HAC in aerobic microorganisms involving oxygenases

In HAC-degrading aerobic bacteria and fungi, a wide range of mono- and dioxygenases catalyze the dearomatization of



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Juliane Breiltgens

Juliane Breiltgens studied Pharmacy at the University of Freiburg, followed by a Diploma in Pharmaceutical and Sustainable Chemistry conducted at the Leuphana University Lüneburg. She completed her PhD (2026) in the research group of Michael Müller focusing on SAM-dependent C-methyltransferases and the chemoselective methylation of aromatic compounds.

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aromatic ring structures by introducing one or both atoms of molecular oxygen into the substrate. The diversity and functional principles of oxygenases involved in aerobic HAC degradation have been extensively investigated over the past six decades,[11] and are therefore only briefly summarized here.

In general, mono- and dioxygenase-catalyzed reactions require activation of molecular oxygen, which, due to its biradical ground state, reacts only sluggishly with aromatic systems. Enzymatic activation is achieved through coordination to metal centers or flavin cofactors, and/or *via* stepwise reduction to more reactive oxygen species bound to these cofactors. Dearomatizing dioxygenases can be broadly divided into two classes based on their mode of action:[12] (i) ring-cleaving dioxygenases and (ii) ring-hydroxylating dioxygenases. Ring-cleaving dioxygenases act on substrates bearing two hydroxyl groups in either *ortho* (*e.g.*, catechol, protocatechuate) or *para* (*e.g.*, gentisate) positions and do not require external electron donor systems.[13] These enzymes catalyze cleavage of the aromatic ring to yield either an unsaturated dicarboxylic acid *via ortho*-cleavage or an unsaturated semialdehyde *via meta*-cleavage pathways. In contrast, ring-hydroxylating dioxygenases are non-heme iron enzymes that depend on NAD(P)H as an electron donor and contain electron-transferring Rieske-type [2Fe–2S] clusters.[14] These enzymes incorporate both oxygen atoms, reduced to the hydroxyl level, into the aromatic substrate to form a *cis*-dihydrodiol intermediate. This intermediate is subsequently rearomatized by an associated dehydrogenase.

Monooxygenases, including cytochrome P450 enzymes[15] and di-iron monooxygenases, can also mediate transient dearomatization through epoxidation of the aromatic ring. In most cases, subsequent spontaneous rearrangement results in rearomatization to a phenolic product. A notable exception is the di-iron monooxygenase system acting on benzoyl-coenzyme A (CoA), known as the BoxAB system, in which 2,3-epoxidation is followed by hydrolytic ring cleavage and release of formate.[16]

Syed Masood Husain

Syed Masood Husain obtained his PhD in 2008 from the University of Münster, Germany, under the supervision of Bernhard Wünsch. He subsequently carried out postdoctoral research with Michael Müller at the University of Freiburg, Germany, where he worked on biocatalysis and natural product biosynthesis. Following his return to India in 2012, he briefly worked with Prof. Yamuna Krishnan at the National Centre for Biological Sciences (NCBS), Bengaluru. Since 2014, he has been an independent faculty member at the Centre of Biomedical Research (CBMR), Lucknow, where he currently serves as an Associate Professor. His research focuses on enzyme catalysis, biocatalysis, chemoenzymatic synthesis, and enzyme engineering.

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Michael Müller obtained his PhD at the University of Munich under the guidance of Wolfgang Steglich. Following a 1-year research exchange at the University of Washington, he became the Group Leader of Bioorganic Chemistry at the Forschungszentrum Jülich. He was appointed to the Professorship for Pharmaceutical and Medicinal Chemistry at the University of Freiburg in 2004. His research interests include chemoenzymatic synthesis, natural products, asymmetric synthesis, as well as sustainability.

3. Dearomatization of HAC in anaerobic microorganisms involving reductases

Anaerobic bacteria that use HAC as carbon and energy source in combination with electron acceptors such as nitrate, sulfate, Fe(III) oxides or CO_2, lack oxygen-dependent enzymes for dearomatization. Instead, these organisms employ fundamentally different dearomatization strategies in which HAC are dearomatized by reduction rather than oxidation. Depending on the electron richness of the aromatic system of the respective HAC substrates, dearomatizing reductases can be broadly classified into two major groups: (i) enzymes acting on 1,3-diol-subsituted HAC and (ii) those targeting aryl-CoA thioesters (Scheme 1):[16–19]

(i) Reductases acting on HAC bearing at least two *meta*-oriented hydroxyl groups are generally capable of catalyzing aromatic ring reduction using common physiological electron donors such as NAD(P)H or reduced ferredoxin (Fd_{red}) (Scheme 1A).[18] In these substrates, *meta*-hydroxyl substituents modify

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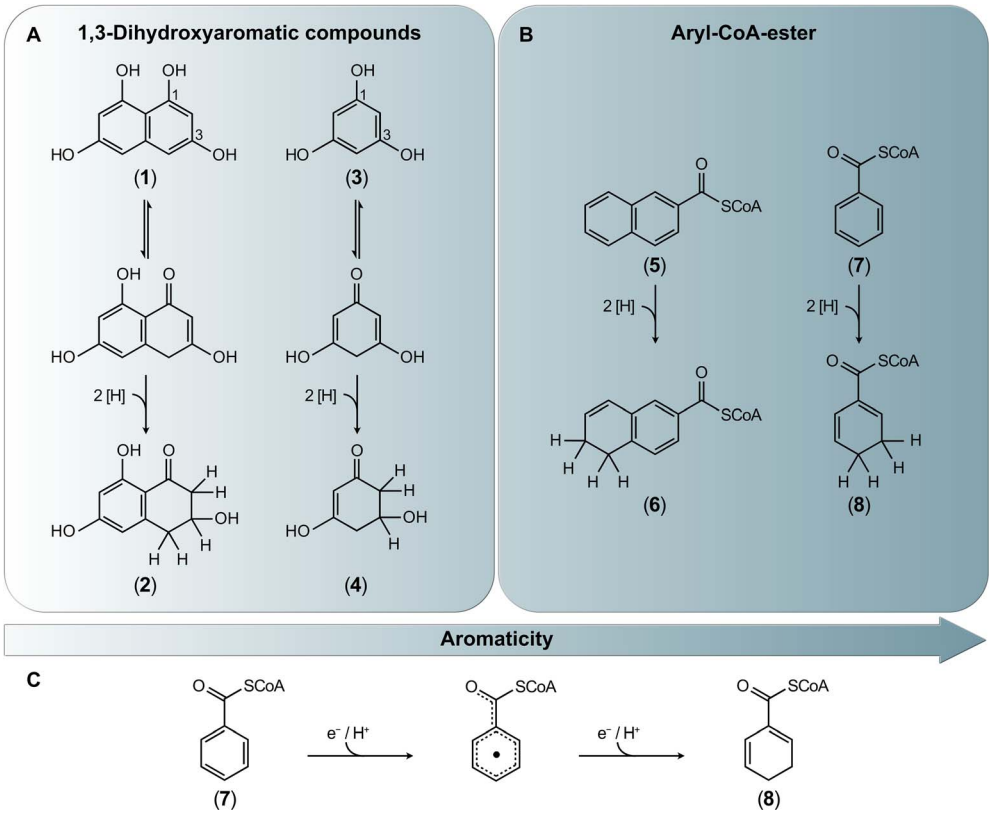
Matthias Boll is a full professor at the Faculty of Biology at the University of Freiburg. For three decades, his research has focused on microbial biochemistry, microbial metabolism and microbial enzymology with a focus on anaerobic bacteria. He has discovered a number of key enzymes involved in the anaerobic degradation of numerous aromatic compounds and steroids that play a crucial role in the degradation of pollutants. Some of these enzymes are being evaluated for biotechnological applications.



