Natural Products

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The Biocatalytic Repertoire of Natural Biaryl Formation

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biaryls · biosynthesis · enzymes · natural products · oxidative coupling

The catalytic and selective construction of carbon–carbon bonds for the generation of complex molecules is one of the most important tasks in organic chemistry. This was clearly highlighted by the 2010 Nobel Prize in Chemistry, which was awarded for the development of Pd-catalyzed cross-coupling reactions. The underlying concept of cross-linking building blocks to generate molecular complexity can also be widely found in natural product biosynthesis. Impressive examples for such natural cross-coupling reactions are biosynthetic processes for the assembly of biaryl moieties in natural products—highly efficient enzymatic reactions that often achieve synthetically yet unmatched selectivities. This Minireview highlights selected examples that showcase these fascinating biotransformations.

1. Introduction

Nature's ability to generate its inexhaustible chemical arsenal of bioactive natural products out of simple building blocks is highly remarkable. By utilizing only a relatively small set of chemical reactions, large families of secondary metabolites with diverse structures are assembled, among them alkaloids, terpenes, polyketides, and (non-)ribosomal peptides.[1] The impressive chemical space that is thereby already opened up gets even extended by further alterations that are catalyzed by tailoring enzymes during or after assembly of the core structure. These biocatalysts decorate metabolites, for example, by alkylation or glycosylation, or a variety of Red./Ox. transformations, including halogenation and hydroxylation reactions.[2]

A particularly interesting oxidative tailoring step gives rise to a large number of medically important secondary metabolites by introducing single or multiple biaryl linkages. Such modifications can be found in many natural product families across phylogenetically diverse producing organisms and occur both inter- and intramolecularly. This results in a plethora of natural biaryl compounds (Figure 1),[3] ranging from isocyclic congeners, for example, true dimers such as (−)-gossypol (**1**)[4] or unsymmetric compounds such as (+)-knipholone (**2**),[5] over biaryls with fused heterocycles, for example, blumeanine (**3**),[6] and heterobiaryls, such as streptonigrin (**4**)[7] or even N,C-fused examples such as murrastifoline F (**5**),[8] to bridged biaryls, including (+)-schizandrin (**6**),[9] cavicularin (**7**),[10] and biphenomycin A (**8**).[11] As a result of the often rewarding biological activities of such molecules, numerous synthetic methods for their preparation have been developed.[12] This is a challenging task, as in many cases it not only requires regioselectivity to be achieved and high steric repulsion to be overcome, for example, for the installation of tetrasubstituted axes, but also control of the stereochemical outcome to select for the correct axial chirality in the case of configurationally stable axes.[13] Most of the current methods to construct biaryls thus require prefunctionalization for substrate activation and/or to achieve regio-/stereoselectivity.[12,13] Much more straightforward would be the direct, yet selective, coupling of two unfunctionalized aryl fragments. Innovative oxidative methods of this type are emerging,[14] but they are still far from being applicable to a broad range of natural product target structures.

But how does nature access such a diverse range of biaryl secondary metabolites? The unifying principle behind the construction of biaryls in natural product biosynthesis is phenol oxidative coupling.[15] This process is commonly thought to involve an oxidative enzyme, such as a laccase, a peroxidase, or a cytochrome P450 enzyme (CYP). The biocatalyst generates a radical at the phenol substituent (or amino group)—either by deprotonation and single-electron transfer or by abstraction of a hydrogen radical—in the coupling substrate, such as **9**, thus creating intermediate **10**. This could either react with a second radical substrate formed by the enzyme (Scheme 1) or by single-radical coupling with

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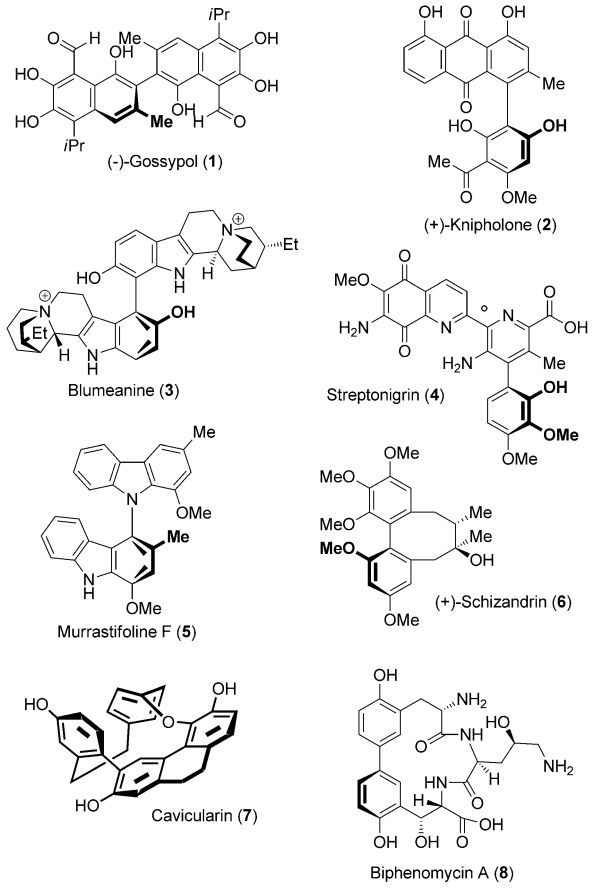
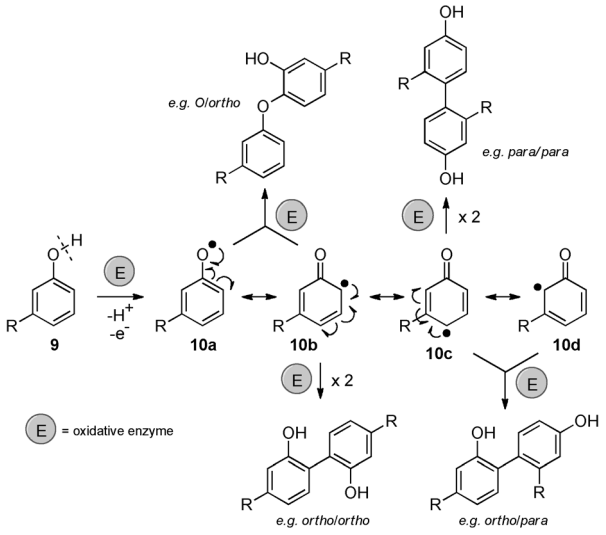


Figure 1. Examples of structurally diverse natural biaryls.



