O-Heterocycles via Laccase-Catalyzed Domino Reactions with O_2 as the Oxidant

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Abstract: Laccase-catalyzed domino reactions of 4-hydroxy-6-methyl-2*H*-pyran-2-one or substituted 4-hydroxy-2*H*-chromen-2-ones with catechols using molecular oxygen as an oxidant afford coumestans and related O-heterocycles with yields ranging from 51% to 99%.

Key words: enzymes, catalysis, oxygen, heterocycles, domino reactions

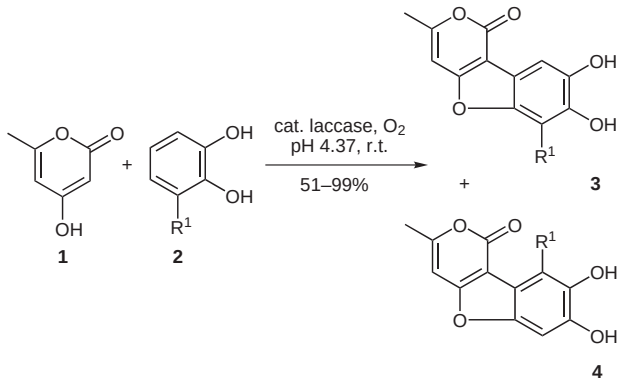
Enzyme-catalyzed reactions play an increasingly important role in organic synthesis.[1] Biotransformations have proved to be particularly valuable for hydrolyzing esters and amides, reducing aldehydes and ketones, oxidizing alcohols and aldehydes as well as various C–C bond forming reactions. Little investigation has been directed though towards the application of enzymes for the synthesis of heterocycles and for domino reactions.[2]

Laccases (benzenediol:O_2 oxidoreductase E.C. 1.10.3.2) are multicopper oxidases that are capable of oxidizing a wide range of substrates while concomitantly reducing O_2.[3] They contain a type 1 (T1) Cu center, a type 2 (T2) Cu center and a type 3 (T3) Cu center; T2 and T3 form a trinuclear Cu cluster. The oxidation of the substrate occurs at the type 1 Cu center. The electrons are transferred to the trinuclear Cu cluster where O_2 is reduced to H_2O.[4]

Laccases are found in some plants, in the majority of fungi, but also prokaryotes. Although they belong to the longest known enzymes, they are still being intensively investigated due to their great importance for the formation of plant lignins,[5] their role as virulence factors involved in fungal diseases and, in particular, their large potential in various industrial oxidative processes such as delignification, dye or stain bleaching, bioremediation and plant fiber modification.[6]

Laccases still play a minor part in organic synthesis, but recently the interest in laccase-catalyzed transformations has been growing steadily.[7] Apart from the oxidation of benzyl alcohols to benzaldehydes performed in the presence of a so-called mediator[8] laccases have been used in the oxidative coupling of phenolic substrates.[9] Also, they have been applied for the oxidative generation of quinoid systems, which can then further react in various subsequent reactions.[10]

The coumestans are comprised of a group of natural products characterized by a 6*H*-benzofuro[3,2-*c*]chromen-6-one skeleton.[11] This group exhibits a number of interesting biological activities, among them phytoestrogenic, antibacterial, antifungal, antihepatotoxic and phytoalexine effects.[12] As a privileged scaffold the coumestans have recently gained increased attention, and, in addition to the already known ones,[13] a number of novel synthetic methods have been developed for constructing the coumestan and related ring systems.[14] One of the most successful methods has been the Wanzlick type of oxidative condensation of 4-hydroxy-2*H*-chromen-2-one with catechols.[15] These reactions are typically performed with inorganic oxidants such as $K_3[Fe(CN)_6]$,[15,16] but can also be accomplished both electrochemically[17] and enzymatically with tyrosinase.[18]



Scheme 1

Table 1 Laccase-Catalyzed Domino Reactions of **1** and **2** in the Presence of O_2 as an Oxidant

Entry	**2**	R^1	Time (h)	Product(s)	Yield (%)
1	**a**	H	3.5	**3a**	76
2	**b**	Me	3.5	**3b**	99
3	**c**	OMe	4.5	**3c**	51
4	**d**	F	7	**3d**, **4d**	76[a]
5	**e**	CO_2Me	4	**4e**, **5**	85[b]

[a] Compounds **3d** and **4d** were obtained in a 1:1 ratio.
[b] Compounds **4e** and **5** were obtained in a 7:3 ratio.

