JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

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Tautomers of Anthrahydroquinones: Enzymatic Reduction and Implications for Chrysophanol, Monodictyphenone, and Related Xanthone Biosyntheses

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Supporting Information

ABSTRACT: Reduction of emodin by sodium dithionite resulted in the formation of two tautomeric forms of emodin hydroquinone. Subsequent conversion by the short-chain dehydrogenase/reductase (SDR) MdpC into the corresponding 3-hydroxy-3,4-dihydroanthracen-1(2*H*)-one implies that deoxygenation is the first step in monodictyphenone biosynthesis. Implications for chrysophanol formation as well as reaction sequences in the related xanthone, ergochrome, and bianthraquinone biosyntheses are discussed.

Xanthones represent a structurally diverse class of natural products and are common among plants, bacteria, and fungi (fungal genera, e.g., *Aspergillus*, *Helminthosporium*, *Penicillium*, and *Pyrenochaeta*).[1] Fungal xanthone rearrangement of emodin (**1**) via a benzophenone intermediate, such as monodictyphenone (**2**)[2] or any derivative thereof, is believed to represent a crucial step in the biosyntheses of ravenelin (**3**),[3] shamixanthone (**4**),[4] tajixanthone (**5**),[3c,4] and ergochromes (e.g., **6**).[3c,5] By analogy to sterigmatocystin formation in aflatoxin biosynthesis,[3c] the formation of **2** has been proposed to proceed by a complex sequence of epoxidation, rearrangement, deoxygenation, Baeyer–Villiger (BV) oxidation, and another deoxygenation.[2c] In previous studies, deoxygenation of **1** to chrysophanol (**7**) has been observed in cell-free and partially purified preparations of *Pyrenochaeta terrestris*.[6] Furthermore, it has been demonstrated that **7** is incorporated into **4**–**6** (Figure 1),[4c,d,5d] suggesting that deoxygenation is the first step in biosynthesis. However, contrary to these reports, it has been assumed that **7** is not an intermediate on the paths to **2** and **4**.[2c,4e] Recently, this issue has been revisited to provide a full rationale of the biosynthetic pathway.[7]

Gene deletion studies have identified five gene products (MdpB, -C, -J, -K, and -L) as essential for downstream processing of **1** to **2** (Figure 1).[2c] For the formation of **4**, the involvement of one further monooxygenase (MdpD), as well as of two prenyl transferases (XptA and -B), and one oxidoreductase (XtpC) distant from the gene cluster has been described.[4e]

MdpB is related to scytalone dehydratase (SD) (55% amino acid similarity)[8a] from *Magnaporthe grisea*, while MdpC shows high similarity to tetrahydroxynaphthalene reductase (T_4HNR) (64%)[8b] and trihydroxynaphthalene reductase (T_3HNR) (70%)[8c] from the same strain. These enzymes catalyze double deoxygenation by a reduction (T_4HNR, T_3HNR) and dehydration (SD) sequence in 1,8-dihydroxynaphthalene (DHN) melanin biosynthesis.[8] Thus, the striking parallels between these enzymes suggested a DHN melanin-like pathway.

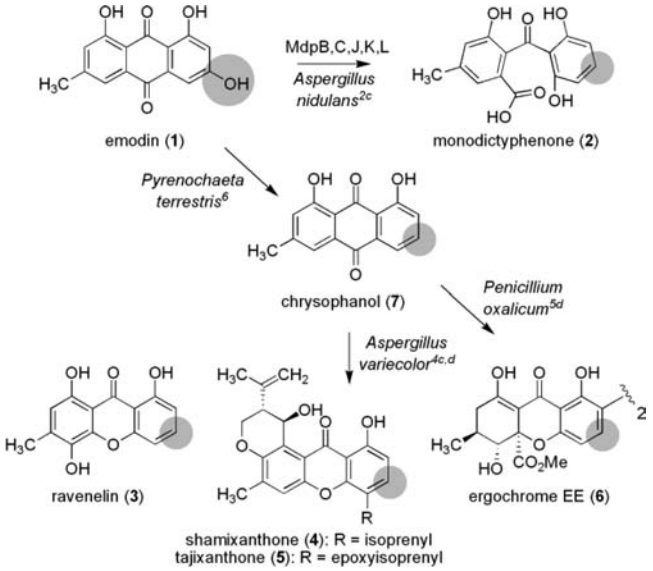


Figure 1. Formations of monodictyphenone (**2**) and related xanthones **3**–**6** proceed by a deoxygenation reaction and BV oxidation.[2−5,7] Chrysophanol (**7**) has been shown to be a precursor of **4**–**6**.[4c,d,5d,7] Each deoxygenation site is highlighted by a circle.

