Oxidative Phenol Coupling

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Synthesis, Biosynthesis, and Absolute Configuration of Vioxanthin**

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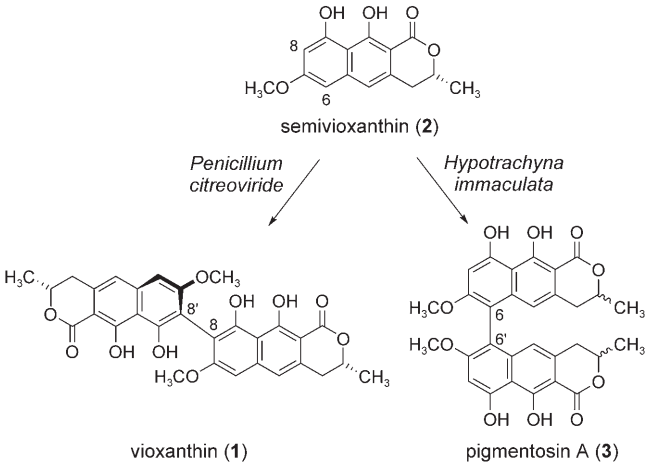
Dedicated to Professor Peter Welzel on the occassion of his 70th birthday

The biaryl axis is a structural element found in a broad variety of bioactive natural products.[1] Moreover, biaryl compounds are important substances in organic synthesis, for example, as versatile auxiliaries for enantioselective synthesis and as ligands in asymmetric catalysis. The biosynthesis of biarylic natural products proceeds through an oxidative phenol coupling, as has been proven for several biarylic secondary metabolites from plants, bacteria, and fungi.[1,2] However, the exact mechanism of the (dehydro)dimerization step through oxidative phenol coupling is still unclear.[3]

In nonenzymatic biaryl synthesis, a control of regio- and stereoselectivity comparable with that of the described biosynthetic process has not been achieved yet.[4,5] Thus, our goal is to identify biocatalysts involved in the regio- and stereoselective intermolecular oxidative phenol coupling. Herein, oxidative phenol coupling in the biosynthesis of vioxanthin is explored to understand how regio- and stereoselectivity is controlled in nature. For this purpose, the elucidation of the absolute configuration as well as the synthesis of vioxanthin (**1**) and its isomers are essential.

The biarylic dihydronaphthopyranone vioxanthin (**1**) was first isolated by Blank et al. from the pathogenic fungus *Trichophyton violaceum*.[6] Zeeck et al. isolated **1** from *Penicillium citreoviride* together with the monomeric compound (*R*)-semivioxanthin ((*R*)-**2**).[7] They determined the absolute configuration of **2** as *R* by comparison of its circular dichroism (CD) spectrum with that of (*R*)-mellein.[7] The CD spectrum of **1** shows positive chirality, thus the absolute configuration of **1** was supposed to be *P*,*R*,*R*.[8] However, this determination solely by chiroptical methods was somewhat ambiguous (see below).

Vioxanthin (**1**) is assumed to be an oxidative phenol coupling product of **2** at position 8 (Scheme 1). Recently, Elix and Wardlaw isolated the regioisomeric compound pigmentosin A (**3**) from the lichen *Hypotrachyna immaculata*, which might be biosynthesized from **2** in a similar way by dimerization at position 6.[9] The occurrence of different regio- and stereoisomeric biphenolic compounds is not unusual. For example, five of six possible regioisomeric bicoumarins have been identified in diverse *Aspergillus* species.[10] Accordingly, we assume that the nonsymmetric 6,8′ dimer of **2** will be identified as a natural product as well.



Scheme 1. Postulated regioselective biosynthetic oxidative phenol coupling of semivioxanthin (**2**) by *Penicillium citreoviride* and by *Hypotrachyna immaculata*.

Herein, we describe the biosynthesis of **1** in *Penicillium citreoviride*, ATCC 42743. The strain was cultivated in complex medium and **2**, **1**, as well as the oxidation products of **1**, xanthomegnin, rubrosulphin, and viomellein, were extracted from the mycelium.[11] Monomer **2** was also isolated from the fermentation broth. Column chromatography of the mycelium extract yielded pure **2** (39 mg/100 mL medium) and **1** (10 mg/100 mL medium).