Scheme 1 Two strategies for reductive enzymatic dearomatizing of HAC. (A) Keto–enol tautomerization of 1,3-diol-substituted HAC enables reduction with physiological electron donors such as NAD(P)H or Fd_{red}. Examples are the reduction of 1,3,6,8-tetrahydroxynaphthalene (**1**) to scytalone (**2**) and of phloroglucinol (**3**) to dihydrophloroglucinol (**4**). (B) Aryl-CoA esters are substantially more electron-rich. While the electron donor for the reduction of 2-naphthoyl-CoA (**5**) to 5,6-dihydro-2-naphthoyl-CoA (**6**) is unknown, reduction of benzoyl-CoA (**7**) to cyclohexa-1,5-dienoyl-CoA (**8**) depends on the coupling to an exergonic reaction. In benzoyl-CoA reductases (BCRs) this is achieved either *via* ATP hydrolysis (class I BCRs) or through flavin-based electron bifurcation (class II BCRs). (C) Proposed mechanism of BCRs involving a substrate radical intermediate stabilized by the thioester carbonyl group.

the electron distribution relative to unsubstituted benzene, thereby promoting keto–enol tautomerization. As the degree of *meta*-hydroxyl substitution increases, this effect progressively enhances the susceptibility of the aromatic ring to reductive dearomatization. For example, resorcinol reductase acting on resorcinol (1,3-dihydroxybenzene, **9**) requires the low-potential electron donor Fd_{red} with $E' < -400$ mV. Conversely, substrates with higher levels of hydroxylation, such as phloroglucinol (1,3,5-trihydroxybenzene, **3**) or 1,3,6,8-tetrahydroxynaphthalene (TH_4N, **1**) exhibit reduced π-electron delocalization, allowing reduction to proceed with higher-potential electron donors such as NAD(P)H.

(ii) By contrast, dearomatizing reductases acting on CoA thioesters of arylcarboxylic acids, including benzoyl-CoA (**7**) and 2-naphthoyl-CoA (**5**), must overcome substantially more electron-rich aromatic systems and therefore require considerably stronger reducing power (Scheme 1B).[17] Indeed, the redox potential of the benzoyl-CoA (**7**)/cyclohexa-1,5-dienoyl-CoA (1,5-dienoyl-CoA, **8**) redox couple is $E^{\circ\prime} = -620$ mV.[20] Thus, even when Fd_{red} is employed as electron donor ($E' \approx -500$ mV),[21] these reductions must be coupled to exergonic processes to render reduction thermodynamically feasible. In class I benzoyl-CoA reductases (BCRs), this is achieved through coupling to stoichiometric ATP hydrolysis.[22,23] Class II BCRs, however, generate low-potential electrons *via* flavin-based electron bifurcation.[24,25] For 2-naphthoyl-CoA reductase, the physiological electron donor system has not been conclusively elucidated.[26] While the thioester functionality (*e.g.*, in benzoyl-CoA (**7**)) does increase the electrophilicity of the carbonyl—potentially further enhanced by partial protonation through active-site amino acid residues—this does not fully explain its biochemical function. In particular, BCRs are proposed to operate *via* radical intermediates, in a mechanism conceptually analogous to the Birch reduction in organic chemical synthesis.[27,28] In this context, the thioester plays a more specific role: it helps stabilize neutral radical intermediates formed after hydrogen atom transfer to the aromatic ring as aryl ketyl-type radical (Scheme 1C).[29,30]

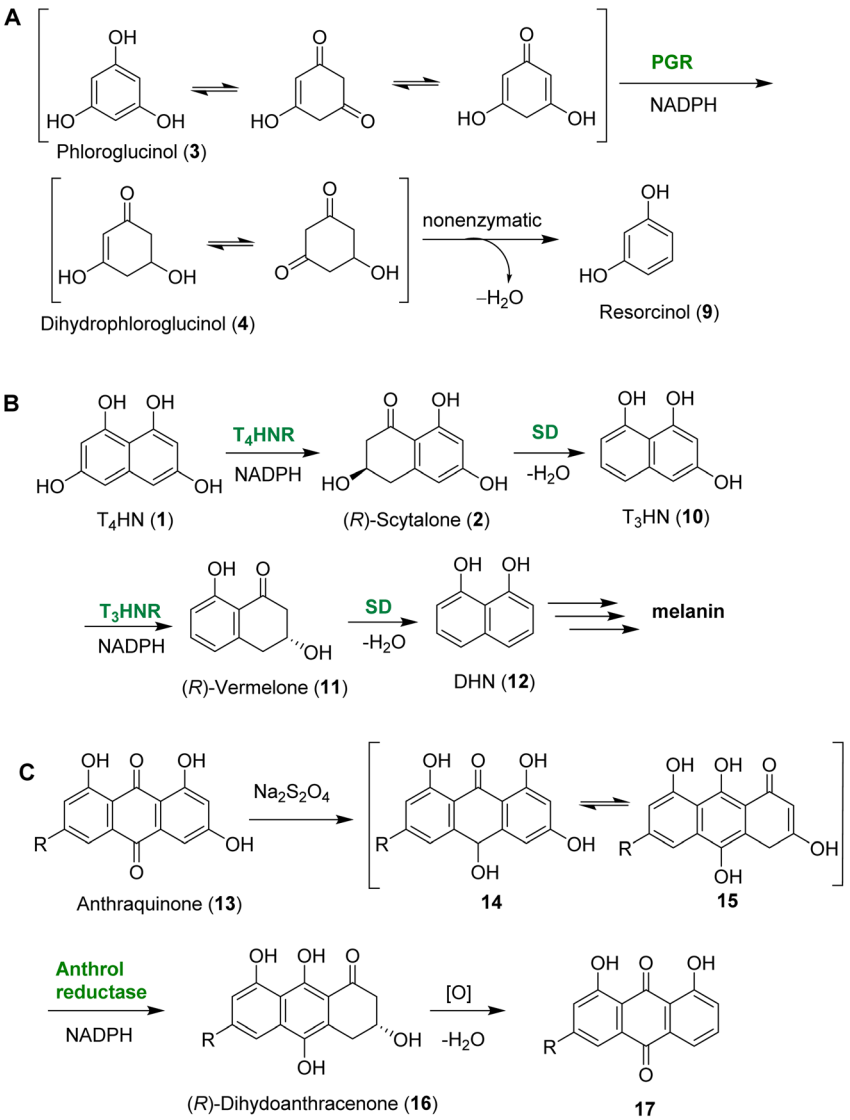
3.1 Reductases acting on aromatic HAC with *meta*-positioned hydroxyl functionalities

Reductases acting on HAC, such as phloroglucinol (**3**) and resorcinol (**9**) bearing *meta*-positioned hydroxyl groups, represent an emerging class of biocatalysts with significant relevance

in green synthesis and aromatic detoxification.[18,19] The compounds phloroglucinol (**3**) and resorcinol (**9**) are key intermediates in the microbial degradation of flavonoids and tannins and can serve as sole carbon sources for anaerobic fermenting bacteria.[31,32] These polyhydroxylated benzenes are intrinsically stable due to their aromaticity and the electron-donating effects of hydroxyl substituents, making their selective reduction challenging. In anaerobic bacteria, the reductive dearomatization of such HAC is mediated by phloroglucinol reductases, which catalyze the regioselective reduction of *meta*-dihydroxylated phenolic substrates.

The bacterial phloroglucinol reductase (PGR) catalyzes the NADPH-dependent reduction of phloroglucinol (**3**) to dihydrophloroglucinol (**4**) (Scheme 2A).[33] Mechanistically, this transformation has been proposed to proceed either *via* classical carbonyl reduction of a keto tautomer or through a Michael-type β-reduction of an α,β-unsaturated intermediate; however, these pathways remain experimentally indistinguishable.[34] This reaction constitutes a key dearomatization step, enabling subsequent ring cleavage and energy conservation under anaerobic conditions.[32] In some cases, the reduced intermediate can undergo dehydration, leading to deoxygenation and formation of resorcinol (**9**) (Scheme 2A), a strategy commonly observed in natural product biosynthesis. Several PGRs, including PGReu (from *Eubacterium* sp. AB3007), PGRcl (from *Clostridium orbiscindens*), and PGRde (from *Desulfosporosinus orientis*), have been shown to catalyze the NADPH-dependent reduction of phloroglucinol (**3**) and related substrates.[35]

Resorcinol (**9**) and hydroxyhydroquinone (HHQ, 1,2,4-trihydroxybenzene) are representative homocyclic aromatic compounds bearing *meta*-positioned hydroxyl groups that undergo degradation in anaerobic microbes *via* reductive dearomatization. Although the reduction of resorcinol (**9**) to the



Scheme 2 (A) Reduction of phloroglucinol (**3**) by PGR using NADPH. (B) Naphthol reductases (T_4HNR and T_3HNR) involved in the reduction of polyhydroxynaphthalene compounds during melanin biosynthesis. (C) Reductive deoxygenation of anthraquinones (**13**) by anthrol reductases.

non-aromatic dihydroresorcinol—thereby disrupting aromaticity and enabling subsequent ring cleavage—has been proposed in fermenting bacteria such as *Clostridium*,[31] no resorcinol reductase has yet been characterized.