Scheme 1. The basic principle of enzymatic oxidative coupling of phenols.

a subsequent renewed overall abstraction of a proton and an electron. Both reaction pathways ultimately lead to the overall abstraction of two protons and two electrons with installation of a biaryl or biaryl–ether bond. The radical **10** not only has spin density at the oxygen atom (**10a**), but also at the

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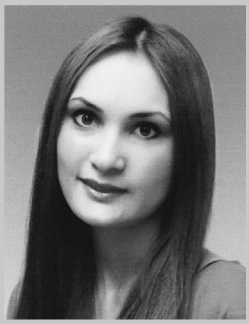
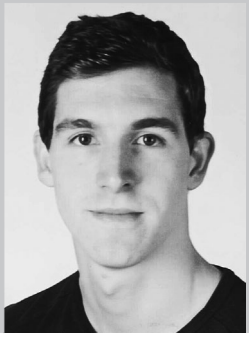


Hülya Aldemir studied chemistry at the University of Cologne and completed her diploma thesis in the field of C–H activation in the group of Prof. Goldfuss in 2010. Thereafter, she started her PhD under the guidance of Prof. Gulder at the University of Bonn, dealing with the chemoenzymatic total synthesis of natural products. Since 2014 she has continued her PhD at the TU München.



René Richarz studied biology at the University of Bonn where he finished his diploma thesis in the field of microbiology in the group of Prof. Deppenmeier. Since 2011 he has been carrying out PhD research with Prof. Gulder in Bonn and at the TU München. He is interested in chemoenzymatic methods for the construction of complex natural products.

ortho or *para* positions (**10b–d**). This gives rise to a large set of possible regiodivergent products with C-O-C (or C-N-C) or C-C linkages. As a mechanistic alternative, a radical-cation-based pathway is often also feasible, where the order of the electron and proton abstraction is reversed, that still leads to the identical outcome of the overall reaction.

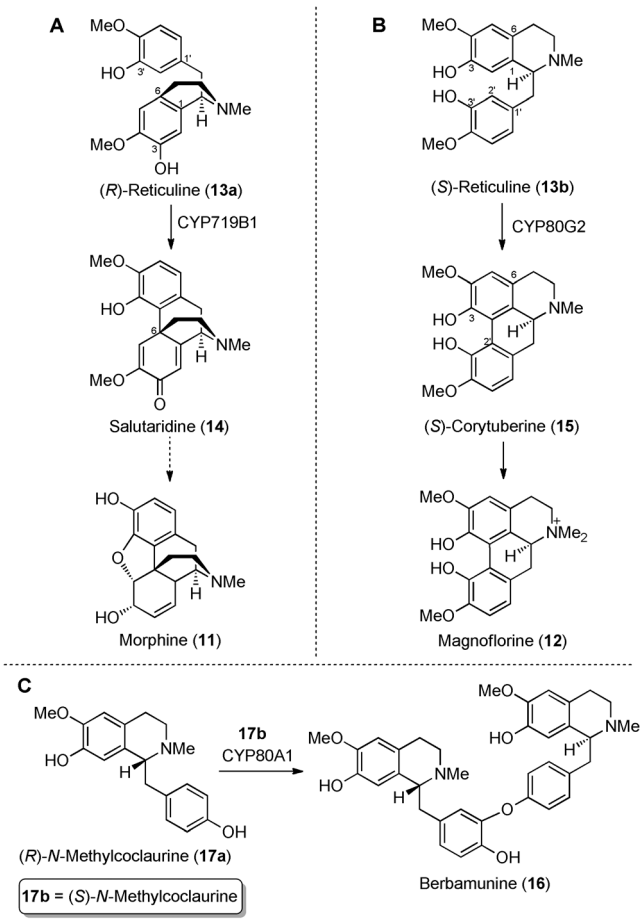
In this Minireview, we strive to highlight the versatility of enzymatic biaryl construction in natural product biosynthesis by discussing intra- and intermolecular variants of this fascinating reaction for a small set of selected examples of biomedically and structurally intriguing secondary metabolites.

2. Intramolecular Enzymatic Biaryl Coupling Reactions

2.1. Formation of Alkaloid Core Structures

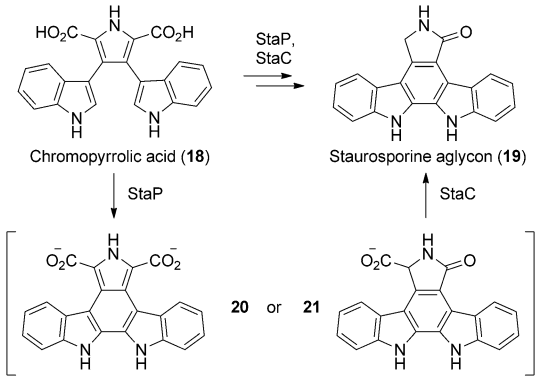
The plant-derived benzylisoquinoline alkaloids constitute a class of natural products that is well known for its pharmaceutical activity, for example, morphine (**11**),[16] which is a narcotic and analgesic drug, and the sedative and anxiolytic magnoflorine (**12**).[17] These structurally complex

compounds are assembled through formation of biaryl and/or biaryl–ether bonds catalyzed by CYP oxidases.[18–20] The intramolecular C–C phenol coupling reaction in morphine biosynthesis in the opium poppy is catalyzed by the highly stereo- and regioselective salutaridine synthase CYP719B1 through a single cycle of iron oxidation.[18] Thereby, (*R*)-reticuline (**13a**) is converted into salutaridine (**14**), a precursor of **11** (Scheme 2). The magnoflorine precursor (*S*)-corytuberine (**15**) is generated by stereospecific oxidation of (*S*)-reticuline (**13b**) by the oxidase CYP80G2.[19] In this case, the coupling is postulated to proceed through a biradical reaction. The biosynthesis of the bisbenzylisoquinoline alkaloid berbamunine (**16**) from *N*-methylcoclaurin (**17**) in *Berberis stolonifera* is an example of a regioselective intermolecular C-O-C phenol coupling reaction (see Section 3) catalyzed by berbamunine synthase CYP80A1.[20]



Scheme 2. Oxidative phenol coupling reactions in the biosynthesis of A) morphine (**14**), B) magnoflorine (**15**), and C) berbamunine (**17**).