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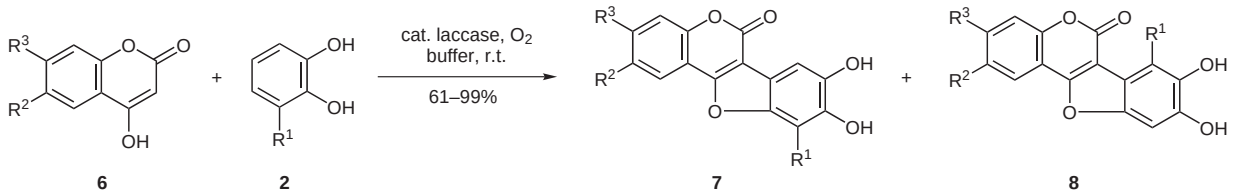


6 **2** **7** **8**

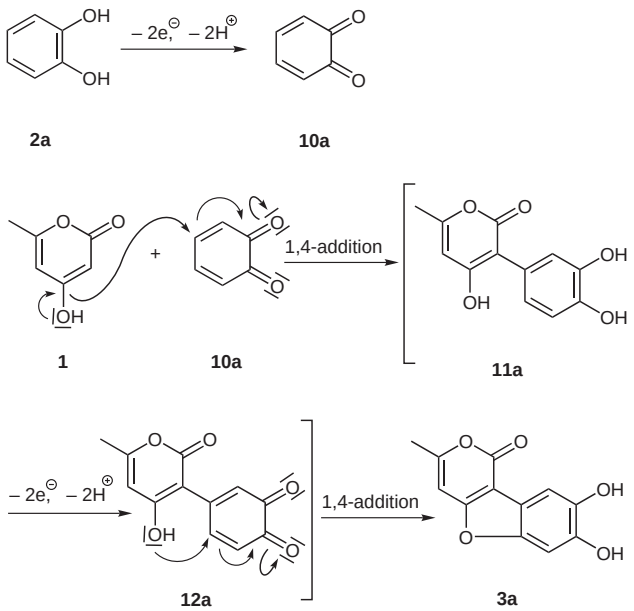
Scheme 2

Here we report for the first time on laccase-catalyzed domino reactions between 4-hydroxy-6-methyl-2*H*-pyran-2-one or 4-hydroxy-2*H*-chromen-2-ones and catechols using molecular oxygen as the oxidating agent.

First, the laccase-catalyzed reaction between 4-hydroxy-6-methyl-2*H*-pyran-2-one (**1**) and catechol (**2a**, R^1 = H) was investigated in the presence of O_2. We used commercially available laccase of *Trametes versicolor* as the enzyme.

It was found that the transformation is best run at room temperature in an acetate buffer at pH 4.37.[19] After 3.5 hours 7,8-dihydroxy-3-methyl-1*H*-pyrano[4,3-*b*]benzofuran-1-one (**3a**) was isolated as the single product in a yield of 76% (Scheme 1; Table 1, entry 1). Correspondingly, the enzyme-catalyzed reaction with 4-hydroxy-2*H*-chromen-2-one (**6a**) exclusively produced 8,9-dihydroxy-6*H*-benzofuro[3,2-*c*]chromen-6-one (**7a**) in 85% yield (Scheme 2; Table 2, entry 1).

We assume that in the first step of the reaction sequence a laccase-catalyzed oxidation of catechol (**2a**) with oxygen takes place to give *o*-benzoquinone **10a**, which then reacts with the nucleophilic 4-hydroxy-6-methyl-2*H*-pyran-2-one (**1**) in an intermolecular 1,4-addition leading to non-isolable **11a** (Scheme 3). After a second laccase-catalyzed oxidation of **11a** to **12a** an intramolecular 1,4-addition occurs giving the heterocycle **3a**. In other words, the entire domino process consists of two oxidations and two 1,4-additions.