In contrast, HHQ reductases are more specialized enzymes identified in anaerobes such as *Desulfovibrio inopinatus*, where they catalyze the initial step in HHQ degradation.[36] These enzymes reduce HHQ to dihydrohydroxyhydroquinone using NADH as an electron donor, typically with a pH optimum around 7.5. Mechanistically, HHQ reductases operate *via* hydride transfer from reduced cofactors (NADH or NADPH), often coupled with protonation steps that facilitate dearomatization. In many cases, they function within multienzyme systems to ensure efficient turnover of otherwise recalcitrant aromatic substrates.

Analogous strategies have evolved in fungi, where reductases of the short-chain dehydrogenase/reductase (SDR) family catalyze regio- and stereoselective reductions of naphthols and anthrols using NADPH.[34,37] These enzymes mediate hydride transfer to the aromatic system, coupled with protonation, to achieve reductive dearomatization. For example, 1,3,6,8-tetrahydroxynaphthalene reductase (T_4HNR) and 1,6,8-trihydroxynaphthalene reductase (T_3HNR), key SDR enzymes in DHN-melanin biosynthesis in *Magnaporthe grisea*, catalyze the reduction of T_4HN (**1**) and T_3HN (**10**) to (*R*)-scytalone (**2**) and (*R*)-vermelone (**11**), respectively. Subsequent dehydration by scytalone dehydratase generates T_3HN (**10**) and 1,8-dihydroxynaphthalene (DHN, **12**) as deoxygenated intermediates (Scheme 2B). Thus, reductive deoxygenation of polyhydroxylated aromatics at *meta*-positions is an important strategy in both biosynthetic and biodegradative pathways.

In addition to mono- and bicyclic phenols, the deoxygenation of anthraquinone-based metabolites such as emodin (R=CH_3, **13**) has been observed in several fungal systems and is proposed to play a crucial role in the biosynthesis of diverse polyketides, including monodictyphenone, xanthones, ergochromes, and aflatoxin B_1.[38] Schätzle *et al.* elucidated the role of the first anthrol reductase, MdpC, in monodictyphenone biosynthesis in *Aspergillus nidulans*. Although MdpC does not directly reduce emodin (**13**) with NADPH, the use of sodium dithionite ($Na_2S_2O_4$) under anoxic conditions generates emodin hydroquinone, enabling MdpC-catalyzed reduction to afford (*R*)-3-hydroxy-3,4-dihydroanthracen-1(2*H*)-one (R=CH_3, **16**). Further studies showed that $Na_2S_2O_4$ converts emodin (**13**) into tautomeric forms of emodin hydroquinone (R=CH_3, **14** and **15**) (Scheme 2C).[38] One of these tautomers is selectively reduced by MdpC in a regio- and stereoselective manner, and the resulting product can undergo spontaneous dehydroxylation and oxidation to form chrysophanol (R=CH_3, **17**), a key intermediate in anthraquinone-derived secondary metabolism.

Subsequent work has identified several related anthrol reductases, including 17β-HSDcl from *Cochliobolus lunatus*,[39] AflM from *Aspergillus parasiticus*,[40] and ARti from *Talaromyces islandicus*.[41] Similar to MdpC, ARti and ARti-2 catalyze the reduction of a range of naturally occurring anthraquinones to (*R*)-configured dihydroanthracenones with >99% ee and high yields.[41,42] These products have been widely utilized in the chemoenzymatic synthesis of complex natural products such as bisanthraquinones[43] and also serve as key intermediates in the biosynthesis of deoxygenated anthraquinone derivatives.

3.2 Reductases acting on HAC with thioester functionalities

3.2.1 Class I benzoyl-CoA reductases. The class I BCR was initially isolated and biochemically characterized from the facultatively anaerobic betaproteobacterium *Thauera* (*T.*) *aromatica* K172.[22] This enzyme catalyzes the endergonic reduction of benzoyl-CoA (**7**) to 1,5-dienoyl-CoA (**8**) using Fd_{red} as the electron donor.[21,44] It couples aromatic ring reduction reaction to stoichiometric ATP hydrolysis forming ADP + P_i, with two ATP molecules consumed per benzoyl-CoA (**7**) reduced.[45] This highly oxygen-sensitive enzyme is a ≈170 kDa heterotetramer composed of two functionally distinct dimeric modules. The ATP-binding BcrAD dimer contains a subunit-bridging [4Fe–4S] cluster and functions as an ATP-dependent electron-activating module. In contrast, the catalytic BcrBC dimer harbors additional Fe–S clusters in each subunit and provides the substrate-binding site.[46]

To date, three subclasses of class I BCRs have been identified.[29,47] Although they share a conserved subunit architecture, they differ in subunit size, complex stability, and substrate specificity. Enzymes of the *T. aromatica*-type subclass form stable complexes between the ATP-binding and catalytic modules, likely due to an enlarged BcrD subunit that contributes to tetramer stabilization. BCRs of the *Azoarcus* subclass, known as the Bzd-type (bzd = benzoate degradation), form only transient interactions between the ATP-binding (BzdPQ) and catalytic (BzdNO) components and exhibit a more symmetric architecture of the ATP-binding dimer. The third subclass, methylbenzoyl-CoA reductases (Mbr-type), is distinguished by a broader substrate spectrum, catalyzing the dearomatization of numerous methyl-, hydroxyl-, and halogen-substituted benzoyl-CoA derivatives.

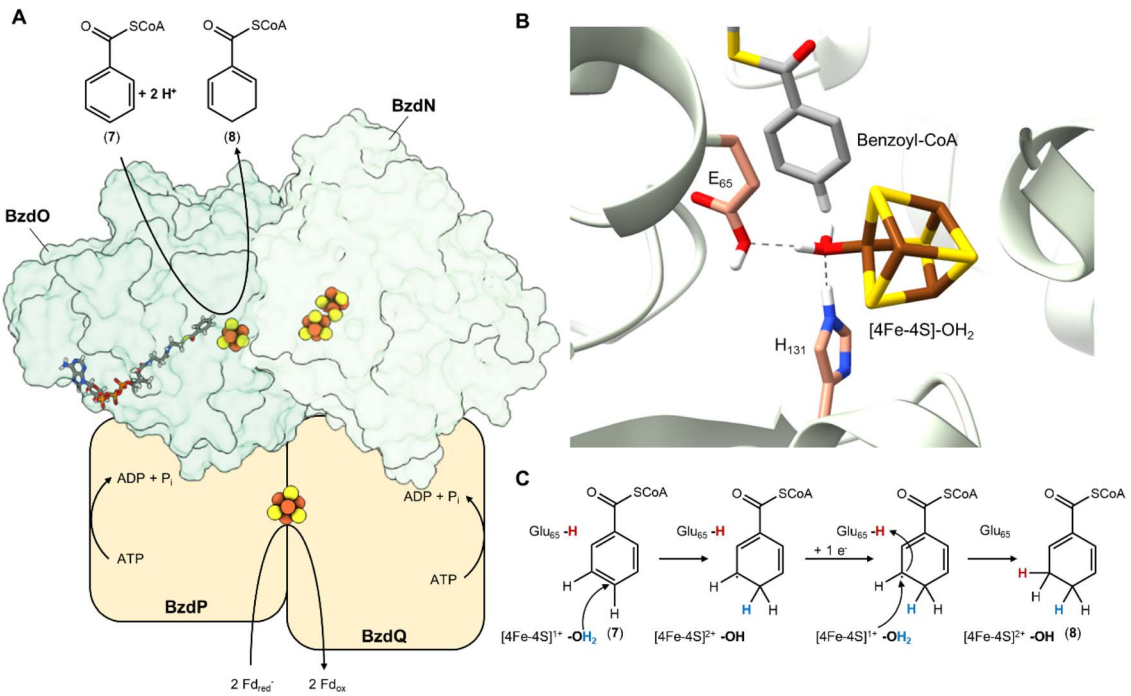
All class I BCRs belong to the broader BCR/2-hydroxyacyl-CoA dehydratase (BCR/HAD) radical enzyme family.[48] In contrast to class I BCRs, HADs do not catalyze redox reactions but instead mediate the chemically demanding elimination of water from 2-hydroxyacyl-CoA substrates.[49] Notably, canonical dehydration reactions in the β-oxidation of fatty acids occur at 3-hydroxyacyl-CoAs, underscoring the mechanistic distinctiveness of HAD-catalyzed reactions. In HAD-systems, the ATP-dependent electron-activating component and the catalytic CoA-ester-binding dimer do not form a stable complex but interact transiently. During catalysis, a single electron is transferred *via* ATP hydrolysis from the electron-activating module to the catalytic dimer, thereby initiating a radical-based dehydration reaction that yields the corresponding enoyl-CoA product. The transferred electron is recycled, enabling multiple catalytic turnovers from a single activation event. A common mechanistic feature of both BCRs and HADs is the essential role of the CoA thioester moiety in stabilizing radical intermediates, such as ketyl radicals, formed during the catalytic cycle.[48]