Another highly interesting class of alkaloids that contain biaryl bonds are the antitumoral indolocarbazoles.[21] The most prominent examples are staurosporine[22] and rebeccamycin.[23] The biaryl coupling reaction that forms these compounds is catalyzed by the CYPs StaP and RebP, respectively.[24] In combination with the flavin-dependent oxidoreductase StaC (or RebC),[25] chromopyrrolic acid (**18**) is transformed into staurosporine aglycon (**19**) in a four-electron oxidation sequence via **20** or **21** (Scheme 3).



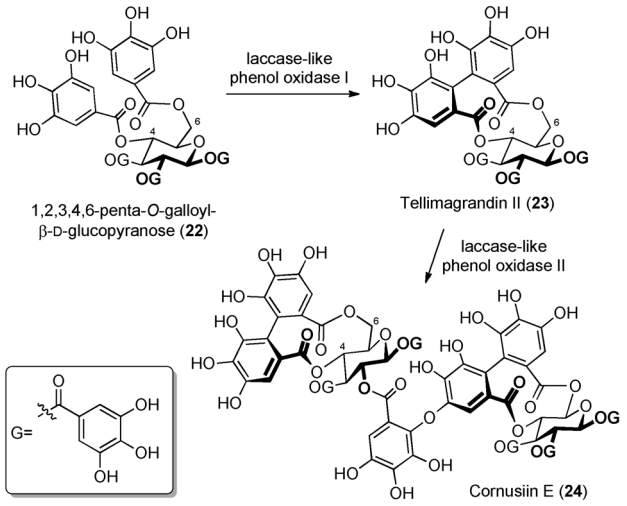
Scheme 3. Oxidative coupling in the biosynthesis of the indolocarbazole staurosporine.

2.2. Oxidative Cross-Coupling in Tannin Biosynthesis

Natural organic tannins form a structurally diverse class of polyphenolic compounds with interesting pharmaceutical properties, such as antimicrobial, antitumoral, or antiviral activities.[26] Tannins can be further classified into hydrolyzable and condensable congeners. The latter consist of polymeric flavonoid compounds, while hydrolyzable tannins contain gallic acid esters with a polyol, typically β-D-glucose. A fully galloylated glucose is represented by the precursor (**22**) of the hydrolyzable ellagitannins and gallotannins. The ellagitannins are structurally characterized by oxidative aryl–aryl coupling between adequately aligned galloyl moieties. The conformation of the central glucose residue thereby defines the regioselectivity of the coupling reaction. 2,3- and 4,6-cross-links are favored if the central glucose unit exists in the energetically preferred $^4C_1$ conformation, while the less-stable $^1C_4$ conformer delivers 1,6-, 3,6-, and 2,4-linkages. The resulting monomeric ellagitannins can be further processed by intermolecular oxidative generation of a biaryl and/or biaryl–ether bond, thus producing a tremendous structural diversity of oligomeric derivatives. Investigations on the biosynthesis of ellagitannins in *Tellima grandiflora* led to the identification of an enzyme that selectively produces tellimagrandin II (**23**)[27] (the pivotal precursor of many ellagitannins[28]) and a second enzyme that oxidatively dimerizes **23** to cornusiin E (**24**)[29] (Scheme 4). The two enzymes are assigned to the subclass of laccases,[26] which catalyze the oxidation of phenols by single-electron transfer and simultaneous reduction of molecular oxygen.[30]

2.3. Formation of Cyclic Peptides by Oxidative Cross-Linking

A number of biomedically important peptides bear biaryl structural elements between aromatic amino acid residues. Such peptides are derived of nonribosomal peptide synthetases (NRPS) or NRPS/polyketide synthase (NRPS/PKS)



Scheme 4. Laccase-mediated oxidative cross-coupling in ellagitannin biosynthesis.

hybrid enzymes. The biaryl and biaryl–ether cross-linking, which has a tremendous influence on the shape and stability of these molecules in vivo and as a consequence is essential for their bioactivity,[31] is performed by CYPs. The most prominent example of this class of natural products is vancomycin (**25**, Figure 2), a glycopeptide antibiotic discovered in the mid-1950s[32] that inhibits cell wall biosynthesis

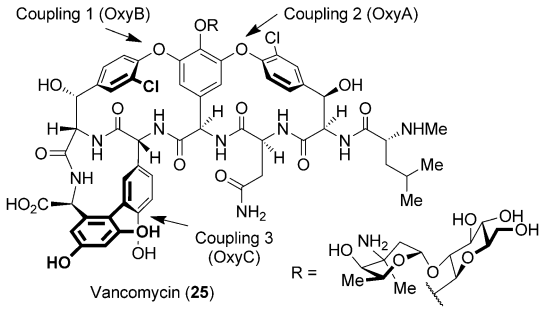


Figure 2. CYP-mediated cross-coupling in vancomycin biosynthesis.

effectively.[33] **25** features three macrocycles formed through installation of biaryl and biaryl–ether bonds. The cross-linking reactions are performed by the three CYPs OxyA, OxyB, and OxyC, which are encoded in the biosynthetic gene cluster.[34] Gene knockout studies in *Amycolatopsis balhimycina*, the producer of the closely related balhimycin, indicate that ring formation is catalyzed in the following order: OxyB→ring 1, OxyA→ring 2, OxyC→ring 3.[31a,35] Until now, the exact timing of the cross-linking reactions during peptide assembly has not been unambiguously elucidated. However, knockout mutants of *A. balhimycina*[31b] and in vitro assays with purified OxyB from the vancomycin producer *Amycolatopsis orientalis* strongly indicate that either the linear hexa- or heptapeptide precursor bound to the penultimate or terminal carrier protein of the NRPS, respectively, is the substrate for biaryl construction, rather than the corresponding free acids.[34a,36]