From a chemical point of view, anthrahydroquinones exhibit a pronounced keto–enol tautomerism,[9] a putative requirement for enzymatic reduction, as described for DHN melanin biosynthesis.[10] Because the cell is in a highly reduced state, which prevents redox cycling of potentially toxic (anthra)-quinones,[11] the hydroquinones and their tautomers might also represent the more prevalent forms in vivo. Hence, we tested MdpC for its capability to reduce emodin hydroquinone (**8**).

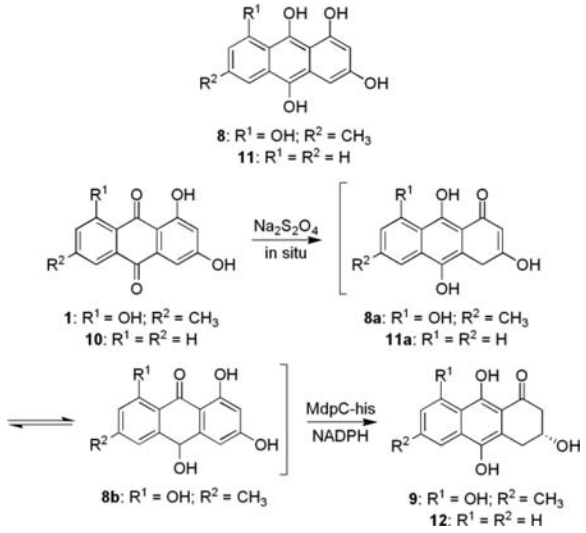
The reduction of **1** by sodium dithionite to generate hydroquinone **8** was investigated first. HPLC–MS analysis revealed the formation of two products, both of which showed a molecular mass corresponding to **8**. Upon exposure to air, these compounds were completely converted back into **1**. Detailed studies of 1- and 2-hydroxyanthrahydroquinones have shown a preferential tautomerism to the oxanthrones.[9] In our

Received: July 21, 2012
Published: August 21, 2012

   dx.doi.org/10.1021/ja307151x | *J. Am. Chem. Soc.* 2012, 134, 14742–14745

case, extraction into acetonitrile-d_3 and NMR analysis revealed the formation of tautomer **8a**[12] along with oxanthrone **8b**, while hydroquinone **8** itself was not detected (Scheme 1).

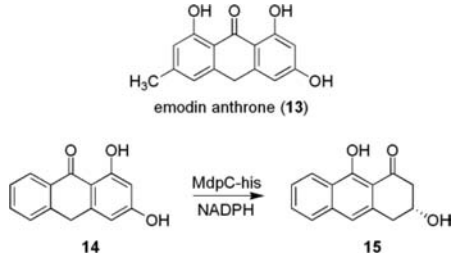
Scheme 1. Tautomers of Anthrahydroquinones and Enzyme-Catalyzed Reduction



As a next step, codon-optimized N-terminally His-tagged *mdpC* was cloned into pET19b, overexpressed, and purified by Ni-NTA affinity chromatography (see the Supporting Information). In situ reduction of **1** with sodium dithionite and subsequent conversion with MdpC-his, using glucose dehydrogenase/D-glucose as an NADPH cofactor regeneration system, gave a 58% yield of 3-hydroxy-3,4-dihydroanthracen-1(2*H*)-one **9** (Scheme 1). The absolute configuration was determined to be *R* using CD spectroscopy. Incubation of **1** with MdpC-his without the addition of sodium dithionite resulted in no conversion. In contrast, Anderson and co-workers have proposed the direct enzymatic reduction of **1** without any involvement of the hydroquinone or its tautomers.[6] As an alternative substrate, 1,3-dihydroxyanthraquinone (**10**) was reduced by sodium dithionite to a single product, which was assigned by NMR analysis as **11a**,[12] a tautomer of hydroquinone **11**. Subsequent conversion by MdpC-his gave **12** in 22% yield.[13]

Anthrones are in tautomeric equilibrium with their aromatic anthrols and thus exhibit the putative requirement for enzymatic reduction.[14] Hence, we tested emodin anthrone (**13**) and 1,3-dihydroxyanthrone (**14**) as substrates for MdpC-his (Scheme 2). Although in both cases the hydroxyanthracene was not detected by NMR analysis in acetone-d_6, we were able

Scheme 2. Enzymatic Reduction of Anthrone 14



to transform **14** into (*R*)-3,9-dihydroxy-3,4-dihydroanthracen-1(2*H*)-one (**15**) in 7% yield. **13** showed no conversion.