Bioinformatic analyses have revealed that members of the BCR/HAD radical enzyme family are widespread across many

bacterial and some archaeal lineages.[47] However, sequence information alone cannot reliably distinguish gene clusters encoding bona fide BCRs, HADs, or as yet unknown members of the BCR/HAD family. A potential distinguishing feature is that all HADs characterized to date contain a single gene encoding the homodimeric ATP-binding activator module, whereas all experimentally verified BCRs possess a heterodimeric ATP-binding module composed of two homologous but distinct subunits.[48]

High-resolution crystal structures of the catalytic BzdNO subunits of class I BCR from the betaproteobacterium *Aromatoleum* sp. CIB have provided detailed insights into cofactor composition and the catalytic mechanism (Scheme 3).[28] The electron-transferring BzdN subunit contains an unusual [8Fe–9S] double-cubane cluster, whereas the catalytically active BzdO subunit harbors an aqua-ligated [4Fe–4S] cluster that functions as the key catalytic cofactor. QM/MM studies support a mechanism in which catalysis is initiated by hydrogen atom transfer from the water ligand coordinated to the [4Fe–4S] cluster, generating a neutral radical intermediate. A subsequent second electron transfer from the same cluster, coupled with protonation by an active-site histidine residue, yields the 1,5-dienoyl-CoA (**8**) product (Scheme 3C).[28] The involvement of a [4Fe–4S]-bound water molecule as a hydrogen atom donor is unprecedented in biological systems. However, it shows notable mechanistic parallels to class II BCRs, in which a tungsten-coordinated water ligand fulfills an analogous role.[27]

Class I BCRs of the *T. aromatica* and MBR subclasses have been shown to reduce a range of substituted benzoyl-CoA (**7**) analogues, particularly those bearing substituents at the *ortho* and *meta* positions.[47] In contrast, substitutions at the *para* position are generally less well tolerated, possibly because this position is involved in the initial hydrogen atom transfer step. Attempts to correlate substrate reactivity with Creary $\sigma^{\bullet}$ substituent constants have been unsuccessful, largely because the relative conversion rates of substituted benzoyl-CoA analogues differ substantially among members of the *T. aromatica* and MBR subclasses. These observations suggest that the reactivity toward substituted benzoyl-CoA (**7**) analogues is governed primarily by intrinsic structural features that vary between BCR types, rather than by the radical-stabilizing or -destabilizing effects of the substituents. Moreover, other steps in the catalytic cycle, most notably ATP-hydrolysis-dependent electron transfer, appear to play a dominant role in determining the overall reaction rate. Consequently, Creary $\sigma^{\bullet}$ constants are not generally suitable predictors of the reactivity of class I BCRs toward substituted benzoyl-CoA (**7**) analogues. Further investigation into the mechanistic details of class I BCR catalysis is required to identify the rate-limiting steps of the overall reaction cycle and to establish a more complete understanding of structure–activity relationships. In particular, it

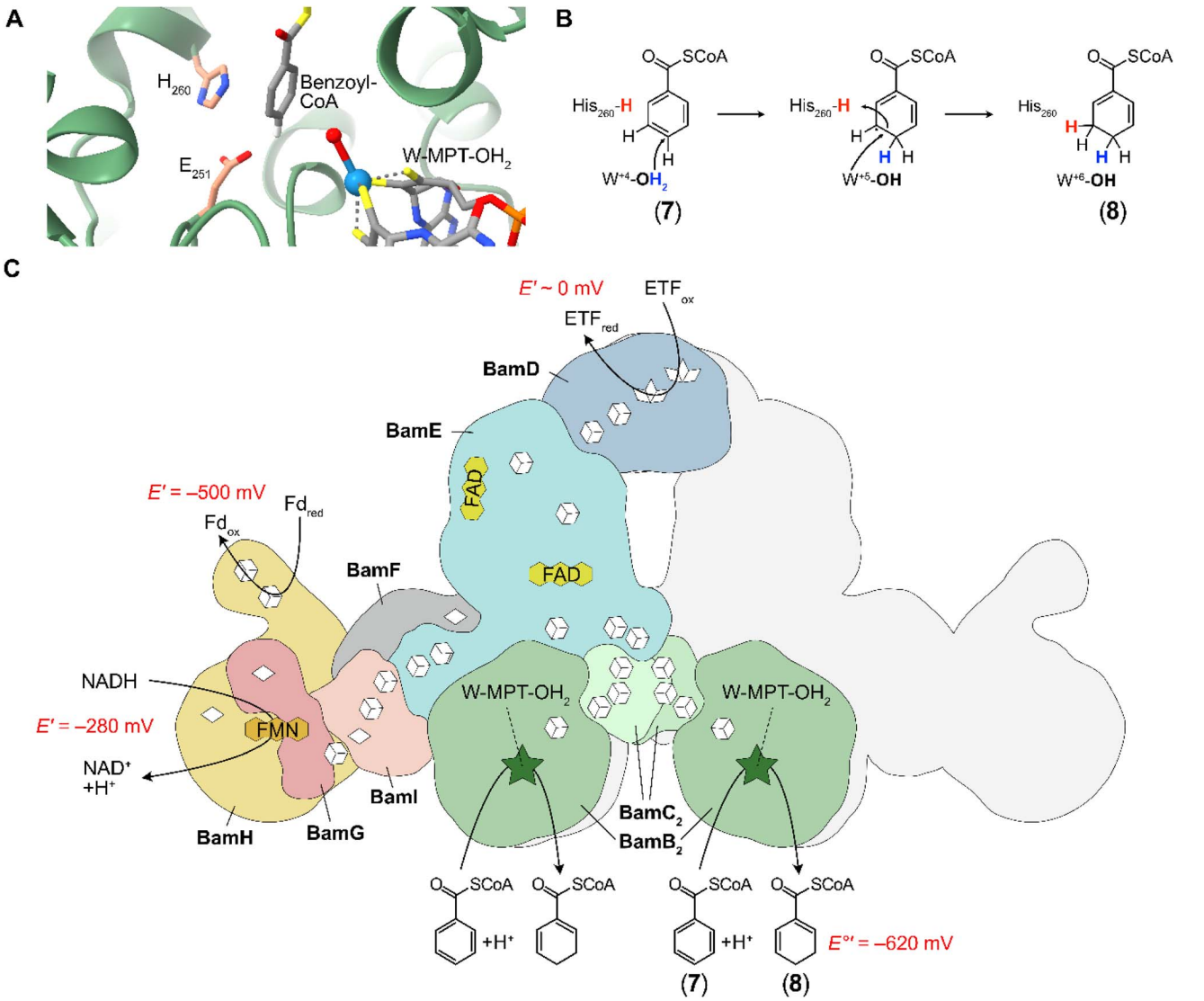


Scheme 3 Structure and function of class I BCRs. (A) General tetrameric architecture of class I BCRs (subunit nomenclature according to *Azoarcus* subclass). Subunit sizes, composition of the FeS cluster in the electron-transferring BzdN subunit, and the extent of stable *versus* transient interactions between the ATP-hydrolyzing and benzoyl-CoA (**7**) reducing subunits vary among subclasses. (B) Active-site of BCR from *Aromatoleum* sp. ClB in the catalytic BzdO-subunit with bound benzoyl-CoA (**7**) and the catalytic aqua-[4Fe–4S] cluster. The cluster-coordinated water molecule is activated by proximal histidine and glutamate residues. (C) QM/MM-derived catalytic mechanism. The water-ligand of the catalytic [4Fe–4S] cluster serves as hydrogen atom donor, generating a neutral radical. Subsequent electron-transfer, coupled to protonation by a catalytic glutamate residue, yields 1,5-dienoyl-CoA (**8**).

remains unclear how ATP hydrolysis is coupled to and drives the otherwise endergonic reduction of the aromatic ring.

