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Biosynthesis of Psoralen: Mechanism of a Cytochrome P450 Catalyzed Oxidative Bond Cleavage

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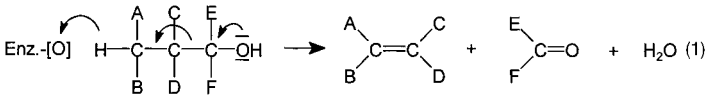
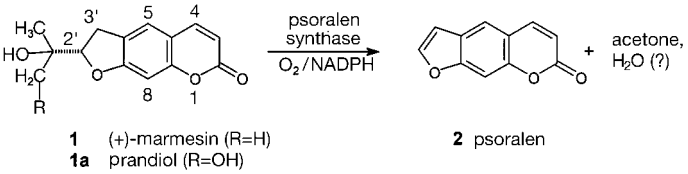
The oxidative cleavage of C–C bonds that leads to olefins and a carbonyl fragment is a common transformation in secondary metabolism and is generally catalyzed by enzymes requiring molecular oxygen as a cofactor. Typical examples are given by the formation of homo- and norterpenes from isoprenoid precursors or by the biosynthesis of 1-alkenes from fatty acids. In general, the resulting unsaturated products are metabolites of precursors that already carry an oxygen atom [Eq. (1); Enz. = enzyme].[1] Such transformations have been



well studied for steroids, where angular methyl groups are oxidatively removed with concomitant introduction of a double bond into the carbon skeleton.[2] The reactions are catalyzed by enzymes belonging to the large family of cytochrome P450. Specific enzymes endowed with multiple catalytic activities catalyze all required transformations, namely, 1) the functionalization of the unactivated methyl group to an alcohol, 2) the subsequent oxidation to an aldehyde, and 3) the actual oxidative bond cleavage generating the unsaturated nor-steroid and formic acid.[3–6]

It is as yet unknown, whether or not this sequential degradation is representative for other oxidative bond cleavage reactions that lead to unsaturated natural products. For example, the dealkylation of (+)-marmesin (**1**) to the phototoxic furocoumarin psoralen (**2**) is achieved by the psoralen synthase (Scheme 1), an enzyme that also belongs to



1 (+)-marmesin (R=H)
1a prandiol (R=OH)
2 psoralen

Scheme 1. Cytochrome P450 catalyzed dealkylation of **1**.

the large family of cytochrome P450 catalysts.[7] This dealkylation is of central importance for the biosynthesis of all other linear furocoumarins, since the resulting psoralen (**2**) serves as an intermediate en route to the other members of this family.[8] Linear and angular furocoumarins are widespread in the plant kingdom, especially in the order of the Apiaceae, where they are produced in response to insect injury and infection.[9] The existence of a natural product such as the diol **1a** (prandiol)[10, 11] could indeed suggest a stepwise degradation of marmesin to psoralen that is analogous to the dealkylation of steroids. Here we disclose that the dealkylation of marmesin indeed does not involve a sequential oxidative degradation of the 2-hydroxy-2-propyl substituent, but proceeds in a single step to yield equimolar amounts of psoralen and acetone. The previously postulated, but now for the first time proven, one-step degradation[12] may be representative for other oxidative transformations that lead to unsaturated nor- and seco-compounds in nature.

Unambiguous evidence for the one-step dealkylation and details of the stereochemical course of the reaction follow from transformations of the specifically deuterated precursors **3** – **7**[13] by microsomes from elicited cell cultures of *Ammi majus* and subsequent analysis of the resulting products.[14] Thus, if the microsomes are treated with [$^2H_7$]marmesin (**7**) in the presence of NADPH and oxygen,[7] equimolar amounts of [8-$^2H_1$]psoralen (**8**) and [$^2H_6$]acetone are formed (Scheme 2). Both products can be identified and quantified by GC-MS. Product **8** is readily distinguished from traces of natural [8-^{1}H]psoralen because of a remaining deuterium label at C8. About 20 – 40 % of the resulting psoralen are further oxidized to bergaptol (5-hydroxypsoralen).[7] [$^2H_6$]Acetone is unambiguously identified and quantified after derivatization to the corresponding imine with pentafluorobenzylhydroxylamine.[15] With respect to the conservation of all six hydrogen isotopes in the C_3 fragment, an intermediate functionalization of the 2-hydroxy-2-propyl substituent is excluded (see **1a**).