2a **10a** **1** **10a** **11a** **12a** **3a**

Scheme 3

Then the domino reactions were performed with 3-substituted catechols. Reactions of **1** and the donor-substituted catechols 3-methyl catechol (**2b**) and 3-methoxy catechol (**2c**) produced heterocycles **3b** and **3c**, respectively, each as the single product (Scheme 1; Table 1, entries 2 and 3). Corresponding results were obtained in transformations with 4-hydroxy-2*H*-chromen-2-one (**6a**), where **7b** and **7c**

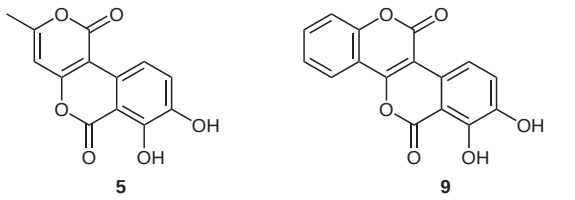
Table 2 Laccase-Catalyzed Domino Reactions of **2** and **6** in the Presence of O_2 as the Oxidant

Entry	**2**	R^1	**6**	R^2	R^3	pH	Time (h)	Product(s)	Yield (%)
1	**a**	H	**a**	H	H	4.37[a]	7	**7a**	85
2	**b**	Me	**a**	H	H	4.37[a]	3	**7b**	99
3	**c**	OMe	**a**	H	H	4.37[a]	5	**7c**	61
4	**e**	CO_2Me	**a**	H	H	4.37[a]	4	**8d**, **9**[c]	89
5	**a**	H	**b**	Me	H	6.0[b]	49	**7e**	96
6	**c**	OMe	**b**	Me	H	6.0[b]	20	**7f**	94
7	**a**	H	**c**	H	OMe	6.0[b]	20	**7g**	99
8	**c**	OMe	**c**	H	OMe	6.0[b]	20	**7h**	88

[a] The reaction was performed using the laccase of *Trametes versicolor*.
[b] The reaction was performed using the laccase of *Agaricus bisporus*.
[c] Compounds **8d** and **9** were obtained in a 71:29 ratio.

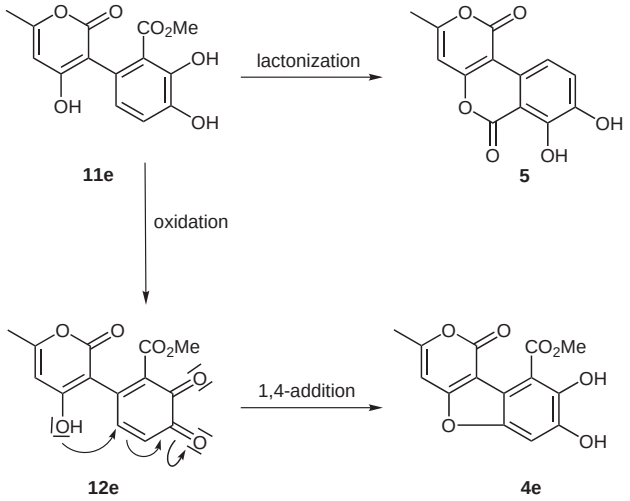
were isolated in 99% and 61%, respectively (Scheme 2; Table 2, entries 2 and 3). The selectivity of these transformations may be explained by assuming that the first 1,4-addition exclusively occurs at the more electrophilic carbon atom at C-5 of the respective *o*-benzoquinones **10**.