3.2.2 Class II benzoyl-CoA reductases. ATP-dependent class I BCRs are absent in strictly anaerobic bacteria, which operate under significant energy constraints.[16,50] Instead, proteomic and transcriptomic analyses of the metal-reducing δ-proteobacterium *Geobacter* (*G.*) *metallireducens* identified the structural *bamBC-DEFGHI* (bam = benzoic acid metabolism) genes, which are induced during growth on aromatic compounds in this organism.[51,52] The catalytic $Bam(BC)_2$ subcomplex of class II BCR was first isolated and characterized.[53] The BamB subunit belongs to the aldehyde: Fd oxidoreductase (AOR) family of metallopterin-binding enzymes, whose tungsten-dependent members catalyze unusual and energetically demanding reactions.[54] BamB contains a tungsten-bis-metallopterin cofactor with a water ligand (W-MPT-OH_2) (Scheme 4A).[27,55] This cofactor serves as the site of benzoyl-CoA (7) reduction that can be observed in the reverse direction as 1,5-dienoyl-CoA: methyl viologen oxidoreductase (DCO) activity.[53] Reduction of benzoyl-CoA (7) is initiated by hydrogen atom transfer from the W-MPT-OH_2 cofactor, followed by electron transfer *via* the cofactor and proton transfer mediated by a conserved histidine residue (Scheme 4B).[27,56] This mechanism proceeds through a kinetically competent radical/W(V)–OH intermediate. The radical is highly delocalized over the substrate, both pyranopterin moieties of the cofactor, and a nearby [4Fe–4S] cluster, thereby enhancing the stabilization of the low-potential reduction intermediate. Consequently, formation of the radical intermediate requires electrons with a less negative redox potential in class II BCRs than in class I BCRs, enabling ATP-independent benzoyl-CoA (7) reduction.[27]

Isolation and characterization of the entire complex from two organisms, *G. metallireducens*[24] and the sulfate-reducing



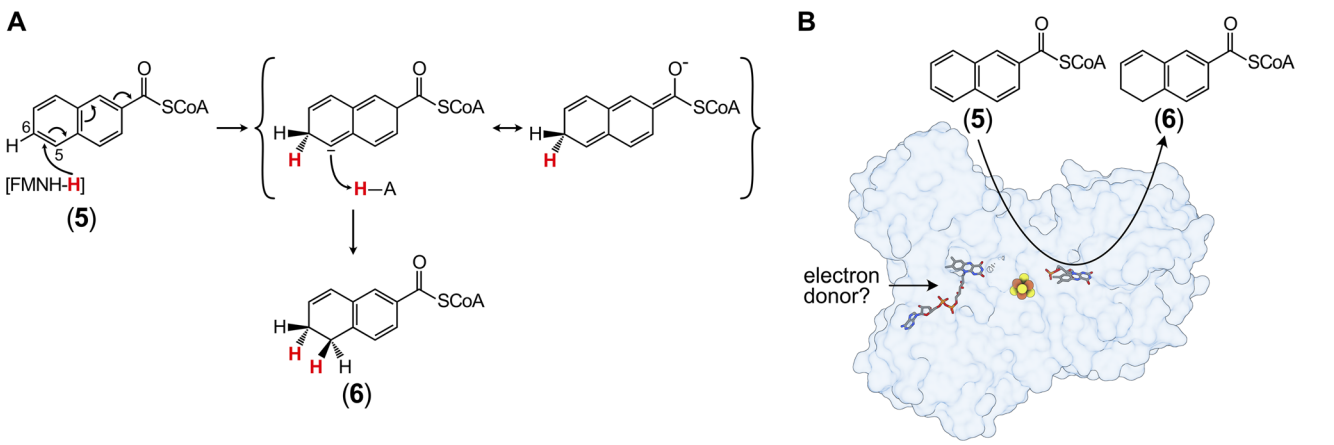
Scheme 4 Structure and mechanism of class II BCR from *G. metallireducens*. (A) Active site in BamB with W-MPT-OH_2 cofactor and benzoyl-CoA bound. (B) Mechanism of benzoyl-CoA (7) reduction to 1,5-dienoyl-CoA (8). (C) Cryo-electron microscopy structure of class II BCR showing subunit organization and cofactors. Benzoyl-CoA reduction is achieved by an electron confurcation in the BamGH subunits by electrons derived from Fd_{red} and NADH, followed by an electron bifurcation to benzoyl-CoA and an electron-transferring flavoprotein (ETF) in the BamE subunit.

Desulfosarcina cetonica,[57] revealed a subunit composition of Bam[(BC)$_2$DEFGHI]$_2$, corresponding to a molecular weight of approximately 1 MDa. In addition to four W-MPT-OH$_2$ cofactors, class II BCRs contain numerous other cofactors, including six flavins, two selenocysteines, six zinc ions, and more than fifty Fe–S clusters, making them one of the most complex metalloenzyme machineries known. The cryo-electron microscopy structure of the class II BCR complex from *G. metallireducens* provided insights into the potential mechanism of electron activation to achieve benzoyl-CoA (7) reduction (Scheme 4C).[25] This mechanism involves flavin-based electron bifurcation (FBEB), a process in which the endergonic reduction of a low-potential electron acceptor by a mid-potential electron donor is coupled to the exergonic reduction of a high-potential electron acceptor; the reverse of this process is termed flavin-based electron confurcation (FBEC).[58,59] In class II BCRs, two distinct FBEB modules are coupled: an electron-bifurcating [FeFe]-hydrogenase-like subcomplex (BamGHI)[25] and an electron-bifurcating heterodisulfide reductase-like core subcomplex (BamDE).[24] The BamF subunit acts as an adapter connecting the two modules. According to the current model of electron activation in class II BCRs, BamGH serves as the electron input module, where electrons from two electron donors, Fd$_{red}$ and NADH, confurcate. The resulting mid-potential electrons are subsequently transferred to the electron-bifurcating FAD in BamE. Following electron bifurcation at this cofactor, low-potential electrons are directed toward BamB, reducing benzoyl-CoA (7) at the W-MPT-OH$_2$ cofactor. The high-potential electrons are transferred to two non-cubane iron-sulfur clusters in the BamD subunit, where they reduce an electron-transferring flavoprotein (ETF).[25] Reduced ETF then transfers electrons to the menaquinone (MK) pool *via* an ETF: MK oxidoreductase (EMO).[60] To date, the *bamB-I* genes have been found in many strictly anaerobic HAC-degrading organisms within the δ-proteobacteria and Firmicutes.[50,57]

Although the basic principles of electron input and output in double-bifurcating class II BCRs are established, many aspects of how this metalloenzyme machinery couples FBEC and FBEB to drive the endergonic reduction of benzoyl-CoA remain unresolved. It is particularly unknown whether and how these two processes are interconnected through dynamic transient conformational changes that open and close electron transfer gates to ensure unidirectional electron flow to ETF and benzoyl-CoA, respectively.