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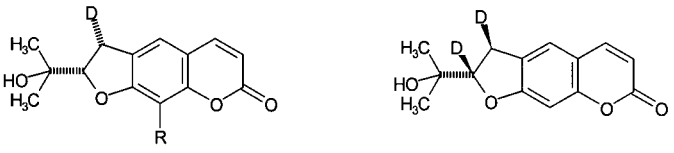
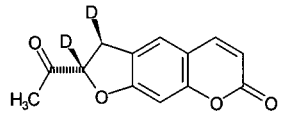


3a R = H
3b R = D

4



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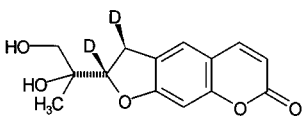
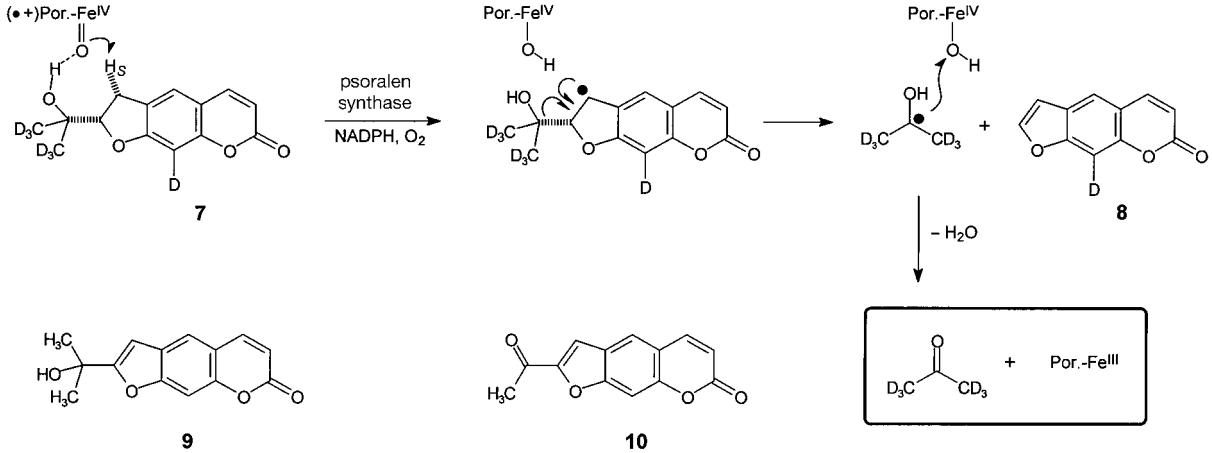


6

presence of iodosylbenzene the metal–porphyrinato complexes successfully convert the labeled marmesins **3a**, **3b**, **4**, and **7** into deuterated psoralens and acetone (Scheme 2). As expected, the model reactions do not show the exclusive *syn* selectivity observed in the enzymatic reaction. On the other hand, the preference for the less hindered *anti* hydrogen atom is also not very pronounced (*anti*:*syn* = 1.6:1).

Unlike for the enzymatic reaction, the metal–oxo complex is not restricted to an attack at benzylic hydrogen atoms at C3′ and may also attack the tertiary hydrogen atom at C2′. The resulting primary radicals are stabilized by resonance with the aromatic system or the neighboring oxygen atom. Following an initial abstraction of a hydrogen atom from C2′, β-cleavage



7 **8**

9 **10**

Scheme 2. Psoralen synthase catalyzed dealkylation of **7**, mechanism, and products **9** and **10** from the model reactions with tetraphenylporphyrinato complexes of Fe^{III} or Mn^{III}. Por. = porphyrinato.