The absolute configuration of isolated **2** was confirmed to be *R* by comparison of its CD spectrum and optical rotation with chemically synthesized (*R*)- and (*S*)-**2**.[12] The enantiomeric excess was determined as 99.0% (^{1}H NMR) upon conversion to the diastereomeric 9-*O*-mandelic acid esters and comparison with the respective esters of chemically synthesized *rac*-**2**. The biaryl axis of **1** in position 8,8′ was confirmed by 2D NMR spectroscopic experiments. The absolute configuration at the chiral axis was proved to be *P* by application of the exciton coupling method (Figure 1). The configuration of the stereogenic centers at C3 and C3′ could not be determined exclusively by spectroscopic methods and

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Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

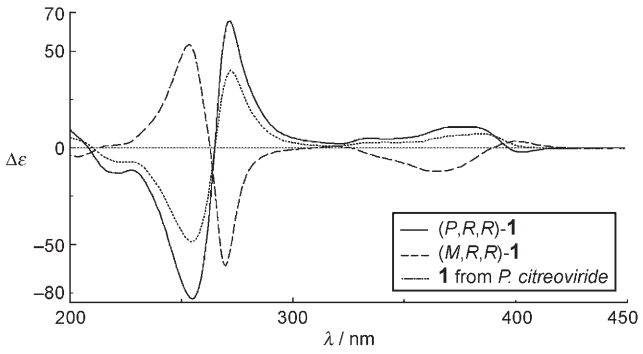
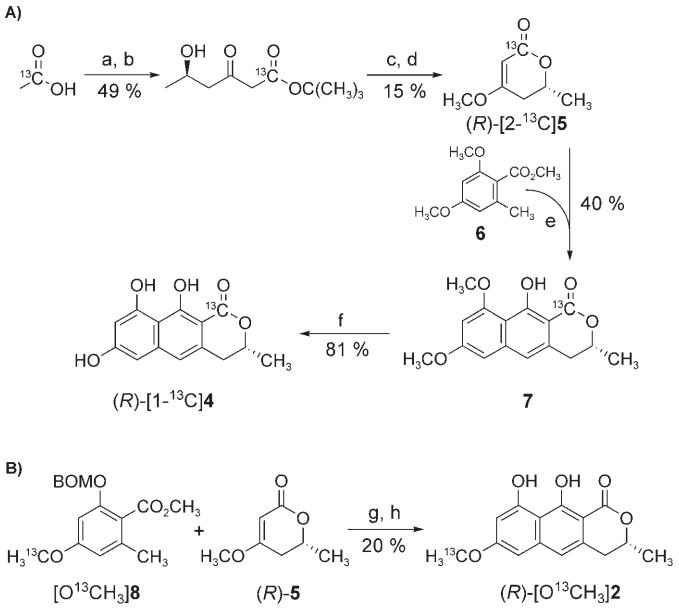


Figure 1. CD spectra (solvent: acetonitrile) of vioxanthin (**1**) isolated from *P. citreoviride*, synthetic (*P*,*R*,*R*)-**1**, and (*M*,*R*,*R*)-**1**.



Scheme 2. A) Synthesis of [1-^{13}C]labeled (*R*)-7-*O*-desmethyl semivioxanthin (**4**): a) *tert*-BuOH, DMAP, DCC, Et_2O; b) LDA, −70 °C, THF, 30 min; ethyl (*R*)-3-hydroxybutyrate, −70 °C, 3 h; AcOH/H_2O; c) TFA, CH_2Cl_2; d) $(MeO)_2SO_2$, K_2CO_3, acetone, Δ; e) LDA, methyl orsellinate **6**, −70 °C, THF, 60 min; addition to [2-^{13}C]**5**, −70 °C, 30 min, EtOH, −70 °C→RT, AcOH; f) BBr_3, CH_2Cl_2. B) Synthesis of (*R*)-[$O^{13}CH_3$]-semivioxanthin ((*R*)-[$O^{13}CH_3$]**2**): g) LDA, methyl orsellinate [$O^{13}CH_3$]**8**, −70 °C, THF, 60 min; addition to (*R*)-**5**, −70 °C, 30 min; EtOH, −70 °C→RT, AcOH; h) H_2, Pd/C, EtOAc/MeOH). Syntheses of the *S* enantiomers and racemates were performed in a similar way. BOM = benzyloxymethoxy, DCC = *N*,*N*′-dicyclohexylcarbodiimide, DMAP = 4-dimethylaminopyridine, LDA = lithium diisopropylamide, TFA = trifluoroacetic acid.