The oxidative cross-linking reactions install three elements of chirality in **25**, one configurationally stable biaryl axis and two biaryl–ether bonds that introduce planar chirality. Although axial chirality has to be controlled by the biaryl coupling step itself, this is less clear when looking at the elements of planar chirality, where the chlorine atoms situated on the tyrosine units are decisive. Nonpolar deletion mutants of OxyABC from the balhimycin gene cluster produced linear, non-chlorinated peptides (Δ*oxyB*) and monocyclic or bicyclic, fully chlorinated peptides (Δ*oxyA* and Δ*oxyC*, respectively).[34b] This, together with the significantly lower turnover of chlorinated hexa- and heptapeptides by vancomycin OxyB in vivo,[37] raises the question of whether fully chlorinated, linear peptides found in earlier knockout studies[31b,35a] are mere shunt products. In this case, it is possible that halogenation occurs after the initial coupling by OxyB,[37] thereby making the halogenation reaction the stereochemically decisive step.

Biaryl and biaryl–ether linkages are a common structural feature across diverse biologically active peptides. Other interesting examples include the antiviral complestatin (**26**)[38] and arylomycin-type antibacterial lipopeptides (e.g. **27**, Figure 3).[39] In all cases, construction of the biaryl unit most likely follows the above scheme,[31c,39d,40] although this has yet to be proven experimentally.

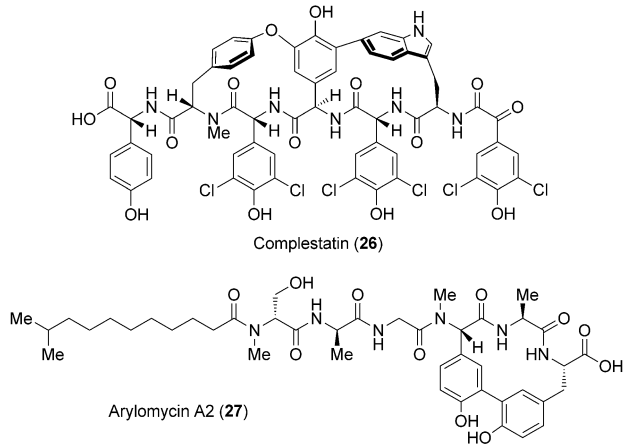
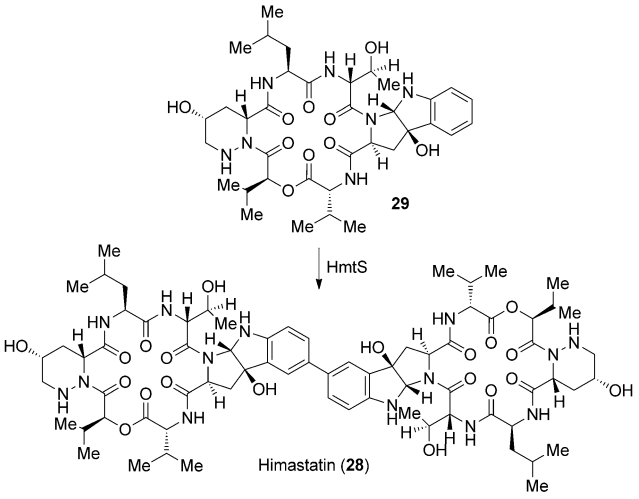


Figure 3. Complestatin (**26**) and arylomycin A2 (**27**).

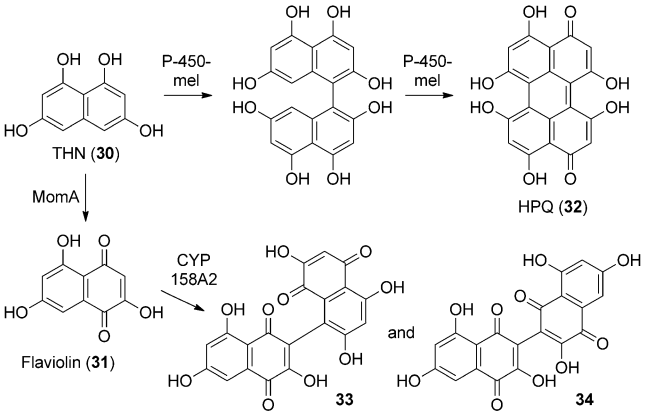
3. Intermolecular Enzymatic Biaryl Coupling Reactions

3.1. Dimerization of Aromatic Peptides

Section 2.3 highlighted biaryl-forming reactions in glycopeptide biosynthesis, which revealed that the resulting cross-links are crucial for the biological activity of the final products. This can also be seen in the intermolecular case of himastatin (**28**).[41] Investigations into the biosynthesis of **28** have shown that the CYP HmtS catalyzes the dimerization of the hexapeptide precursor **29** (Scheme 5).[42] This is a remarkable reaction, as the coupling substrates are free—that is, not bound to a carrier protein. Interestingly, the candidate



Scheme 5. Oxidative dimerization towards himastatin (**28**).



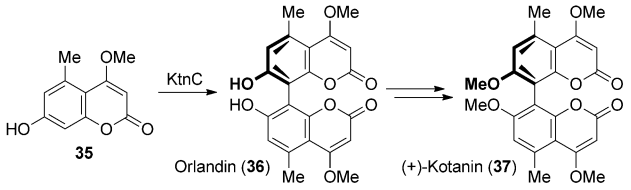
Scheme 6. Biocatalytic formation of THN (**30**) and flaviolin dimers **32**–**34**.

enzyme HmtS is closely related to KtzM, a protein that is thought to be responsible for the oxidative transformation of a tryptophan moiety into hexahydropyrroloindole in kutzneride biosynthesis;[43] this structural feature is also found in **28**. In the himastatin pathway, however, HmtS performs the oxidative dimerization, while the formation of the indole portion is catalyzed by HmtT.[44]