3.2.3 2-Naphthoyl-CoA reductase and related enzymes. Anaerobic degradation of naphthalene and other polycyclic aromatic hydrocarbons (PAHs) is initiated by carboxylation[61] and subsequent thioesterification, generating aryl-CoA esters that serve as substrates for dearomatizing aryl-CoA reductases.[62] The key enzyme mediating dearomatization of the naphthyl ring system, 2-naphthoyl-CoA reductase (NCR), has been studied from the naphthalene-degrading, sulfate-reducing enrichment culture N47 in detail, and is regarded as a prototypical enzyme of the anaerobic PAH degradation pathway.[26,63] NCR catalyzes the highly enantioselective two-electron reduction of 2-naphthoyl-CoA (NCoA, **5**) to 5,6-dihydro-2-naphthoyl-CoA (DHNCoA, **6**), producing exclusively the (5*S*, 6*S*)-configured product when the reaction is performed in D_2O (Scheme 5A).[64]

The monomeric enzyme belongs to the Old Yellow Enzyme (OYE) family of flavoproteins. It adopts a three-domain architecture comprising a catalytic FMN-binding domain, a [4Fe–4S] cluster-containing linker domain, and an FAD-binding domain (Scheme 5B).[65] The standard redox potential of the reaction ($E^{o\prime}$ = −493 mV) is substantially more positive than that of the benzoyl-CoA (7)/1,5-dienoyl-CoA (**8**) redox couple.[63] Consequently, a hydride-transfer mechanism has been proposed in place of the radical-based mechanism characteristic of BCRs. QM/MM calculations based on the crystal structure further support a hydride-transfer mechanism for NCR, despite operation at an exceptionally low redox potential, which likely approaches the lower energetic limit for flavin-mediated hydride transfer.[65] Structural analyses additionally demonstrate how the protein environment tunes the active-site FMN cofactor into a potent hydride donor capable of driving the



Scheme 5 Structure and function of NCR. (A) Mechanism of enantioselective hydride transfer with an active site FMNH$_2$ serving as low-potential hydride donor ($E^{o\prime}$ ≈ −493 mV). (B) Structure of the NCR-monomer showing the electron-transferring FAD and [4Fe–4S] cofactors and the catalytic FMN.

reductive dearomatization of the naphthyl ring system. Although reduced methyl viologen can function as artificial electron donor for NCR *in vitro*, the physiological electron donor has not been identified and is presumed to be Fd_{red}.

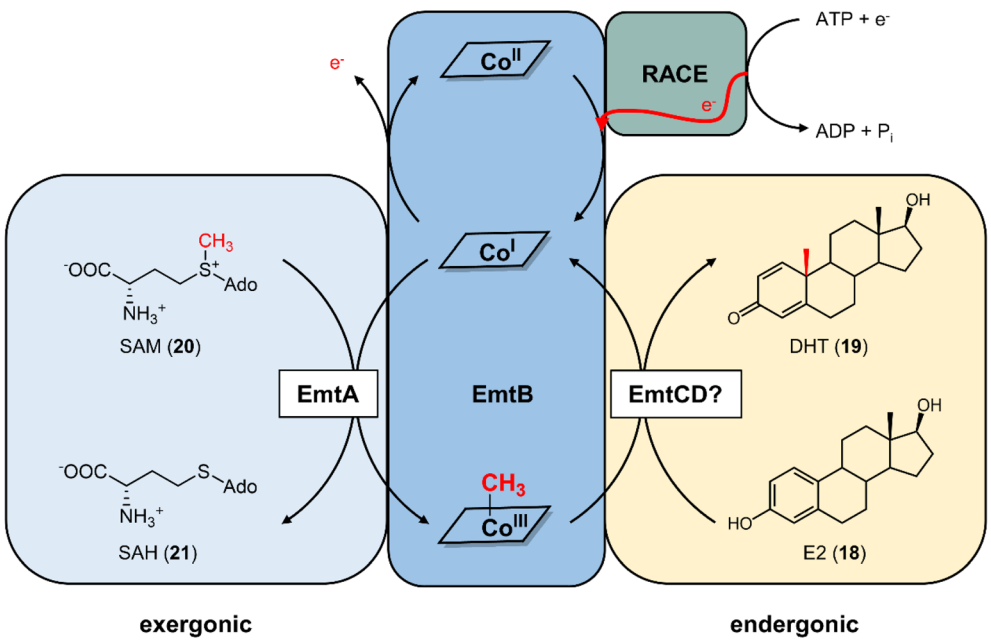
Three-domain NCR-like proteins of the OYE family are widespread in anaerobic PAH-degrading bacteria and mediate multiple dihydro additions during the stepwise reduction of polycyclic ring systems.[62,63] A dearomatizing 2-phenanthroyl-CoA reductase containing FMN, FAD, and a [4Fe–4S] cluster has been characterized from a phenanthrene-degrading sulfate-reducing enrichment culture;[66] similar to NCR, it also catalyzes dihydro-addition to the aryl-CoA substrates. In general, OYE-like reductases dearomatize polycyclic aryl-CoA esters by hydride transfer to positions outside the CoA-activated ring, whereas reduction of the remaining CoA-bound aromatic ring (*e.g.*, in 5,6,7,8-tetrahydro-2-naphthoyl-CoA, THNCoA), is likely catalyzed by an ATP-dependent class I benzoyl-CoA reductase.[67]

4. Dearomatization of phenols *via* *S*-adenosyl-L-methionine-dependent methyltransferases

4.1 Estrogen methyltransferase

Estrogens are steroid sex hormones derived from cholesterol and include the three major endogenous estrogens estrone (E1), 17β-estradiol (E2, **18**) and estriol (E3). These hormones regulate reproductive functions and the development of secondary sexual characteristics, primarily in females.[68] Estrogens are biosynthesized from testosterone by the oxygen- and NADPH-dependent aromatase cytochrome P450.[69] They are classified as Group I carcinogens by the International Agency for Research on Cancer (IARC).[70] Their aromatic A ring and low water solubility contribute to persistence in aquatic environments, where they originate from sources such as hospital effluents, livestock manure, agricultural runoff, and pharmaceutical waste streams.[71,72] To date, three denitrifying bacterial strains have been reported to grow on E2 (**18**) as the sole carbon source: *Denitratisoma* sp. strain DHT3,[73] *Steroidobacter denitrificans*[74] and *Denitratisoma oestradiolicum*.[75]

In *Denitratisoma* sp. strain DHT3, the conversion of ^{13}C-labelled E1/E2 (**18**) into the 1-dehydrotestosterone (DHT, **19**) by C10 methylation was demonstrated. This reaction represents a dearomatization of the phenolic A ring through methyl transfer and can formally be considered the reverse of the aromatase reaction. The *emtABCD* (emt: estradiol methylation) gene cluster responsible for estrogen methylation was identified and sequence comparisons suggested the following functional assignments: (i) EmtA is a cobalamin-dependent methyltransferase sharing highest sequence homology with MtmB, the catalytic subunit of monomethylamine methyltransferase from methanogenic archaea; (ii) EmtB represents the cobamide-binding subunit most similar to the corresponding dimethylamine methyltransferase component in *Methanosarcina* spp.; (iii) EmtC encodes a hypothetical protein; and (iv) EmtD is similar to a flavin-dependent oxidoreductase, likely involved in the reductive regeneration of the cobalamin cofactor. Notably, cob(I)alamin cofactors spontaneously oxidize to inactive cob(II)alamin even under anaerobic



