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COMMUNICATION

Laccase-catalyzed stereoselective oxidative ring opening of 2,5-dialkylfurans into 2-ene-1,4-diones using air as an oxidant†

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The laccase-catalyzed ring opening of 2,5-dimethylfuran using air as an oxidant stereoselectively yields (*Z*)- or (*E*)-3-hexene-2,5-dione depending on the mediator employed: with TEMPO the (*Z*)-3-hexene-2,5-dione is formed, while a combination of TEMPO and violuric acid gives (*E*)-3-hexene-2,5-dione. The (*Z*)-selective ring cleavage was extended to a variety of symmetrical and unsymmetrical 2,5-dialkylfurans.

Laccases that belong to the blue multicopper oxidases are widely distributed in nature.[1] They have several biological functions including wound response and lignification in plants and lignin degradation in fungi. In laccases the four-electron reduction of O_2 to H_2O is coupled with four concomitant one-electron oxidations of organic substrate molecules. The resulting substrate radicals can undergo oxidative couplings with formation of dimers, oligomers and polymers, or can be further oxidized. The use of air, which is the cheapest and greenest oxidant available, the independence from cofactors and the formation of H_2O as the only by-product have stimulated the development of laccase-catalyzed processes in the pulp and paper, textile, food and cosmetic industries as well as in the field of bioremediation.[2]

Due to their broad substrate spectrum, the possibility to extend their redox potential by mediators, their high stability and mild reaction conditions laccases have received increasing attention in organic synthesis.[3] Laccases or laccase/mediator systems have been used to oxidize aromatic methyl groups,[4] alcohols,[5] ethers,[6] benzyl amines and hydroxylamines.[7] The oxidation of catechols and hydroquinones to the corresponding benzoquinones and related transformations are also known.[8] We have reported on laccase-initiated domino reactions between catechols and 1,3-dicarbonyls allowing for the efficient preparation of heterocyclic systems.[9] It has been shown that laccases catalyze the oxidative coupling of phenolics.[10] The laccase-catalyzed reaction of *o*-phenylenediamine with benzaldehydes provides access to 2-aryl-1-*H*-benzimidazoles.[11]

Over the last few years considerable effort has been devoted to develop alternative liquid fuels for transportation as well as alternative feedstocks for the chemical industry from renewable resources.[12] Several furans have been identified as platform molecules that can be obtained from renewable resources.[13] In this respect, 2,5-dimethylfuran (**1a**) is of great interest as it has been considered a serious candidate for the substitution of fossil liquid fuel.[14] In contrast to the chemistry of some of these furans,[15] only little is known about the reactions of 2,5-dimethylfuran (**1a**).

The oxidative ring opening of furans provides the most important synthetic access to unsaturated 1,4-diones.[16] Due to the high density of functional groups 2-ene-1,4-diones are highly versatile substrates which can be transformed in multiple ways. This is why the oxidative ring opening of furans to 2-ene-1,4-dicarbonyls has been performed with a great number of reagents. The selection of suitable oxidizing agents includes reagents as different as Br_2,[17a] *meta*-chloroperbenzoic acid (*m*-CPBA),[17b] Cr(VI) reagents,[17c] $^1O_2$,[17d] ceric ammonium nitrate (CAN),[17e] dimethyldioxirane,[17f] H_2O_2/TS-1,[17g] $NaClO_2/NaH_2PO_4$,[17h] and magnesium monoperoxyphthalate (MMPP).[17i] So it is all the more surprising that the ring opening of furans has not yet been performed using air which is not only the cheapest and most easily accessible oxidant but also allows for oxidations to be performed in an environmentally friendly manner.

We started with the observation that the laccase-catalyzed oxidation of 2,5-dimethylfuran (**1a**) with air in the presence of 10 mol% TEMPO (**4a**) as a mediator (Fig. 1) yielded 47% (*Z*)-2-hexene-2,5-dione (**2a**) (*Z* : *E* = 90 : 10) (Scheme 1). When instead the oxidation was performed with violuric acid (**5**) (*E*)-2-hexene-2,5-dione (**3a**) (*E* : *Z* = 88 : 12) was isolated with 35% yield.

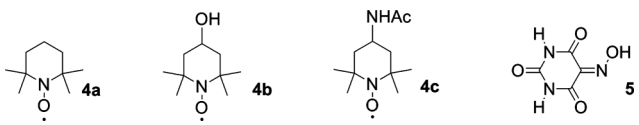

Fig. 1 Structures of mediators used for laccase-catalyzed reactions.