3.2. Biaryl Coupling Reactions with Aromatic Polyketides

A biosynthetically and structurally diverse class of metabolites are the melanins.[45] These macromolecules are generally formed by oxidative polymerization of phenolic or indolic compounds. One of the most important classes of fungal melanins are the so-called DHN melanins (from 1,8-dihydroxynaphthalene). The monomeric precursor 1,3,6,8-tetrahydroxynaphthalene (**30**, THN) is assembled by a fungal type I polyketide synthase (PKS I),[46] transformed into DHN, and the latter oxidatively dimerized/polymerized by a laccase.[45] Interestingly, **30** can also be biosynthesized by a PKS III system in diverse Streptomycetes.[47] The PKS gene is often flanked by a monooxygenase homologue *momA* and a CYP homologue *P-450mel*.[48] Although MomA is a quinone-forming enzyme, for example, oxidizing **30** to flaviolin (**31**),[48] P-450mel catalyzes the dimerization of **30** by double biaryl coupling to give hexahydroxyperylenequinone (**32**, HPQ) in *Streptomyces griseus* (Scheme 6).[49] Remarkably, the homologous CYP158A2 in *Streptomyces coelicolor* A3(2) in turn accepts **31** as a substrate, which results in the formation of dimeric and trimeric flaviolin coupling products, such as, **33** and **34**.[50]

The dimerization of **31** by CYP158A2 lacks any regioselectivity (no investigations on the stereoselectivity have been reported). By contrast, the regio- and stereochemical outcome of the coupling reaction is controlled efficiently in many other polyketide-derived natural products.[3] Particularly impressive examples are the bicoumarins.[51] Hüttel and Müller illuminated the biosynthetic sequence from the monomethylated precursor **35** via orlandin (**36**) to (+)-kotanin (**37**; Scheme 7).[52] The CYP KtnC was shown to catalyze the biaryl coupling reaction in vivo.[53] Docking experiments with a homology model of KtnC furthermore gave the first structural insights into the control of regio- and stereoselectivity during the formation of **37**. Feeding experiments for phlegmacin-type fungal polyketides suggest similar biosynthetic pathways for these metabolites.[54]

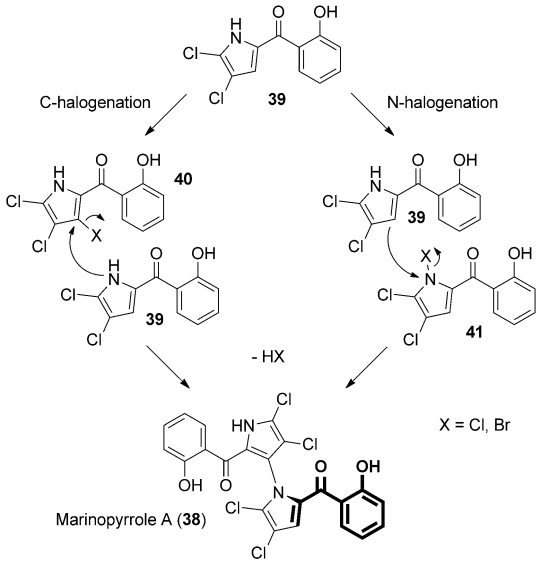


Scheme 7. Biaryl coupling in the biosynthesis of kotanin (**37**).

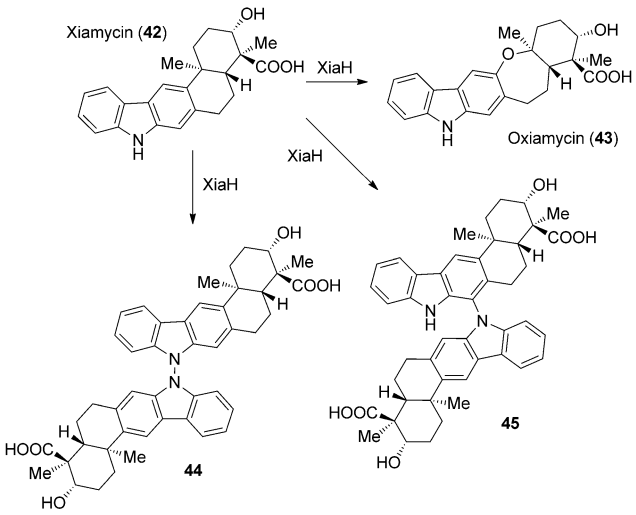
3.3. New Biaryl Coupling Principles in the Biosynthesis of Indoloterpenes and Pyrrols

Fenical and co-workers recently reported on the marinopyrroles, for example, marinopyrrole A (**38**), structurally novel compounds with remarkable antibiotic activities from the marine *Streptomyces* strain CNQ-418.[55] These metabolites feature an *M*-configured biaryl axis that is biosynthetically installed with perfect stereocontrol. Investigations into the biosynthesis of **38** revealed that two flavoproteins related to $FADH_2$-dependent halogenases are involved in construction of the biaryl unit.[56] Besides the common radical mechanisms, this makes an entirely different coupling mechanism feasible. Starting from monomeric monodeoxypyoluteorin (**39**), C- or N-halogenation could lead to **40** or **41**, which after N- or C-nucleophilic attack, respectively, by a second equivalent of **39** could ultimately yield **38** with formal loss of HX (Scheme 8).

Another highly interesting novel class of natural products in this context are the indolosesquiterpenes, for example, xiamycin (**42**)[57] and oxiamycin (**43**; Scheme 9).[58] Investigations on the gene clusters encoding these metabolites not only



Scheme 8. Proposed biaryl coupling mechanisms in the biosynthesis of **38**.



Scheme 9. Product diversity of the single flavoprotein XiaH.

revealed an unprecedented cyclization sequence,[59] but also allowed the increased production of these compounds in a heterologous host, thereby facilitating the characterization of unique N,N (e.g. **44**) and C,N dimers (e.g. **45**) of xiamycin (**42**).[59b] Remarkably, Hertweck and co-workers identified a single flavoprotein, the FAD-dependent aromatic ring hydroxylase XiaH, as the sole enzyme that not only forms all the biaryl derivatives by radical coupling, but also catalyzes the oxidative rearrangement of **42** to **43**.[60]

4. Conclusions and Outlook

The above examples clearly showcase the impressive biocatalytic toolkit nature has evolved to efficiently conduct the (oxidative) cross-coupling of aromatic compounds to form biaryl and biaryl–ether bonds. In the biosynthesis of small secondary metabolites, laccases and in particular CYPs seem to be the main players to construct these privileged structural features. Despite intensive research in natural product biosynthesis, the new biochemistry of the flavoenzymes presented in Section 3.3 clearly shows that an enormous yet untapped enzymatic repertoire is still likely to be discovered.