When reacting **1** with methyl 2,3-dihydroxybenzoate (**2e**) a mixture of **4e**[20] and **5** (Figure 1)[21] was isolated in a 70:30 ratio and 85% yield and separated by HPLC (Scheme 1; Table 1, entry 5). We assume that the formation of both products starts with the selective 1,4-addition of **1** at the more electrophilic carbon atom at C-4 of the *o*-benzoquinone **10e** and yields the non-isolable intermediate **11e** (Scheme 4). The OH group of the enolized 1,3-dicarbonyl system may then either react directly with the ester group in a lactonization reaction of **11e** to yield **5** or, following oxidation of **11e** to give **12e**, undergo an intramolecular 1,4-addition leading to **4e**. Correspondingly, **2e** was reacted with **6a**, giving a 71:29 mixture of tetracycles **8d** and **9** (Figure 1) with 89% yield (Scheme 2; Table 2, entry 4). Here, the reaction products could also by separated by HPLC.



5 **9**

Figure 1

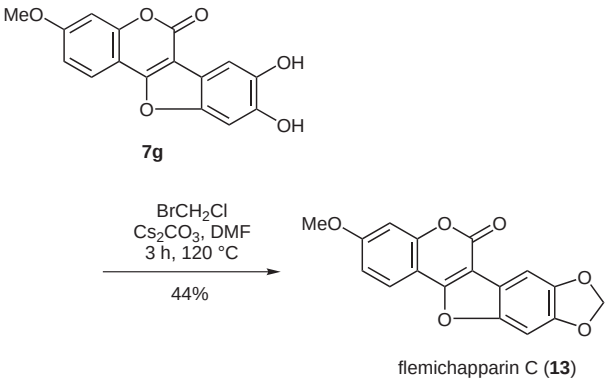


Scheme 4

Quite unexpectedly, the laccase-catalyzed transformation between **1a** and 3-fluorocatechol (**2d**) proceeds unselectively, yielding approximately equal amounts of **3d** and **4d** (Scheme 1; Table 1, entry 4).

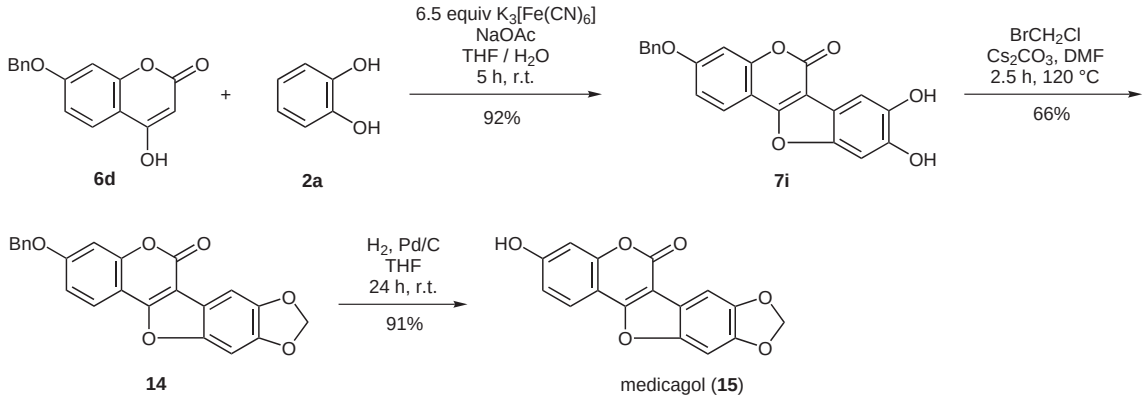
Subsequently, we investigated domino processes of 4-hydroxy-6-methyl-2*H*-chromen-2-one (**6b**) and 4-hydroxy-7-methoxy-2*H*-chromen-2-one (**6c**), which are both poorly water-soluble at pH 4.37, with catechol (**2a**) and 3-methoxy catechol (**2c**). When using laccase of *Trametes versicolor* under different conditions no reaction occurred. After several experiments we found that transformations are catalyzed by laccase of *Agaricus bisporus* in a phospate buffer at pH 6.0. Thus heterocycles **7e**–**h**[22] were obtained selectively with yields between 88% and 99% (Scheme 2; Table 2, entries 5–8).