Control experiments clearly showed that the oxidative ring cleavage of **1a** does not proceed in the absence of either laccase from *Trametes versicolor* or a mediator (**4a** or **5**). In all these cases

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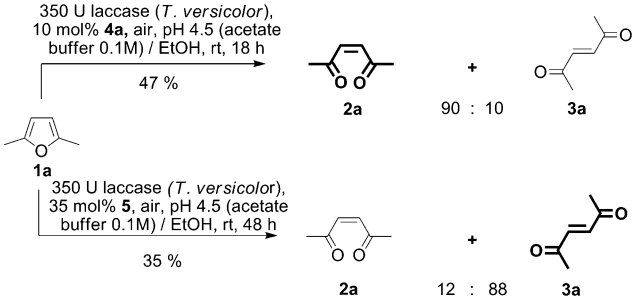
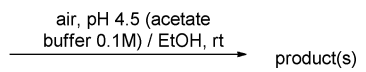


Scheme 1 Initial results for the laccase-catalyzed ring opening of **1a** with air in the presence of different mediators.

Table 1 Control experiments with **2a** and **3a**



Entry	Substrate	U (Laccase)	Mediator (mol%)	*t* (h)	Product (**2a**:**3a**[a])
1	**2a**	350	**4a** (10)	18	92 : 8
2	**2a**	—	**4a** (10)	18	94 : 6
3	**2a**	350	**5** (35)	48	5 : 49
4	**2a**	—	**5** (35)	48	9 : 91
5	**3a**	350	**4a** (10)	18	0 : 100
6	**3a**	—	**4a** (10)	18	0 : 100
7	**3a**	350	**5** (35)	48	0 : 100
8	**3a**	—	**5** (35)	48	0 : 100

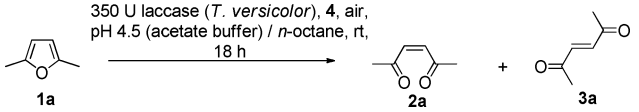
[a] Ratio determined by ^{1}H NMR

only the substrate **1a** was detected after 18 h at rt. With argon instead of air only traces of **2a** and **3a** were formed. These results demonstrate that this transformation requires the triple action of a laccase, a mediator and air. When the amount of laccase was reduced from 350 U to 250 U and 100 U, respectively, the yields of **2a** and **3a** decreased substantially.

To further corroborate these findings a number of control experiments were conducted with **2a** and **3a**. When the pure *Z*-isomer **2a** was reacted with 350 U laccase‡ and 10 mol% TEMPO (**4a**), **2a** and **3a** were isolated in a 92 : 8 ratio (Table 1, entry 1). A similar result was obtained when **2a** was stirred in the presence of 10 mol% **4a** alone (Table 1, entry 2). When **2a** was reacted with violuric acid (**5**) as a mediator, isomerization to **3a** (which is considered to be thermodynamically more stable than **2a**) was observed to a more or less extensive degree (Table 1, entry 3 and 4). Interestingly, isomerization to **3a** is nearly complete with **5** alone (Table 1, entry 4). In the presence of laccase and **5**, **2a** undergoes only partial isomerization (Table 1, entry 3). We concluded that violuric acid (**5a**) was the responsible agent. Control experiments with the pure *E*-isomer **3a** and the mediators **4a** or **5** in the presence/absence of laccase demonstrated that an isomerization of **3a** to **2a** does not occur in any case (Table 1, entries 5–8). These observations confirmed the assumption that **3a** is thermodynamically more stable than **2a**.

With these results in hand we were encouraged to optimize the (*Z*)-selective ring opening of furan **1a**. Experiments with a number of organic solvents demonstrated that a two-phase system consisting of acetate buffer and *n*-octane (10 : 1) was the most suitable. Further experiments studied the influence of different concentrations of the mediators **4a–c** as well as different

Table 2 Optimization of the selective oxidation of **1a** to **2a**[a]



Entry	**4** (mol%)	Buffer (M)	Yield (%)	**2a**:**3a**[b]
1	**a** (10)	0.1	57	98 : 2
2	**a** (10)	0.01	61	98 : 2
3	**a** (20)	0.01	72	94 : 6
4	**b** (10)	0.1	73	97 : 3
5	**b** (10)	0.01	79	99 : 1
6	**b** (20)	0.01	74	100 : 0
7	**c** (10)	0.1	75	94 : 6
8	**c** (10)	0.01	73	99 : 1
9	**c** (20)	0.01	72	90 : 10

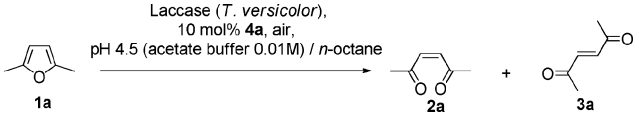
[a] 2 mmol of **1a** were reacted. [b] Ratio determined by ^{1}H NMR.

acetate buffer concentrations on both yield and stereoselectivity (Table 2).