In addition, besides oxidative strategies for biaryl biosynthesis, further natural concepts to form this structural element exist. A remarkable example is the formation of the biphenyl phytoalexins of the Pyrinae catalyzed by biphenyl synthase, which elongates a benzoate starter unit by three malonate units with subsequent decarboxylative cyclization of the resulting polyketo chain to yield the second aromatic portion of the biaryl system.[61] Taking into consideration that even long-known, for example, CYP-mediated oxidative cross-coupling reactions, are far from being mechanistically well understood, chemically utilized, or even biotechnologically exploited, exciting decades of modern natural product biomolecular research in this field are to be expected.

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[1] P. M. Dewick, *Medicinal Natural Products*: *A Biosynthetic Approach*, *3rd ed.*, Wiley, Chichester, **2009**.
[2] For reviews, see the following articles and references cited therein: a) C. T. Walsh, H. Chen, T. A. Keating, B. K. Hubbard, H. C. Losey, L. Luo, C. G. Marshall, D. A. Miller, H. M. Patel, *Curr. Opin. Chem. Biol.* **2001**, *5*, 525 – 534; b) P. Wang, X. Gao, Y. Tang, *Curr. Opin. Chem. Biol.* **2012**, *16*, 362 – 369.
[3] G. Bringmann, C. Günther, M. Ochse, O. Schupp, S. Tasler, *Progress in the Chemistry of Organic Natural Products*, *Vol. 82* (Eds.: W. Herz, H. Falk, G. W. Kirby, R. E. Moore, C. Tamm), Springer, Wien, **2001**, pp. 1 – 249.
[4] L. Marchlewski, *J. Prakt. Chem.* **1899**, *60*, 84 – 90.
[5] E. Dagne, W. Steglich, *Phytochemistry* **1984**, *23*, 1729 – 1731.
[6] D. Arbain, Dachriyanus, Firmansyah, M. V. Sargent, B. W. Skelton, A. H. White, *J. Chem. Soc. Perkin Trans. 1* **1998**, 2537 – 2540.
[7] K. V. Rao, W. P. Cullen, *Antibiot. Annu.* **1959 – 1960**, 950 – 953.
[8] C. Ito, Y. Thoyama, M. Omura, I. Kajiura, H. Furukawa, *Chem. Pharm. Bull.* **1993**, *41*, 2096 – 2100.
[9] Y. Ikeya, H. Taguchi, H. Mitsuhashi, S. Takeda, Y. Kase, M. Aburada, *Phytochemistry* **1988**, *27*, 569 – 573.
[10] M. Toyota, T. Yoshida, Y. Kan, S. Takaoka, Y. Asakawa, *Tetrahedron Lett.* **1996**, *37*, 4745 – 4748.
[11] I. Uchida, N. Shigematsu, M. Ezaki, M. Hashimoto, H. Aoki, H. Imanaka, *J. Antibiot.* **1985**, *38*, 1462 – 1468.
[12] For selected reviews, see a) J. Hassan, M. Sévignon, C. Gozzi, E. Schulz, M. Lemaire, *Chem. Rev.* **2002**, *102*, 1359 – 1470; b) G. Bringmann, A. J. P. Mortimer, P. A. Keller, M. J. Gresser, J. Garner, M. Breuning, *Angew. Chem.* **2005**, *117*, 5518 – 5563;