as such, a combination of synthesis and feeding experiments proved helpful (see below).

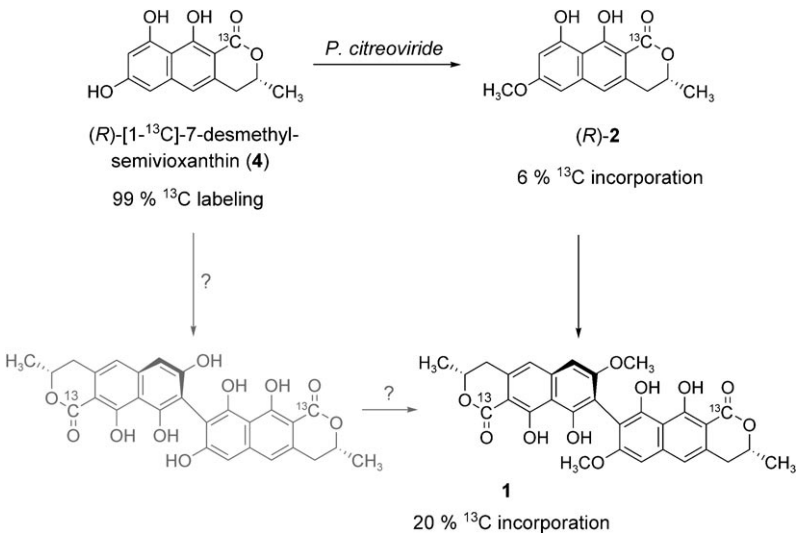
The putative biosynthesis of **1** through oxidative phenol coupling of a monomeric compound should be proved by feeding of a ^{13}C-labeled monomeric substrate to *P. citreoviride*. Besides **2**, 7-*O*-desmethyl semivioxanthin (**4**) might also be a biogenetic precursor of **1**, although it has not been identified as a metabolite of *P. citreoviride*.[13] For most polyketide natural products, the carbon skeleton is built up prior to further modification of functional groups by tailoring reactions, like *O*-methylation.[14] In the biosynthesis of the dimeric coumarin kotanin in *Aspergillus niger*, the oxidative phenol coupling takes place on a monomeric compound with an unprotected phenolic group to give the dimeric product (orlandin), which is then methylated to yield kotanin.[10] Moreover, if a permethylated phenylether is fed to *A. niger*, which produces kotanin, one methyl ether is cleaved selectively prior to dimerization.

For feeding experiments, 7-*O*-desmethyl **4** was used as the ^{13}C-labeled substrate. It was synthesized according to a method for (*R*)- and (*S*)-**2**[12] through a tandem Michael–Dieckmann condensation of the Michael acceptor (*R*)-[2-^{13}C]**5** and methyl orsellinate **6**, followed by complete demethylation of intermediate **7** (Scheme 2 A). The ^{13}C label was introduced by the use of [1-^{13}C]acetic acid as a precursor of the Michael acceptor **5**. (*R*)- and (*S*)-[1-^{13}C]**4** were synthesized through this sequence. Methoxy-^{13}C-labeled (*R*)-[$O^{13}CH_3$]**2** was obtained by a tandem Michael–Dieckmann condensation of the Michael acceptor (*R*)-**5** and methyl 2-benzyloxymethoxy-4-[$O^{13}CH_3$]-methoxy-6-methyl-benzoate ([$O^{13}CH_3$]**8**; Scheme 2 B).