In addition to the observed overall transformation of **1**, deoxygenation as an initial step in the biosyntheses of **2** and related xanthones is supported by several previously reported observations: (i) Deletion of *mdpC* led to a strong increase in **1** and oxidized derivatives (e.g., ω-hydroxyemodin), whereas the deoxygenated products (**2**, **7** and xanthones) were absent. (ii) Deletion of *mdpL* (a putative BV monooxygenase) led to accumulation of both **1** and the deoxygenated products **7** and aloe-emodin. Moreover, **2** and related xanthones (e.g., **4**) were absent.[2c,4e] Altogether, this argues that deoxygenation precedes BV oxidation.

Accordingly, we propose the following reaction sequence for the biosyntheses of chrysophanol (**7**), monodictyphenone (**2**) and related xanthones (Figure 2): Tailoring of the putative

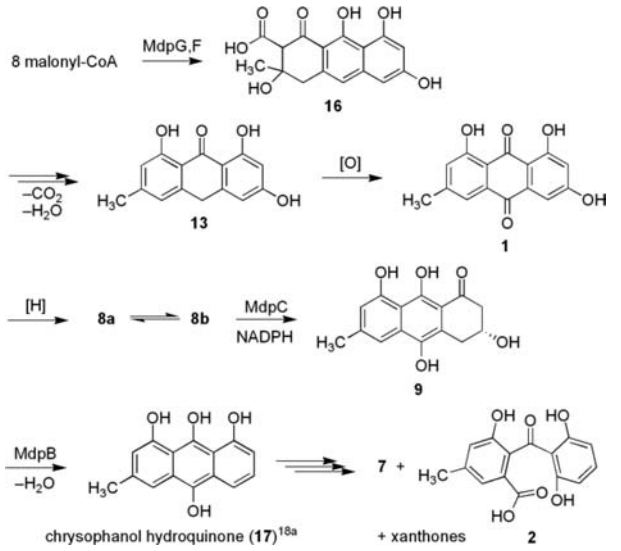


Figure 2. Proposed biosynthetic pathway of **2** in *Aspergillus nidulans* FGSC A4. Tailoring of PKS-derived **16** to give **8a**/**8b** and conversion by MdpC into **9** putatively represent the initial steps in the biosyntheses of **7**, **2**, and related xanthones.

polyketide synthase (PKS) product atrochrysone carboxylic acid (**16**) yields emodin anthrone (**13**) and emodin (**1**).[2c,15] Reduction of **1** leads to **8a**/**8b**, the tautomeric forms of emodin hydroquinone (**8**). The enzymes responsible for the tailoring steps, with the exception of an emodin anthrone oxygenase,[16] have not yet been characterized. The oxidation of **13** might also lead directly to **8a**/**8b**. As in melanin biosynthesis,[10,17] MdpC-catalyzed addition of a hydride to any tautomeric form of **8** yields 3-hydroxy-3,4-dihydroanthracen-1(2*H*)-one **9**. Dehydration of **9** by MdpB to chrysophanol hydroquinone (**17**) and oxidation leads to chrysophanol (**7**).[18] The further biosynthetic course remains unknown. In analogy to aflatoxin biosynthesis, epoxidation of **7** before BV oxidation has been proposed in the formation of **3**, **5**, and **6**;[3c] however, this mechanism cannot explain the rearrangement of **7** to **2**. The same is valid for arugosin F, the aldehyde derivative of **2** and probable intermediate in the biosynthesis of **4** and **5**. Here, **2** seems to represent a shunt metabolite.[4d,7]