Angew. Chem. Int. Ed. **2005**, *44*, 5384 – 5427; c) D. Alberico, M. E. Scott, M. Lautens, *Chem. Rev.* **2007**, *107*, 174 – 238.
[13] G. Bringmann, T. Gulder, T. A. M. Gulder, M. Breuning, *Chem. Rev.* **2011**, *111*, 563 – 639.
[14] For selected reviews, see a) M. C. Kozlowski, B. J. Morgan, E. C. Linton, *Chem. Soc. Rev.* **2009**, *38*, 3193 – 3207; b) J. A. Ashenhurst, *Chem. Soc. Rev.* **2010**, *39*, 540 – 548.
[15] The oxidative phenol coupling reaction was first described by R. Pummerer, P. Puttfarcken, P. Schopflocher, *Ber. Dtsch. Chem. Ges.* **1925**, *58*, 1808 – 1820.
[16] P. R. Blakemore, J. D. White, *Chem. Commun.* **2002**, 1159 – 1168.
[17] J. B. de la Pena, H. L. Lee, S. Y. Yoon, G. H. Kim, Y. S. Lee, J. H. Cheong, *J. Nat. Med.* **2013**, *67*, 814 – 821.
[18] a) M. H. Zenk, R. Gerardy, R. Stadler, *J. Chem. Soc. Chem. Commun.* **1989**, 1725 – 1727; b) R. Gerardy, M. H. Zenk, *Phytochemistry* **1992**, *32*, 79 – 86; c) A. Gesell, M. Rolf, J. Ziegler, M. L. D. Chávez, F.-C. Huang, T. M. Kutchan, *J. Biol. Chem.* **2009**, *284*, 24432 – 24442.
[19] N. Ikezawa, K. Iwasa, F. Sato, *J. Biol. Chem.* **2008**, *283*, 8810 – 8821.
[20] a) R. Stadler, M. H. Zenk, *J. Biol. Chem.* **1993**, *268*, 823 – 831; b) P. F. X. Kraus, T. M. Kutchan, *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 2071 – 2075.
[21] K. S. Ryan, C. L. Drennan, *Chem. Biol.* **2009**, *16*, 351 – 364.
[22] S. Omura, Y. Iwai, A. Hirano, A. Nakagawa, J. Awaya, H. Tsuchya, Y. Takahashi, R. Masuma, *J. Antibiot.* **1977**, *30*, 275 – 282.
[23] J. A. Bush, B. H. Long, J. J. Catino, W. T. Bradner, K. Tomita, *J. Antibiot.* **1987**, *40*, 668 – 678.
[24] a) A. R. Howard-Jones, C. T. Walsh, *J. Am. Chem. Soc.* **2006**, *128*, 12289 – 12298; b) A. R. Howard-Jones, C. T. Walsh, *J. Am. Chem. Soc.* **2007**, *129*, 11016 – 11017; c) M. Makino, H. Sugimoto, Y. Shiro, S. Asamizu, H. Onaka, S. Nagano, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 11591 – 11596; d) Y. Wang, H. Chen, M. Makino, Y. Shiro, S. Nagano, S. Asamizu, H. Onaka, S. Shaik, *J. Am. Chem. Soc.* **2009**, *131*, 6748 – 6762.
[25] K. S. Ryan, A. R. Howard-Jones, M. J. Hamill, S. J. Elliott, C. T. Walsh, C. L. Drennan, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 15311 – 15316.
[26] R. Niemetz, G. G. Gross, *Phytochemistry* **2005**, *66*, 2001 – 2011.
[27] R. Niemetz, G. G. Gross, *Phytochemistry* **2003**, *62*, 301 – 306.
[28] E. Haslam, Y. Cai, *Nat. Prod. Rep.* **1994**, *11*, 41 – 66.
[29] R. Niemetz, G. G. Gross, *Phytochemistry* **2003**, *64*, 1197 – 1201.
[30] N. Santhanam, J. M. Vivanco, S. R. Decker, K. F. Reardon, *Trends Biotechnol.* **2011**, *29*, 480 – 489.
[31] a) D. Bischoff, S. Pelzer, A. Höltzel, G. J. Nicholson, S. Stockert, W. Wohlleben, G. Jung, R. D. Süssmuth, *Angew. Chem.* **2001**, *113*, 1736 – 1739; *Angew. Chem. Int. Ed.* **2001**, *40*, 1693 – 1696; b) D. Bischoff, B. Bister, M. Bertazzo, V. Pfeifer, E. Stegmann, G. J. Nicholson, S. Keller, S. Pelzer, W. Wohlleben, R. D. Süssmuth, *ChemBioChem* **2005**, *6*, 267 – 272; c) X. Jin, M. Rao, W. Wei, M. Ge, J. Liu, D. Chen, Y. Liang, *Biotechnol. Lett.* **2012**, *34*, 2283 – 2289.
[32] M. H. McCormick, W. M. Stark, G. E. Pittenger, R. C. Pittenger, J. M. McGuire, *Antibiot. Annu.* **1955 – 1956**, 606 – 611.
[33] D. Kahne, C. Leimkuhler, W. Lu, C. T. Walsh, *Chem. Rev.* **2005**, *105*, 425 – 448.
[34] a) K. Zerbe, O. Pylypenko, F. Vitali, W. Zhang, S. Rouset, M. Heck, J. W. Vrijbloed, D. Bischoff, B. Bister, R. D. Süssmuth, S. Pelzer, W. Wohlleben, J. A. Robinson, I. Schlichting, *J. Biol. Chem.* **2002**, *277*, 47476 – 47485; b) E. Stegmann, S. Pelzer, D. Bischoff, O. Puk, S. Stockert, D. Butz, K. Zerbe, J. Robinson, R. D. Süssmuth, W. Wohlleben, *J. Biotechnol.* **2006**, *124*, 640 – 653.
[35] a) R. D. Süßmuth, S. Pelzer, G. Nicholson, T. Walk, W. Wohlleben, G. Jung, *Angew. Chem.* **1999**, *111*, 2096 – 2099; *Angew. Chem. Int. Ed.* **1999**, *38*, 1976 – 1979; b) D. Bischoff, S. Pelzer, B. Bister, G. J. Nicholson, S. Stockert, M. Schirle, W. Wohlleben, G. Jung, R. D. Süssmuth, *Angew. Chem.* **2001**, *113*, 4824 – 4827; *Angew. Chem. Int. Ed.* **2001**, *40*, 4688 – 4691.
[36] a) K. Zerbe, K. Woithe, D. B. Li, F. Vitali, L. Bigler, J. A. Robinson, *Angew. Chem.* **2004**, *116*, 6877 – 6881; *Angew. Chem. Int. Ed.* **2004**, *43*, 6709 – 6713; b) K. Woithe, N. Geib, K. Zerbe, D. B. Li, M. Heck, S. Fournier-Rousset, O. Meyer, F. Vitali, N. Matoba, K. Abou-Hadeed, J. A. Robinson, *J. Am. Chem. Soc.* **2007**, *129*, 6887 – 6895.
[37] P. C. Schmartz, K. Wölfel, K. Zerbe, E. Gad, El S. El Tamany, H. K. Ibrahim, K. Abou-Hadeed, J. A. Robinson, *Angew. Chem.* **2012**, *124*, 11635 – 11639; *Angew. Chem. Int. Ed.* **2012**, *51*, 11468 – 11472.
[38] I. Kaneko, K. Kamoshida, S. Takahashi, *J. Antibiot.* **1989**, *42*, 236 – 241.
[39] a) J. Schimana, K. Gebhardt, A. Höltzel, D. G. Schmid, R. Süssmuth, J. Müller, R. Pukall, H. P. Fiedler, *J. Antibiot.* **2002**, *55*, 565 – 570; b) A. Höltzel, D. G. Schmid, G. J. Nicholson, S. Stevanovic, J. Schimana, K. Gebhardt, H. P. Fiedler, G. Jung, *J. Antibiot.* **2002**, *55*, 571 – 577; c) P. Kulanthaivel, A. J. Kreuzman, M. A. Strege, M. D. Belvo, T. A. Smitka, M. Clemens, J. R. Swartling, K. L. Minton, F. Zheng, E. L. Angleton, D. Mullen, L. N. Jungheim, V. J. Klimkowski, T. I. Nicas, R. C. Thompson, S. B. Peng, *J. Biol. Chem.* **2004**, *279*, 36250 – 36258; d) W. T. Liu, R. D. Kersten, Y. L. Yang, B. S. Moore, P. C. Dorrestein, *J. Am. Chem. Soc.* **2011**, *133*, 18010 – 18013.
[40] H. T. Chiu, B. K. Hubbard, A. N. Shah, J. Eide, R. A. Fredenburg, C. T. Walsh, C. Koshla, *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 8548 – 8553.
[41] T. M. Kamenecka, S. J. Danishefsky, *Chemistry* **2001**, *7*, 41 – 63.
[42] J. Ma, Z. Wang, H. Huang, M. Luo, D. Zuo, B. Wang, A. Sun, Y.-Q. Cheng, C. Zhang, J. Ju, *Angew. Chem.* **2011**, *123*, 7943 – 7948; *Angew. Chem. Int. Ed.* **2011**, *50*, 7797 – 7802.
[43] D. G. Fujimori, S. Hrvatin, C. S. Neumann, M. Strieker, M. A. Marahiel, C. T. Walsh, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 16498 – 16503.
[44] H. Zhang, J. Chen, H. Wang, Y. Xie, J. Ju, Y. Yan, H. Zhang, *FEBS Lett.* **2013**, *587*, 1675 – 1680.
[45] For melanin biosynthesis, see the following reviews and literature cited therein: a) P. M. Plonka, M. Grabacka, *Acta Biochim. Pol.* **2006**, *53*, 429 – 443; b) K. Langfelder, M. Streibel, B. Jahn, G. Haase, A. A. Brakhage, *Fungal Genet. Biol.* **2003**, *38*, 143 – 158.
[46] a) Y. Takano, Y. Kubo, K. Shimizu, K. Mise, T. Okuno, I. Furusawa, *Mol. Gen. Genet.* **1995**, *249*, 162 – 167; b) I. Fujii, Y. Mori, A. Watanabe, Y. Kubo, G. Tsuji, Y. Ebizuka, *Biochemistry* **2000**, *39*, 8853 – 8858.
[47] a) N. Funa, Y. Ohnishi, I. Fujii, M. Shibuya, Y. Ebizuka, S. Horinouchi, *Nature* **1999**, *400*, 897 – 899; b) M. B. Austin, M. Izumikawa, M. E. Bowman, D. W. Udwary, J.-L. Ferrer, B. S. Moore, J. P. Noel, *J. Biol. Chem.* **2004**, *279*, 45162 – 45174.
[48] N. Funa, M. Funabashi, E. Yoshimura, S. Horinouchi, *J. Biol. Chem.* **2005**, *280*, 14514 – 14523.
[49] N. Funa, M. Funabashi, Y. Ohnishi, S. Horinouchi, *J. Bacteriol.* **2005**, *187*, 8149 – 8155.
[50] B. Zhao, F. P. Guengerich, A. Bellamine, D. C. Lamb, M. Izumikawa, L. Lei, L. M. Podust, M. Sundaramoorthy, J. A. Kalaitzis, L. M. Reddy, S. L. Kelly, B. S. Moore, D. Stec, M. Voehler, J. R. Falck, T. Shimada, M. R. Waterman, *J. Biol. Chem.* **2005**, *280*, 11599 – 11607.
[51] H. Hussain, J. Hussain, A. Al-Harrasi, K. Krohn, *Tetrahedron* **2012**, *68*, 2553 – 2578.
[52] W. Hüttel, M. Müller, *ChemBioChem* **2007**, *8*, 521 – 529.
[53] C. Gil Girol, K. M. Fisch, T. Heinekamp, S. Günther, W. Hüttel, J. Piel, A. A. Brakhage, M. Müller, *Angew. Chem.* **2012**, *124*, 9926 – 9929; *Angew. Chem. Int. Ed.* **2012**, *51*, 9788 – 9791.
[54] a) C. Elsworth, M. Gill, A. Giménez, N. M. Milanovic, E. Raudies, *J. Chem. Soc. Perkin Trans. 1* **1999**, 119 – 126; b) M.