8,9-Dihydroxy-3-methoxy-6*H*-benzofuro[3,2-*c*]chromen-6-one (**7g**), which is available in 99% yield, can easily be reacted with bromochloromethane to give the natural product flemichapparin C (**13**) (Scheme 5). The synthesis of flemichapparin C was thus effected in just two steps and a total yield of 43%. Flemichapparin C was first isolated from the roots of *Flemingia chappar*[23] and has been synthesized with total yields ranging from 4% to 19%.[14d,24]

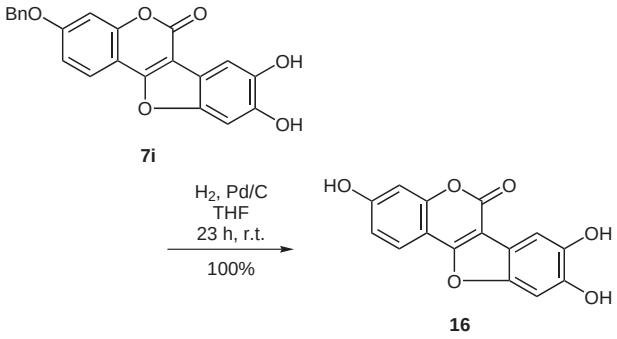


Scheme 5

Laccase-catalyzed synthesis of medicagol (**15**) was then tackled. This natural product was first isolated from *Medicago sativa*[25] and has been synthesized repeatedly.[12a,14d,26] To effect synthesis of medicagol (**15**) a laccase-catalyzed reaction between 7-benzyloxy-4-hydroxy-2*H*-chromen-2-one (**6d**) and **2a** was attempted. It turned out, though, that neither laccase of *Trametes versicolor* nor laccase of *Agaricus bisporus* were able to catalyze this transformation. The reasons remain unclear. But we succeeded to construct **7i** according to Wanzlick[15] by reacting **6d** and **2a** with 6.5 equivalents of $K_3[Fe(CN)_6]$ in THF–H_2O with 92% yield (Scheme 6). Subsequent introduction of the methylenedioxy group by reacting **7i** with bromochlormethane in 66% yield and the final debenzylation of **14** with H_2, Pd/C led to the formation of the natural product. Starting from **2a** and **6d** the synthesis of medicagol (**15**) was thus effected in 3 steps and a total yield of 55%. Compound **7i** can also be used to form – via debenzylation with H_2, Pd/C – trihydroxy coumestan (**16**), another compound demonstrating interesting biological activity (Scheme 7).[12d]



Scheme 6



Scheme 7

The structures of all the products described in this paper have been elucidated unambiguously by NMR spectroscopic methods.

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(19) **General Procedure for Laccase-Catalyzed Domino Reactions.**
4-Hydroxy-2*H*-pyran-2-one (**1**, 1 equiv, 3.0 mmol), 4-hydroxy-2*H*-chromen-2-one (**6**, 1 equiv, 3.0 mmol), and catechol **2** (1.1 equiv, 3.4 mmol) were dissolved in 200 mL acetate buffer (pH 4.37, 0.2 M) and vigorously stirred under air at r.t. in the presence of 25 mg laccase (19 U/mg) of *Trametes versicolor* until the substrates had been fully consumed, as judged by TLC. The reaction mixture was saturated with NaCl and filtered on a Buchner funnel. The filter cake was washed with a solution of 200 mL 15% NaCl and 10 mL H_2O. The crude products obtained after drying exhibited a purity of 90–95% (NMR). Analytically pure products could be obtained by recrystallization. Transformations with laccase (320 U/mg) of *Agaricus bisporus* were performed in a phosphate buffer (pH 6.0, 0.2 M).