The stereochemical course of the oxidative bond cleavage was determined from the transformations of the metabolic probes **3a**/**3b** and **4**, which are labeled by deuterium atoms in *syn* or *anti* orientations relative to the 2-hydroxy-2-propyl substituent.[13] In both cases an exclusive abstraction of the hydrogen isotope *syn* to the C_3 substituent is observed. While the transformation of **3a** proceeds with loss of the deuterium atom from C3′ to yield unlabeled psoralen, **4** is analogously converted into a product with retention of the deuterium atoms at C3′ and C8. Following administration of equimolar amounts of the precursor pairs **3a** and **4** or **3b** and **1**, the substrates **1** and **4** possessing a hydrogen atom in *syn* orientation relative to the C_3 substituent are preferably transformed. From the intensity distribution of the molecular ions of the isotopomeric psoralens, a kinetic isotope effect of $k_H/k_D \approx 4$ ($T = 20\,^{\circ}C$) can be calculated.[16]

Concerning the substrate tolerance of the psoralen synthase, it is interesting to note that also the ketone **5** is deacylated by the microsomal enzyme to give [2′,3′-$^2H_2$]psoralen. In contrast, the diol **6** ([$^2H_2$]prandiol)[10] is not transformed. Since the stereochemistry of the deacylation of **5** is in line with the findings for the dealkylation of marmesin, both reactions are probably catalyzed by the same enzyme.

The enzymatic dealkylation of marmesin can be reproduced in a biomimetic fashion by tetraphenylporphyrinato complexes of Fe^{III}[17] or Mn^{III},[18] both of which can act as chemical models for cytochrome P450 enzymes. In the will yield exclusively didehydromarmesin (**9**), while a primary radical formed on C3′ can yield **2** and **9**. Accordingly, the oxidative degradation of marmesin by the active porphyrinato complexes furnishes the products **2** and **9** in approximately equal amounts. The oxidation of the ketone **5** yields acetylpsoralen (**10**) together with a very small amount of **2**, reflecting the pronounced resonance stabilization of the initial radical at C2′ of **5**.[19]

In agreement with the findings from the enzymatic reactions and the model reactions, the dealkylation of marmesin is assumed to proceed as outlined in Scheme 2. First, a reactive porphyryl-Fe^{IV}-O radical cation attacks C3′-H_S of the substrate **7**, leading to a benzylic radical that decays by β-cleavage into [8-^{2}H]psoralen (**8**) and a 2-hydroxy-2-propyl radical. Following rapid "oxygen rebound"[20] to the neighboring porphyryl-Fe^{IV}-OH center, formally the hydrate of [$^2H_6$]acetone is produced. By analogy, the ketone **5** may be deacylated to yield psoralen and acetic acid via an intermediate acyl radical. The above results disprove mechanistic alternatives assuming hydroxylation and subsequent formation of a carbenium ion at C3′, as proposed by Birch et al.[21] Hydroxylated intermediates were observed neither during the enzymatic transformations nor in the course of the model reactions. Furthermore, the heterolytic fragmentation of a hypothetical 3′-hydroxymarmesin should proceed as an *anti* elimination. Instead, the almost exclusive *syn* elimination of an alkoxy or acyl residue together with a vicinal hydrogen

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atom[1, 4] is in good agreement with the radical abstraction and recombination process outlined in Scheme 2.[20] In the course of the disproportionation of the benzylic radical (β-cleavage) into the olefin, the ensuing alkyloxy or acyloxy radical will immediately recombine with the neighboring porphyryl-Fe^{IV}-OH center to avoid formation and escape of free radicals.