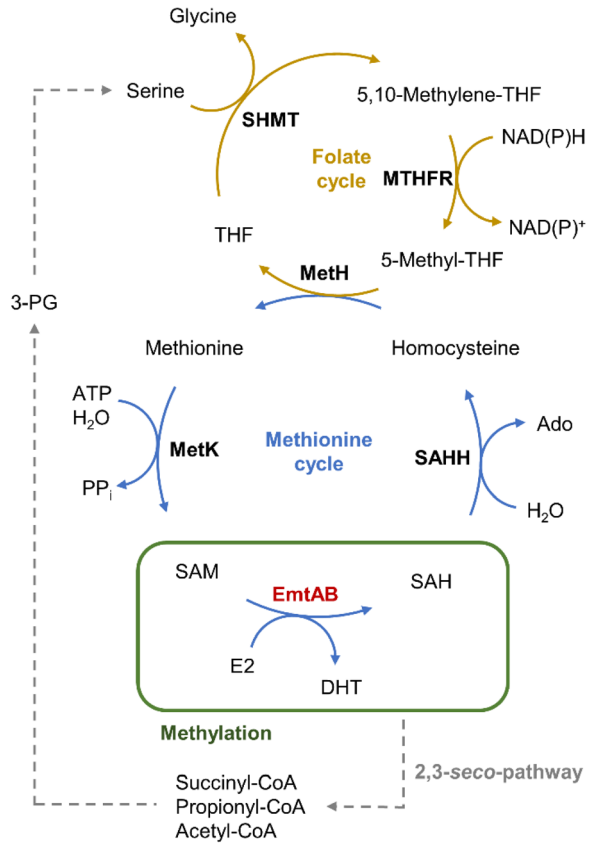
Scheme 6 Proposed reaction mechanism for the unusual SAM-and cobalamin-dependent estrogen methyltransferase EmtABCD complex from *D. oestradiolicum*. Two partial reactions are proposed: an exergonic methyl transfer from SAM (**20**) to cob(I)alamin, yielding methylcob(III)alamin (shown in dark and soft blue), and an endergonic methyl transfer from methyl(III)cobalamin to E2 (**18**) (shown in dark blue and yellow). Regeneration of the active cob(I)alamin cofactor is mediated by a RACE-like protein (shown in green) *via* ATP hydrolysis. Emt: estrogen methyltransferase, SAM: *S*-adenosyl-L-methionine, SAH: *S*-adenosyl-L-homocysteine (**21**), Co: cobalamin cofactor in its respective redox state, E2: 17β-estradiol, DHT: 1-dehydrotestosterone (**19**), RACE: reductive activator of corrinoid enzyme.

conditions. In the vicinity of the emtABCD gene cluster, a gene encoding a putative RACE (reductive activator of corrinoid enzyme) was identified that is supposed to be involved in ATP-dependent cob(I)alamin regeneration.[73]

The activity of the putative EmtABCD complex was further studied in *D. oestradiolicum* cells grown anaerobically on E2 (**18**) as carbon source and nitrate as electron acceptor. *S*-adenosyl-L-methionine (SAM, **20**) was identified as methyl donor for estrogen methylation, and two partial reactions were assigned to a SAM: estrogen methyltransferase complex: (i) exergonic methyl transfer from SAM (**20**) to cob(I)alamin, yielding methylcob(III)alamin, and (ii) an endergonic methyl transfer from methylcob(III)alamin to E1/E2 (**18**). The latter partial reaction could only be followed in the reverse reaction, the demethylation of DHT (**19**) to E2 (**18**). Although ring A dearomatization through methyl transfer from methylcob(III)alamin is thermodynamically highly unfavorable, the first exergonic methyl transfer step from SAM (**20**) to cob(I)alamin is proposed to drive the second one forward through a yet unknown coupling mechanism. The reaction depends on the artificial low-potential electron donor Ti(III)citrate to keep the cofactor in EmtB in its active cob(I)alamin state (Scheme 6).[76]

To date, the combination of SAM (**20**) and cobalamin as methyltransferase cofactors have been described for radical SAM enzymes, where SAM (**20**) may have a dual function: (i) generation of a 5′-deoxyadenosyl radical for hydrogen-atom abstraction from the substrate, and (ii) methyl group transfer to regenerate methylcob(III)alamin.[77] In contrast, the estrogen methyltransferase system EmtABCD does not belong to radical SAM superfamily but instead represents a distinct methyltransferase system in which SAM (**20**) functions solely as the methyl donor, cobalamin acts as intermediate methyl carrier, and the estrogen growth substrate serves as the terminal methyl acceptor.

The use of SAM (**20**) as a stoichiometric methyl donor to initiate a catabolic pathway is unprecedented and imposes a substantial cellular demand for this cofactor. To meet these elevated SAM (**20**) requirements, *D. oestradiolicum* harbors two genomic copies of genes involved in SAM (**20**) regeneration. Notably, one of these gene sets is strongly upregulated during growth on E2 (**18**) as the sole carbon source, thereby ensuring sufficient stoichiometric SAM (**20**) availability for each degradation cycle (Scheme 7). Consequently, anaerobic growth on E2 (**18**) relies not only on a specialized methyltransferase system but also on an efficient channeling of C1 units towards SAM (**20**) regeneration to sustain pathway initiation. This metabolic adaptation highlights a potentially valuable *in vivo* strategy for enhancing biotechnologically relevant SAM-dependent methylation reactions, including applications in pharmaceutical synthesis.[76]

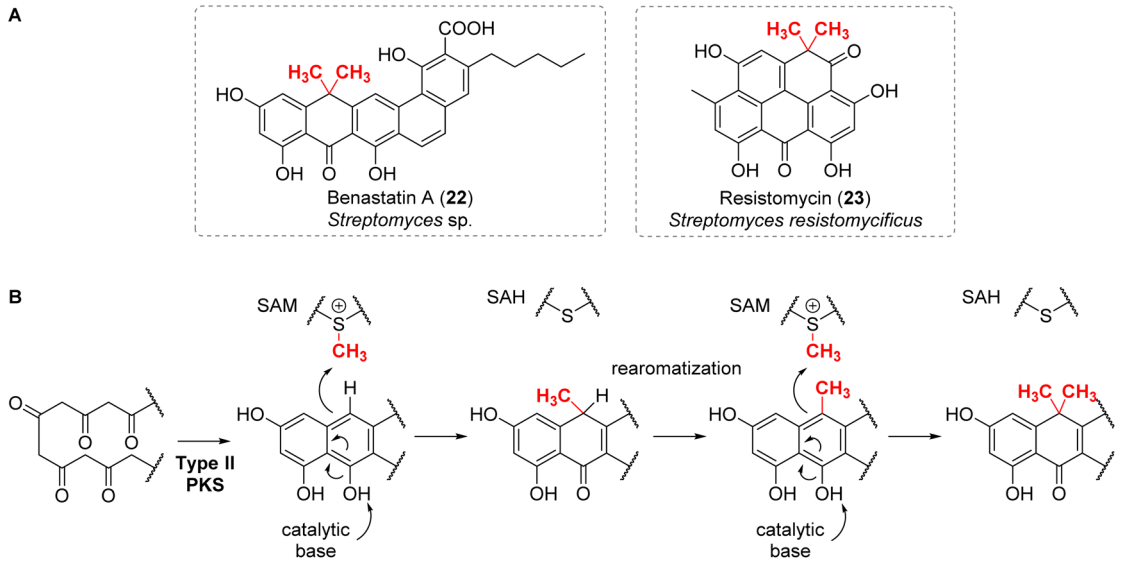


Scheme 7 Regeneration of SAM during growth with E2. Gene encoding indicated enzymes are upregulated in cells grown on E2 (**18**) compared with acetate. E2: 17β-estradiol, DHT: 1-deyhdrotestosterone, SAM: *S*-adenosyl-L-methionine, SAHH: *S*-adenosyl-L-homocysteine (SAH) hydrolase, Ado: adenosine, MetK: L-methionine adenosyltransferase, MetH: L-methionine synthase, MTHFR: 5,10-methylenetetrahydrofolate reductase, SHMT: L-serine hydroxymethyltransferase, 3-PG: 3-phosphoglycerate.

4.2 Geminal dimethylation in polyketide tailoring

Nature's dearomatization strategy *via* SAM-dependent methylation is also observed in the biosynthesis and diversification of aromatic polyketides. Nonoxidative tailoring reactions of aromatic polyketide synthase (PKS)-derived products include rare geminal C-dimethylation. This motif is found, for example, in the unusual type II polyketides benastatin (**22**) and resistomycin (**23**) from Streptomyces (Scheme 8A).[10,78] Gene inactivation and complementation experiments have demonstrated that the SAM-dependent C-methyltransferases BenF as well as RemG, catalyze the sequential installation of two methyl groups at the same carbon center within the pentacyclic aromatic scaffolds. This modification leads to dearomatization and formation of stable ketones. Mechanistically, SAM-dependent aromatic C-methylation typically proceeds *via* an S_N2-type reaction, in which a nucleophilic carbon of the electron-rich aromatic system attacks the electrophilic methyl group of SAM (**20**). The resulting transiently dearomatized keto intermediate undergoes rearomatization to yield the methylated aromatic product (Scheme 8B).[79–83] Consequently, a second methylation at the same position prevents the rearomatization by generating a quaternary carbon center.