The best results were obtained when the laccase-catalyzed transformation of **1a** was performed with 10 mol% 4-hydroxy-TEMPO (**4b**) in 0.01 M acetate buffer/*n*-octane (10 : 1).§ Under these conditions 79% (*Z*)-2-hexene-2,5-dione (**2a**) (**2a**:**3a** = 99 : 1) were isolated (Table 2, entry 5).¶ Yields and selectivity were in the same range when 4-acetamido-TEMPO (**4c**) was employed as a mediator (Table 2, entry 8). Now an enzyme-catalyzed procedure for the oxidative ring opening of **1a** with air is available for the first time, which delivers (*Z*)-2-hexene-2,5-dione (**2a**) with both high yield and outstanding (*Z*)-selectivity.

All oxidations presented so far were performed on a 2 mmol scale. To demonstrate the usefulness of this laccase-catalyzed reaction in preparative organic chemistry the transformation was scaled up to the 10 mmol scale (Table 3). In these experiments the mediator 4-hydroxy-TEMPO (**4b**) was replaced by the cheaper TEMPO (**4a**). The yields of **2a** strongly depend on the amount of laccase used. With 350 U of laccase a maximum of 40% product could be isolated (Table 3, entry 1); with 500 U laccase the yield increased to 54% (Table 3, entry 2). A further increase to 66 and 71% was achieved by extending the reaction time from 18 to 36 h and 48 h, respectively (Table 3, entry 5 and 7). No further improvement could be achieved by increasing the amount of the laccase to 700 U (Table 3, entry 6). Raising the

Table 3 Scale-up experiments[a]



Entry	U (Laccase)	*T* (°C)	*t* (h)	Yield (%)	**2a**:**3a**[b]
1	350	r.t.	18	40	96 : 4
2	500	r.t.	18	54	97 : 3
3	500	40	18	46	95 : 5
4	500	60	18	9	92 : 8
5	500	r.t.	36	66	98 : 2
6	700	r.t.	36	65	98 : 2
7	500	r.t.	48	71	97 : 3

[a] 10 mmol of **1a** were reacted. [b] Ratio determined by ^{1}H NMR.

reaction temperature from rt to 40 °C and 60 °C led to decreasing yields (Table 3, entry 3 and 4). To sum up, the stereoselective transformation of **1a** into **2a** can easily be scaled up to the 10 mmol scale by appropriately adjusting the reaction conditions.

The next set of experiments was devoted to find out whether the laccase-catalyzed (*Z*)-selective ring opening of **1a** can be extended to other 2,5-disubstituted furans. The required substrates **1b–g** (Fig. 2) were obtained by deprotonation of the corresponding 2-alkylfurans with *n*-BuLi and subsequent alkylation with the corresponding alkyl iodides.[18]

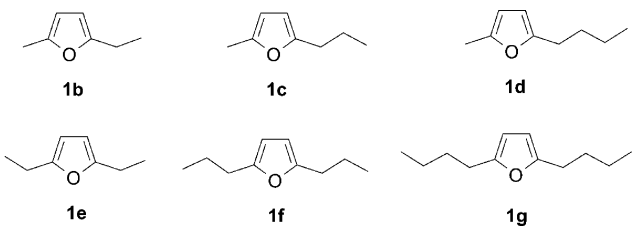
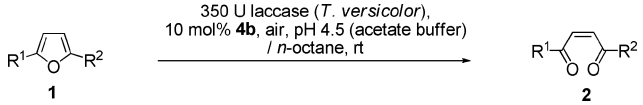
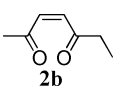
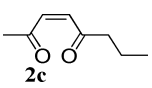
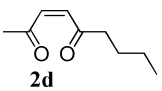
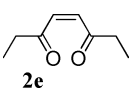
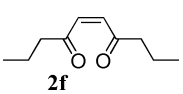


Fig. 2 Structures of 2,5-dialkylfurans **1b–g**.