A substructure similar to that of **9**, albeit in a different oxidation state, can also be found in dihydrocatenarin B (**18**), isolated from *Penicillium islandicum* (Figure 3). The rather unstable **18** easily decomposes to islandicin (**19**),[19] which in

dx.doi.org/10.1021/ja307151x | J. Am. Chem. Soc. 2012, 134, 14742–14745

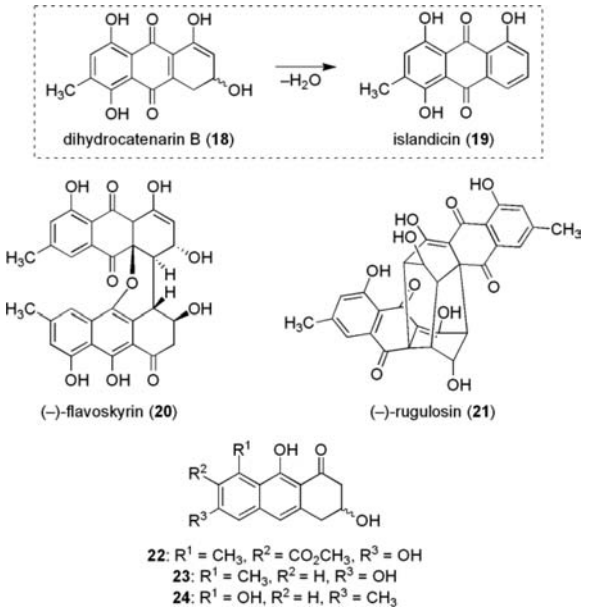


Figure 3. Natural products derived from a 3,9-dihydroxy-3,4-dihydroanthracen-1(2*H*)-one substructure.[19,20,22] Dashed box: dehydration of **18** leading to islandicin (**19**).

turn has been shown to represent a precursor for the formation of ergochrome EE (**6**), albeit with a lower incorporation rate than for **7**.[5d] Although the timing of A-ring hydroxylation is uncertain, the involvement of homologous enzymes in the biosynthesis of **6** is suggested.

The substructure mentioned can also be found as a monomeric unit of bianthraquinones, such as (−)-flavoskyrin (**20**) and (−)-rugulosin (**21**), which suggests the same biosynthetic course.[20] The biomimetic synthesis of bianthraquinones from similar monomeric units has been described.[21] Several natural products also exhibit an anthrone-derived substructure, such as aloesaponol I (**22**), aloesaponol II (**23**), and germichrysone (**24**).[22]

In summary, we have shown that MdpC from the putative monodictyphenone gene cluster converts the tautomers of emodin hydroquinone (**8**) into the 3-hydroxy-3,4-dihydroanthracen-1(2*H*)-one derivative **9**. The occurrence of this transformation instead of reduction of the quinone illustrates the importance of considering the reduced state of the cell in biosynthesis. Although strong hints imply a reaction sequence starting with an initial reduction, **9** and chrysophanol (**7**) or the respective hydroquinone/oxanthrone remain to be confirmed as the precursors for subsequent transformations. Homologous enzymes showing high similarity to MdpC, such as AflM of the aflatoxin gene cluster (77% similarity),[23] might be involved in analogous transformations. Accordingly, the identification of the function of MdpC will broaden the scope of catalytic asymmetric dearomatization reactions.[17,24]

■ ASSOCIATED CONTENT

Supporting Information

Experimental details and characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This paper is dedicated to Prof. Dr. G. Bringmann on the occasion of his 60th birthday. Financial support of this work by the Deutsche Forschungsgemeinschaft (IRTG 1038) is gratefully acknowledged. We thank O. Fuchs for skillful technical support, V. Brecht for measurement of NMR spectra, and Prof. Dr. G. Fuchs and Prof. Dr. H. G. Floss for helpful discussions.

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(12) In analogy to DHN melanin biosynthesis,[10b] we assume the formation of the 1-keto tautomers **8a** and **11a** instead of the corresponding 3-keto tautomers. This is due to the possibility of stabilization by hydrogen bonding (also see the Supporting Information).
(13) Deoxygenation of **10** has been observed with a partially purified extract of *P. terrestris*. See: Ichinose, K.; Kiyono, J.; Ebizuka, Y.; Sankawa, U. *Chem. Pharm. Bull.* **1993**, *41*, 2015.
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(18) (a) For **17**, keto−enol tautomerism to the oxanthrone is proposed. (b) Alternatively, oxidation of **9** might occur prior to dehydration to **7**.
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■ NOTE ADDED AFTER ASAP PUBLICATION

This paper was published ASAP on August 28, 2012. The table of contents graphic has been updated. The revised version was posted on August 30, 2012.