The oxidative bond cleavage reaction described here may also be representative for ring-cleavage reactions in the biosynthesis of seco compounds that are, as yet, not systematically addressed on mechanistic grounds. For example, the transformation of loganin into secologanin, a central step in the biosynthesis of indole and chinchona alkaloids, apparently proceeds without intermediates and is catalyzed by a cytochrome P450.[22] Comparable reactions take place in the oxidative cleavage of ring A of the triterpenoid β-amyrin to provide nyctanthic acid[23] or the oxidative degradation of acyclic geranylacetone to 4,8-dimethyl-1,3,7-nonatriene. The latter transformation is exceptionally widespread in higher plants in response to herbivory.[24] The recent finding that the acyclic geranylacetone is degraded by *syn* elimination[24, 25] strongly supports the significance of this unique stereochemical feature of cytochrome P450 catalyzed oxidative bond cleavage reactions.

Experimental Section

Enzymatic conversions were performed with microsomes from elicited cell cultures of *Ammi majus*.[14] The labeled marmesin derivatives[13] (40.0 nmol) were dissolved in Tris/HCl buffer (25.0 μL, 50.0 mM, pH 7.5) containing EDTA (1.0 mM) and NADPH (10.0 μL of a 10.0 mM solution in the same buffer), and a suspension of the microsomes (20.0 μL) was added. After 30 min at 20 °C the reaction products were isolated by solid-phase microextraction (SPME, fiber coated with polymethyldisiloxane).[26] Equilibrium was reached after 30 min of extraction, and the products were then evaporated (250 °C) from the fiber in the injection port of the GC-MS instrument (GC-MS: Fisons MD 800, column: SE 30, 10 m × 0.31 mm, carrier gas: He, temperature program: 50 °C (2 min) to 280 °C at 20 °C min^{-1}, interface: 270 °C, mass range: 35–350 Da sec^{-1}). In each experiment about 5% of the substrates **3a**, **3b**, **4**, or **7** were transformed. To facilitate the analysis of acetone and [$^2H_6$]acetone, the reaction mixture was treated prior to extraction with 5.0 μL of a 0.1 mM solution of pentafluorobenzylhydroxylamine in Tris/HCl buffer.[15] Formation of the amine was complete after 30 min. Following derivatization, acetone and psoralen could be quantified by GC-MS. Calibration was achieved with authentic references prepared from acetone and [$^2H_6$]acetone.

In the model reactions solutions of the metalloporphyrinato complex (Fe^{3+} or Mn^{3+}, 1.4 mmol) and the deuterated (±)-marmesins **3a**, **3b, 4**, and **7** (8.1 mmol) in dichloromethane (3.0 mL) were stirred with iodosylbenzene (22.0 mmol) at 4 °C for 8 h. The products were analyzed by GC-MS without further workup. About 10% of the substrate was transformed. The carbonyl fragment was detected as described above as the pentafluorobenzylhydroxylamine.[15]

Received: May 9, 1997
Revised version: October 7, 1998 [Z 10426 IE]
German version: *Angew. Chem.* **1999**, *111*, 413–415

Keywords: biosynthesis · cytochrome · eliminations · furocoumarins · porphyrinoids

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Transmetalation of Tetraalkynyltin Compounds with Grignard Reagents: Access to Mono- and Dialkyltin Products

Pascale Jaumier, Bernard Jousseaume,* and Mohammed Lahcini

The transmetalation of organotin compounds with lithium reagents[1] provides access to allyl-[1], vinyl-,[2] and α-heteroalkyllithium reagents,[3] especially for applications in organic synthesis.[4] Recent examples of other such transmetalation reactions involved palladium,[5] copper,[6] and boron compounds.[7] In these reactions [Eq. (1)], the organic group that

$$R^1_3SnR^2 + R^3M \longrightarrow R^2M + R^1_3SnR^3 \quad (1)$$

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