The resulting 2,5-dialkylfurans **1b–g** were then reacted with 350 U laccase in the presence of 10 mol% **4b** as the mediator under the conditions given in Table 2, entry 5, resulting in the exclusive formation of the (*Z*)-substituted enediones **2b–f** (Table 4). Not even traces of the (*E*)-substituted enediones could be detected. The results clearly demonstrate that both symmetrically and asymmetrically substituted dialkylfurans can be successfully ring-opened to yield the (*Z*)-substituted enediones **2b–f** in a highly diastereoselective manner. A comparison of the individual transformations indicates that decreasing yields of **2** are observed when the length of the side chains of the furans increases. The only substrate that could not be oxidized at all was 2,5-dibutylfuran (**1g**) (Table 4, entry 6).

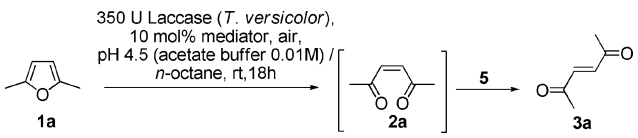
Table 4 Laccase-catalyzed ring opening of 2,5-dialkylfurans **1b–g** to (*Z*)-enediones **2b–f**[a]



Entry	**1**	*t* (h)	Product	Yield (%)	Ratio **2**:**3**
1	**b**	72	 **2b**	52	100 : 0[b]
2	**c**	72	 **2c**	59	100 : 0[b]
3	**d**	96	 **2d**	33	100 : 0[b]
4	**e**	72	 **2e**	65	100 : 0[b]
5	**f**	72	 **2f**	22	100 : 0[b]
6	**g**	96	—		

[a] 2 mmol of **1** were reacted. [b] In the ^{1}H NMR no traces of **3** could be detected.

Table 5 Optimization of the selective oxidation of **1a** to **3a**[a]



Entry	**4**	**5** (mol%)	*T*/°C	*t* (isom., h)	Yield (%)	**2a**:**3a**[b]
1	**b**	50	25	42	50	0 : 100
2	**b**	50	50	4	50	0 : 100
3	**b**	—	50	4	70	99 : 1
4	**a**	50	25	42	67	0 : 100
5	**a**	37.5	25	42	62	0 : 100
6	**a**	25	25	48	52	0 : 100
7	**a**	—	50	6	—	94 : 6[c]
8	**a**	50	50	6	61	0 : 100
9	**a**	25	50	6	70	0 : 100
10	**a**	10	50	15	60	0 : 100

[a] 2 mmol of **1a** were reacted. [b] Ratio determined by ^{1}H NMR. [c] Crude product.

Finally, our focus was on the (*E*)-selective oxidative ring opening of **1a** into **3a**.‖ We clearly needed to improve the transformation presented in Scheme 1 with respect to both yield and selectivity. On the basis of the results given in Tables 1 and 2, we assumed that this goal could be achieved by combining the laccase-catalyzed (*Z*)-selective oxidative ring opening of **1a** to **2a** with **4** as a mediator and the isomerization of **2a** to **3a** using violuric acid (**5**). This assumption was confirmed by the experiments summarized in Table 5. The best results were obtained when **1a** was first reacted using 350 U laccase, 10 mol% of **4a** and air for 18 h at rt and the reaction mixture was stirred for 6 h at 50 °C after adding 25 mol% of **5**. Using this protocol, stereoisomerically pure (*E*)-2-hexene-2,5-dione (**3a**) could be isolated with 70% yield (Table 5, entry 9). Decreasing the amount of mediator **5** to 10 mol%, isomerically pure **3a** could be isolated with 60% yield (Table 5, entry 10). Control experiments established that the selective formation of **3a** is impossible when **5** is absent (Table 5, entries 3,7).

Due to their molecular symmetry the magnetically equivalent vinylic protons of **2a** and **3a** resonate as a singlet each. Thus it was impossible to directly determine the *E*/*Z*-configuration of the double bonds on the basis of the size of the vicinal coupling constants. Replacement of one olefinic ^{12}C by ^{13}C (approx. 1% natural abundance), however, abolishes the magnetic equivalence, and an ABX spin system with two doublets of doublets is formed (Fig. 3). The size of $^3J_{HH}$ in these isotopomers can be easily derived from the outer doublets which show no signal overlap with the central proton singlet because of $^1J_{CH} \gg {}^2J_{CH}$. Vicinal coupling constants of 11.7 Hz and 16.5 Hz unambiguously establish the double bond configuration to be *Z* for **2a** and *E* for **3a**, respectively.