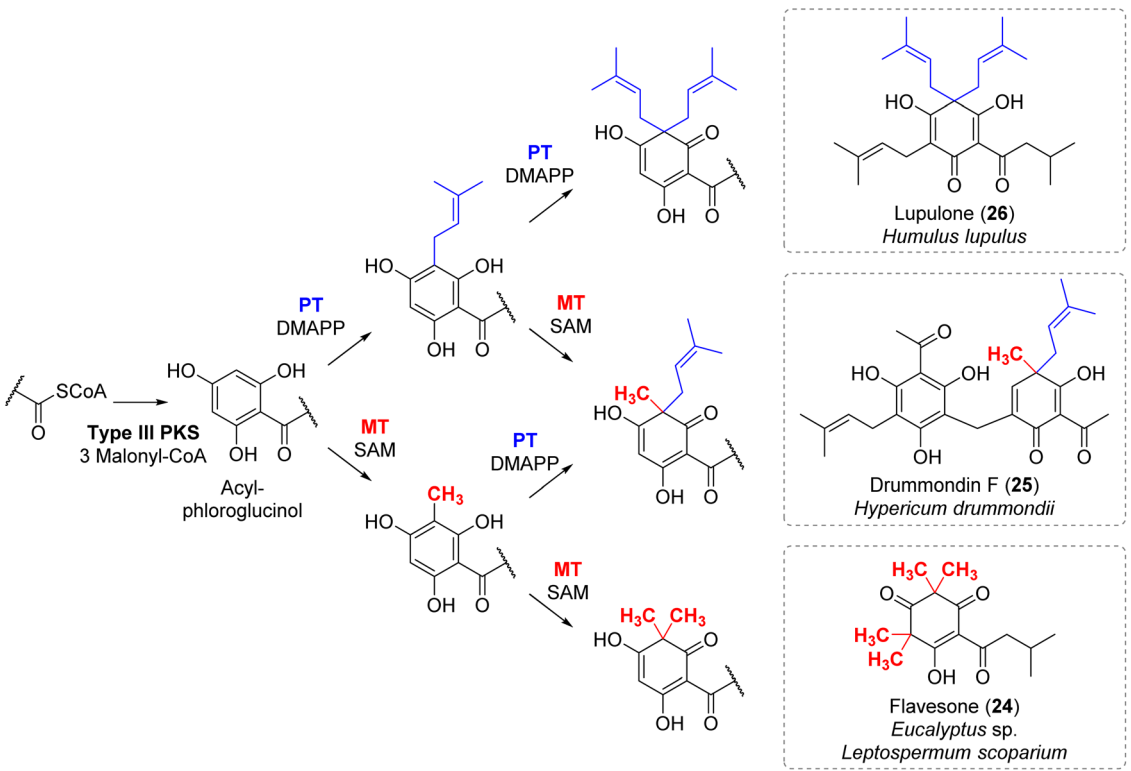
The enhanced stability of the resulting ketones may explain the biological rationale for this modification. Knockout experiments have shown that mutants lacking remG produce an unstable, non-methylated precursor of resistomycin (**23**).[10]



Scheme 8 (A) Structures of benastatin A (**22**) and resistomycin (**23**). (B) Proposed mechanism of dearomatization of type II aromatic polyketides by SAM-dependent geminal dimethylation. SAM: *S*-adenosyl-L-methionine. SAH: *S*-adenosyl-L-homocysteine.

Similarly, deletion of benF leads to the formation of non-methylated, anthrone-like intermediates that are prone to side reactions, including oxidation and substrate dimerization.[78] Thus, in the biosynthesis and tailoring of aromatic polyketides, geminal C-methylation appears to serve, *e.g.*, a protective role by preventing alternative oxidative reaction pathways.

Similar dimethylation patterns are found in type III PKS-derived polyphenols in plants. In this context, geminal dimethylation of acylphloroglucinols results in the formation of



Scheme 9 Diversification of type III PKS-derived polyphenols by geminal alkylation and examples of dearomatized acylphloroglucinols in plants. PKS: polyketide synthase. MT: *S*-adenosyl-L-methionine (SAM)-dependent methyltransferase. PT: dimethylallyl diphosphate (DMAPP)-dependent prenyltransferase.

stable β-triketones, such as flavesone (**24**) from Myrtaceae (Scheme 9). The enzymes responsible for these modifications remain elusive, and different biosynthetic routes are conceivable, including post-PKS methylation of the aromatic intermediate,[84,85] incorporation of methylmalonyl-CoA as a PKS building block,[86,87] or methylation during PKS assembly.[88–90] Notably, geminal alkylation in plant polyketides extends beyond methylation. Mixed substitution by methylation and prenylation is observed, for example, in drummondins (**25**) from *Hypericum drummondii*, while geminal diprenylation occurs in hops β-bitter acids such as lupulone (**26**) from *Humulus lupulus* (Scheme 9). In the latter case, a complex of two aromatic prenyltransferases (HlPT1 and HlPT2) catalyze three sequential prenylation steps, with the final geminal prenylation inducing dearomatization of the acylphloroglucinol scaffold.[91]

Thus, dearomatization through stable ketone formation, achieved by C-methylation and/or C-prenylation, expands the structural diversity of plant polyketides. The variety of these dearomative alkylation pathways forms a matrix of biosynthetic routes that generate structural variation. This diversity reflects the broad enzymatic and chemical flexibility of natural biosynthetic systems, consistent with the "screening hypothesis" of Firn and Jones.[92]

5. Conclusions

Oxygen-independent enzymatic dearomatization pathways exhibit extraordinary diversity in both their catalytic mechanisms and their biological functions. The underlying enzymatic strategies differ substantially depending on the producing organism, the degree and type of substrate aromaticity, and the physiological or ecological context in which the transformation occurs. Across microbial primary metabolism and specialized secondary metabolism, these reactions fulfill a broad spectrum of biological roles, including aromatic compound degradation, carbon assimilation, detoxification, and the structural tailoring or diversification of bioactive metabolites. A particularly intriguing aspect of these transformations is the dual functional consequence of disrupting aromaticity. In many catabolic pathways, dearomatization increases substrate reactivity and thereby facilitates subsequent bond cleavage and degradation steps. Conversely, in other metabolic settings, especially for polyhydroxylated aromatic compounds, dearomatization may enhance metabolite stability by preventing undesired oxidative reactions and protecting sensitive intermediates from spontaneous oxidation. This functional versatility highlights the sophisticated evolutionary adaptation of anaerobic and oxygen-limited metabolic systems.

Nature has evolved two fundamentally distinct oxygen-independent strategies for arene dearomatization: reductive dearomatization and SAM-dependent methylation. Both strategies effectively complement the classical oxygenase-dependent enzymatic toolbox and expand the biochemical repertoire available for aromatic compound transformation under anaerobic conditions. The underlying oxygen-independent pathways enhance metabolic flexibility in diverse ecological niches.

Beyond their biological significance, oxygen-independent dearomatization enzymes hold considerable promise for biotechnological and industrial applications. Their ability to selectively functionalize highly stable aromatic compounds offers attractive opportunities for the synthesis of value-added chemicals from HAC precursors. In addition, they are increasingly relevant for sustainable biomass valorization, including the large-scale conversion of lignocellulose feedstocks into central platform chemicals for bioplastics production, as well as the anaerobic transformation of plant biomass into biogas or other renewable energy carriers. Continued mechanistic and structural investigation of these enzymes will provide powerful new tools for green chemistry, synthetic biology, and circular bioeconomy strategies.

6. Author contributions

Conceptualization: M. B., M. M. writing – original draft: M. B. (Section 1–3 and Conclusions), T. Z. (Section 4.1), J. B. (Section 4.2), L. P. (Section 3.2.1), L. A. (Section 3.2.2), S. M. H. (Section 3.1). Writing – review & editing: all authors. All authors have read and approved the final version of the manuscript.

7. Conflicts of interest

There are no conflicts of interests to declare.

8. Data availability

No primary research results, software or code have been included and no new data were generated or analysed as part of this review.

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