(20) Selected data for **4e**: IR (ATR): 3405, 3119, 1703, 1440, 1296, 1220, 1050, 839 cm^{-1}. UV (MeCN): λ_{max} (lg ε) = 233 (4.28), 335 nm (4.18). ^1H NMR (300 MHz, DMSO-d_6): δ = 2.36 (s, 3 H, CH_3), 3.83 (s, 3 H, OCH_3), 6.95 (s, 1 H, 4-H), 7.20 (s, 1 H, 6-H), 9.51 (br s, 2 H, 7-OH, 8-OH). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 20.51 (CH_3), 52.44 (OCH_3), 96.36 (C-4), 100.04 (C-6), 103.02 (C-9b), 110.94 (C-9a), 114.45 (C-9), 142.02, 146.40, 148.43 (C-5a, C-7 or C-8), 158.72 (C-1), 162.76 (C-3), 164.31 (C-4a), 166.73 (C=O). MS (EI, 70 eV): *m/z* (%) = 290 (20) [M^+], 258 (100) [$M^+ - CH_4O$], 229 (4), 202 (16), 174 (11), 69 (7), 43 (19). HRMS: *m/z* calcd for $C_{14}H_{10}O_7$: 290.04266; found: 290.04251.

(21) Selected data for **5**: IR (ATR): 3440, 3092, 2500, 1693, 1636, 1273, 1252, 1046, 832 cm^{-1}. UV (MeCN): λ_{max} (lg ε) = 225 (4.00), 244 (4.08), 347 nm (4.04). ^1H NMR (300 MHz, acetone-d_6): δ = 2.42 (s, 3 H, CH_3), 6.53 (s, 1 H, 4-H), 7.49 (d, $^3J_{9\text{-H, }10\text{-H}}$ = 8.7 Hz, 1 H, 9-H or 10-H), 8.39 (d, $^3J_{10\text{-H, }9\text{-H}}$ = 8.7 Hz, 1 H, 9-H or 10-H), 8.74 (br s, 1 H, OH), 10.87 (br s, 1 H, OH). ^{13}C NMR (75 MHz, acetone-d_6): δ = 19.27 (CH_3), 98.95 (C-4), 99.72 (C-10b), 106.14 (C-6a or C-10a), 116.58 (C-9 or C-10), 124.46 (C-9 or C-10), 124.50 (C-6a or C-10a), 145.42 (C-7 or C-8), 149.31 (C-7 or C-8), 160.13 (C-1, C-4a or C-6), 160.15 (C-1, C-4a or C-6), 163.15 (C-3), 164.33 (C-1 or C-6). MS (EI, 70 eV): *m/z* (%) = 260 (100) [M^+], 245 (4) [$M^+ - CH_3$], 232 (4), 189 (5), 176 (11), 161 (6), 120 (15), 43 (25). HRMS: *m/z* calcd for $C_{13}H_8O_6$: 260.03207; found: 260.03162.

(22) Selected data for **7f**: IR (ATR): 3504, 3207, 1685, 1459, 1318, 1254, 1085, 848, 817 cm^{-1}. UV (MeCN): λ_{max} (lg ε) = 215 (4.57), 250 (4.14), 343 nm (4.21). ^1H NMR (300 MHz, DMSO-d_6): δ = 2.43 (s, 3 H, CH_3), 4.11 (s, 3 H, OCH_3), 7.06 (s, 1 H, 7-H), 7.44 (s, 2 H, 3-H, 4-H), 7.82 (s, 1 H, 1-H), 9.38 (br s, 2 H, 8-OH, 9-OH). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 21.01 (CH_3), 61.35 (OCH_3), 100.04 (C-7), 106.12 (C-6a or C-6b), 112.66 (C-11b), 115.01 (C-6a or C-6b), 117.49 (C-3 or C-4), 121.51 (C-1), 133.05 (C-3 or C-4), 134.16 (C-10), 135.16 (C-2), 138.16, 142.41, 146.55 (C-8, C-9 or C-10a), 151.32 (C-4a), 158.29 (C-6 or C-11a), 158.67 (C-6 or C-11a). MS (EI, 70 eV): *m/z* (%) = 312 (100) [M^+], 297 (45) [$M^+ - CH_3$], 269 (17), 185 (7), 156 (9), 139 (11), 128 (16), 77 (12). Anal. Calcd for $C_{17}H_{12}O_6$ (312.27): C, 65.39; H, 3.87. Found: C, 65.11; H, 4.10.

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