Müller, K. Lamottke, W. Steglich, S. Busemann, M. Reichert, G. Bringmann, P. Spiteller, *Eur. J. Org. Chem.* **2004**, 4850 – 4855.
[55] a) C. C. Hughes, A. Prieto-Davo, P. R. Jensen, W. Fenical, *Org. Lett.* **2008**, *10*, 629 – 631; b) C. C. Hughes, C. A. Kauffman, P. R. Jensen, W. Fenical, *J. Org. Chem.* **2010**, *75*, 3240 – 3250; c) N. M. Haste, C. C. Hughes, D. N. Tran, W. Fenical, P. R. Jensen, V. Nizet, M. E. Hensler, *Antimicrob. Agents Chemother.* **2011**, *55*, 3305 – 3312.
[56] K. Yamanaka, K. S. Ryan, T. A. M. Gulder, C. C. Hughes, B. S. Moore, *J. Am. Chem. Soc.* **2012**, *134*, 12434 – 12437.
[57] a) L. Ding, J. Münch, H. Goerls, A. Maier, H.-H. Fiebig, W.-H. Lin, C. Hertweck, *Bioorg. Med. Chem. Lett.* **2010**, *20*, 6685 – 6687; b) L. Ding, A. Maier, H.-H. Fiebig, W.-H. Lin, C. Hertweck, *Org. Biomol. Chem.* **2011**, *9*, 4029 – 4031.
[58] Q. Zhang, A. Mándi, S. Li, Y. Chen, W. Zhang, X. Tian, H. Zhang, H. Li, W. Zhang, S. Zhang, J. Ju, T. Kurtán, C. Zhang, *Eur. J. Org. Chem.* **2012**, 5256 – 5262.
[59] a) H. Li, Q. Zhang, S. Li, Y. Zhu, G. Zhang, H. Zhang, X. Tian, S. Zhang, J. Ju, C. Zhang, *J. Am. Chem. Soc.* **2012**, *134*, 8996 – 9005; b) Z. Xu, M. Baunach, L. Ding, C. Hertweck, *Angew. Chem.* **2012**, *124*, 10439 – 10443; *Angew. Chem. Int. Ed.* **2012**, *51*, 10293 – 10297.
[60] M. Baunach, L. Ding, T. Bruhn, G. Bringmann, C. Hertweck, *Angew. Chem.* **2013**, *125*, 9210 – 9213; *Angew. Chem. Int. Ed.* **2013**, *52*, 9040 – 9043.
[61] See: C. Chizzali, L. Beerhues, *Beilstein J. Org. Chem.* **2012**, *8*, 613 – 620 and references cited therein.