In summary, combinations of a laccase and a mediator were demonstrated to catalyze the efficient and sustainable oxidative ring opening of 2,5-dialkyl furans to the corresponding enediones using air as an oxidant. Depending on the mediator

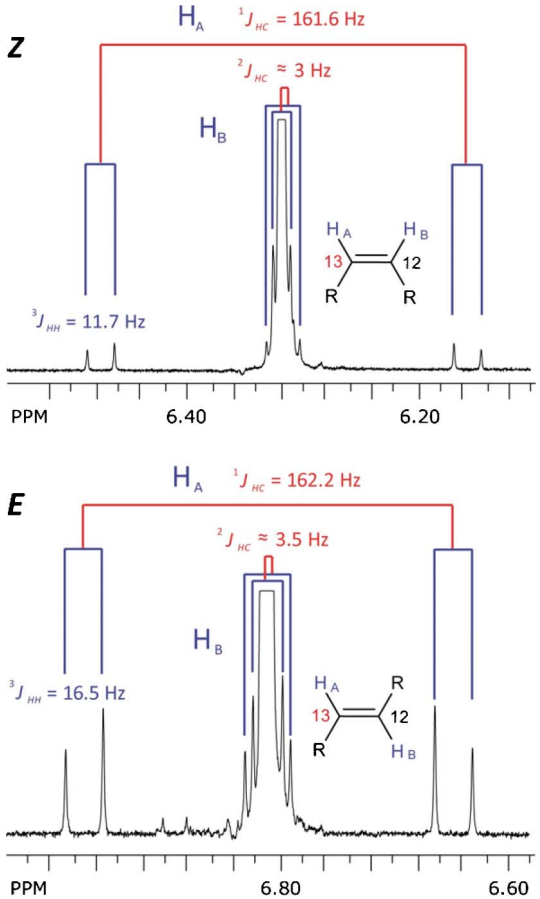


Fig. 3 ^{13}C satellites of the vinylic protons of (*Z*)-3-hexene-2,5-dione (**2a**) and (*E*)-3-hexene-2,5-dione (**3a**) as well as multiplet pattern analysis.

used the (*Z*)- or (*E*)-enediones are formed in a different ratio. This work represents not only the first biocatalytic example of the selective ring opening of 2,5-dialkylfurans but also the first example of a laccase-catalyzed oxidative ring opening of aromatic heterocycles.

Acknowledgements

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Notes and references

‡ Determination of laccase activity according to ref. 19: A 0.1 M solution of ABTS (0.3 mL) in 0.01 M acetate buffer (pH = 4.5) was diluted with 0.01 M acetate buffer (2.6 mL, pH = 4.5) and treated with a solution of laccase in the same buffer (0.1 mL). The change in absorption was followed *via* UV-spectroscopy ($\lambda = 414$ nm). One unit was defined as the amount of laccase (*Trametes versicolor*, Fluka) that converts 1 mmol of ABTS per minute at pH = 4.5 at rt.

§ General procedure for the synthesis of enediones **2a–f**: A 250 ml round bottomed flask with a magnetic stirrer bar was charged with 2 mmol 2,5-dialkylfuran **1**, 1 mL *n*-octane, 10 mL 0.01 M acetate buffer pH 4.5, 0.2 mmol 4-hydroxy-TEMPO (**4b**) and 350 U laccase (*Trametes versicolor*, Fluka) and closed with a septum. The mixture was stirred at room temperature for the time given in Table 4. The aqueous phase was saturated with 1 g NaCl and extracted with CH_2Cl_2 (4 × 25 mL). The combined organic phases were dried over Na_2SO_4. After removal of the solvents *in vacuo* the crude product was purified by flash chromatography on SiO_2 (diethyl ether/*n*-pentane = 1 : 1).

¶ Selected analytical data for (*Z*)-3-hexene-2,5-dione (**2a**): R_f 0.19 (diethyl ether/*n*-pentane = 1 : 1); δ_H (300 MHz; $CDCl_3$) 2.28 (s, 6H, 1-H_3 and 6-H_3), 6.29 (s, 2H, 3-H and 4-H); δ_C (75 MHz; $CDCl_3$) 29.72 (C-1 and C-6), 135.67 (C-3 and C-4), 200.40 (C-2 and C-5).

‖ Selected analytical data for (*E*)-3-hexene-2,5-dione (**3a**): R_f 0.29 (diethyl ether/*n*-pentane = 1 : 1); δ_H (300 MHz; $CDCl_3$) 2.36 (s, 6H, 1-H_3 and 6-H_3), 6.78 (s, 2H, 3-H and 4-H); δ_C (75 MHz; $CDCl_3$) 28.00 (C-1 and C-6), 137.81 (C-3 and C-4), 198.46 (C-2 and C-5).

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