In the feeding experiments, the monomeric precursors (15 mg) were adsorbed on Amberlite XAD-7 resin (150 mg) prior to addition to the culture (100 mL, complex medium). The resin was used owing to the low solubility of the substrates in aqueous media. Additionally, we observed increased production of **1** in cultures supplemented with resin. Incubation experiments with (*R*)-[1-^{13}C]**4**, after extraction and column chromatography, yielded 19 mg of **2** and 11 mg of **1**. Both metabolites showed incorporation of the ^{13}C-labeled substrate as determined by ^{13}C NMR spectroscopy and MS analysis. The incorporation rate deduced by MS analysis was 6.0 % for **2** and 20.2 % for **1** (Scheme 3). An analogous feeding experiment with (*R*)-[$O^{13}CH_3$]**2** revealed that homochiral (*R*)-**2** is also a substrate for oxidative phenol coupling in *P. citreoviride*. The incorporation rate measured by MS was 16.6 % for **2**, 14.2 % for single-labeled **1**, and additionally 1.2 % for double-labeled **1**. These results clearly demonstrate that **4** is methylated to **2**, which is then dimerized to **1**. No evidence for dimerization of **4** could be found by MS or NMR spectroscopic analysis.

Further feeding experiments with the ^{13}C-labeled *S* enantiomer and the racemic form of 7-*O*-desmethyl semivioxanthin (*S*-[1-^{13}C]**4** and *rac*-[1-^{13}C]**4**, respectively) revealed that these substrates are also incorporated into metabolites **1** and **2**.[15] In comparison with naturally occurring **1**, the product isolated from these experiments consisted of several diastereomers of **1**. The incorporation rate measured by MS analysis was 80.3 % for unlabeled **1**, 19.1 % for single-labeled **1**, and 0.6 % for double-labeled **1**. These diastereomers resulted in ^{13}C NMR signals of the carbonyl groups at $\delta = 171.61$ ppm and $\delta = 171.57$ ppm. The signal at $\delta = 171.57$ ppm is identical to the carbonyl signal of natural vioxanthin (**1**).

Combining the results of the feeding experiments and the CD and NMR spectroscopic analysis, the absolute configuration of **1** was elucidated as follows: 1) The absolute configuration of **2** isolated from *Penicillium citreoviride* is *R* (*ee* 99.0 %) and was determined by optical rotation, CD, and chemical synthesis of (*R*)- and (*S*)-**2**. 2) The configuration of

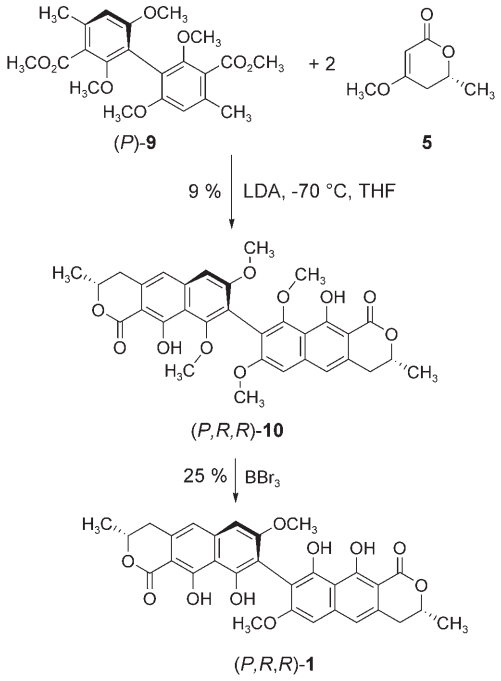


Scheme 3. Feeding experiments with (*R*)-[1-^{13}C]**4** in *P. citreoviride*.

the stereogenic centers C3 and C3′ in **1** is not presupposed to be *R* as (*S*)-**2** might be incorporated into **1** by *P. citreoviride*, resulting in monomeric unreacted (*R*)-**2** being enriched in the mycelium. Thus, only the configuration of the axis could be determined with certainty to be *P* by CD (exciton-coupling method).[16] 3) For the assignment of the absolute configuration at stereogenic centers C3 and C3′ in **1**, feeding experiments were essential. Feeding of (*S*)-[1-^{13}C]**4** yields **1** whose ^{13}C NMR spectra showed two discrete carbonyl signals for the diastereomers *P*,*R*,*R*, *P*,*R*,*S*, and *P*,*S*,*S*. After feeding of (*R*)-[1-^{13}C]**4**, the ^{13}C NMR spectrum of isolated **1** showed only one enriched signal for the carbonyl C, which resonates at exactly the same frequency as was seen in **1** isolated from *P. citreoviride*. Consequently, **1** isolated from *P. citreoviride* has the *P*,*R*,*R* configuration.

To verify these results, we prepared (*M*,*R*,*R*)- and (*P*,*R*,*R*)-**1** by using a similar strategy as for the synthesis of monomeric **2**. In a tandem Michael–Dieckmann reaction, the dimeric orsellinate **9** was condensed twice with the Michael acceptor **5**, followed by selective demethylation of intermediate **10** to yield **1** (Scheme 4). Compound **9** was synthesized as a pure atropisomer in six steps according to the literature.[17,18] The tandem Michael–Dieckmann condensation was optimized with monomeric and dimeric racemic orsellinates. We observed that sterically less-demanding protecting groups for the C2 hydroxy group of the orsellinate resulted in higher yields of condensation products. Thus, the methyl ether was chosen as a protecting group. For the tandem Michael–Dieckmann condensation, the dianion of (*M*)- and (*P*)-**9**, generated with 3.5 equivalents of LDA, was reacted with an excess of the Michael acceptor (*R*)-**5**. Addition of ethanol at −70 °C and rapidly warming the mixture to room temperature afforded **10** in 9 % yield.[19] The intermediate **10** had to be deprotected regioselectively at the C9/C9′ methoxy groups, whereas the methoxy groups at C7/C7′ has to be retained. This selective deprotection was feasible by using one equivalent of BBr_3 in dichloromethane.[20] The selectivity of this demethylation is probably due to coordination with the C10/C10′ hydroxy group.[21] A similar selectivity is seen with chelation by an adjacent carbonyl group.[22] (*P*,*R*,*R*)-**1** was synthesized in nine steps from methyl 4-*O*-methyl-orsellinate and ethyl (*R*)-3-hydroxybutyrate. This represents the first enantioselective synthesis of a natural dimeric dihydronaphthopyranone.

The ^{1}H NMR spectra of the (*P*,*R*,*R*)- and (*M*,*R*,*R*)-**1** thus obtained are identical. The ^{13}C NMR spectra (100 MHz, $CDCl_3$) of (*M*,*R*,*R*)-**1** and (*P*,*R*,*R*)-**1** exhibit very small differences in the chemical shift of C1 (carbonyl group) only.[23] However, identical parameters are essential for comparison of two different samples. Small changes, for example, in temperature, cause signal shifts that prevent comparison. The CD spectra of the two diastereomers are in accordance with the known configuration of the axis in the starting material **9** (determined by X-ray crystal structure analysis). As (*M*,*R*,*R*)-**1** and (*P*,*R*,*R*)-**1** show almost mirror-image CD spectra for the entire UV range, it is not possible to determine the absolute configuration of the stereogenic centers at C3 and C3′ merely from the CD spectra (Figure 1).[24]



Scheme 4. Synthesis of (*P*,*R*,*R*)-vioxanthin ((*P*,*R*,*R*)-**1**).

Thus, the results of the feeding experiments and spectroscopic analysis of the synthesized compounds confirm the conclusions for the absolute configuration of natural **1**. The CD spectra and the ^{13}C NMR shift for C1 of isolated **1** are superimposeable to that of synthesized (*P*,*R*,*R*)-**1**. Vioxanthin (**1**) obtained from a feeding experiment with (*R*)-**4** also shows the same ^{13}C NMR spectroscopic carbonyl shift.

In summary, we could show that vioxanthin (**1**) is biosynthesized through a regio- and stereoselective (dehydro)dimerization of monomeric semivioxanthin (**2**). This highly interesting selectivity is unprecedented in nonenzymatic chemical transformations. Our results set the stage for further studies to identify the biocatalysts responsible for the regio- and stereoselective oxidative phenol coupling.

Experimental Section

(*P*,*R*,*R*)-Vioxanthin ((*P*,*R*,*R*)-**1**): ^{1}H NMR: (400 MHz, $CDCl_3$, 24 °C): $\delta = 1.55$ (d, $^3J_{HH} = 6.2$ Hz, 6H, 2 × CH_3), 3.01 (m, 4H, 2 × CH_2), 3.84 (s, 6H, 2 × OCH_3), 4.76 (m, 2H, 2 × CH), 6.70 (s, 2H, 2 × 6-H_{ar}), 6.95 (s, 2H, 2 × 5-H_{ar}), 9.69 (d, $J_{HH} = 1.0$ Hz, 2H, 2 × 9-OH), 13.79 (d, $J_{HH} = 1.0$ Hz, 2H, 2 × 10-OH). ^{13}C NMR: (100 MHz, $CDCl_3$, 25 °C): $\delta = 20.7$ (CH_3), 34.7 (CH_2), 56.0 (OCH_3), 76.5 (CH), 98.1 (C6), 99.3 (C10a), 108.1 (C8), 108.5 (C9a), 116.1 (C5), 132.8 (C4a), 140.0 (C5a), 155.4 (C9), 161.4 (C7), 162.8 (C10), 171.57 ppm (C=O, C1). CD: (acetonitrile): λ [nm] (Mol. CD) = 402 (−2.16), 374 (10.94), 322 (2.26), 272 (65.74), 255 (−78.20), 228 (−11.39), 221 (−13.12), 192 (15.55).

(*M*,*R*,*R*)-Vioxanthin ((*M*,*R*,*R*)-**1**): ^{1}H NMR: (400 MHz, $CDCl_3$, 25 °C), $\delta = 1.55$ (d, $^3J_{HH} = 6.2$ Hz, 6H, 2 × CH_3), 3.01 (m, 4H, 2 × CH_2), 3.84 (s, 6H, 2 × OCH_3), 4.76 (m, 2H, 2 × CH), 6.70 (s, 2H, 2 × 6-H_{ar}), 6.95 (s, 2H, 2 × 5-H_{ar}), 9.69 (s, 2H, 2 × 9-OH), 13.79 ppm (s, 2H, 2 × 10-OH). ^{13}C NMR: (100 MHz, $CDCl_3$, 26 °C): $\delta = 20.7$ (CH_3), 34.7 (CH_2), 56.0 (OCH_3), 76.5 (CH), 98.1 (C6), 99.3 (C10a), 108.1 (C8), 108.5 (C9a), 116.1 (C5), 132.8 (C4a), 140.0 (C5a), 155.4 (C9), 161.4 (C7), 162.8 (C10), 171.61 ppm (C=O, C1). CD: (acetonitrile): λ [nm] (Mol. CD) = 400 (3.48), 363 (−12.09), 320 (0.85), 270 (−61.01), 253 (53.30).

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[23] All other ^{13}C NMR signals were not distinguishable for the two diastereomers. A mixture of synthesized (*P*,*R*,*R*)-**1**, (*P*,*S*,*S*)-**1**, and (*P*,*R*/*S*,*R*/*S*)-**1** showed similar ^{13}C NMR spectra with two carbonyl signals at $\delta = 171.61$ ppm and $\delta = 171.57$ ppm.
[24] Although the correct *P*,*R*,*R* configuration for natural vioxanthin (**1**) from *P. citreoviride* has been proposed by Zeeck et al. (reference [7]), their approach to confirm this is not valid: The CD spectra of **1** in the range of 300–450 nm is not predominantly determined by the chirality of the stereogenic centers, but rather by the axial chirality.

