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An oxidation of alcohols by oxygen with the enzyme laccase and mediation by TEMPO

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Abstract—A simple and efficient oxidation of alcohols to carbonyl compounds by oxygen at room temperature is described; it requires the laccase/TEMPO mediator system as the catalyst. A possible mechanistic explanation is provided.

Laccases are multi-copper oxidases expressed under ligninolytic conditions by white-rot fungi.[1] In view of the low redox potential, native laccases can oxidise only phenolic fragments of lignin,[2] with the concomitant reduction of O_2. However, the oxidation of non-phenolic substrates can also take place on mediation by appropriate substances.[3] We have recently found that one such mediator is 2,2′,6,6′-tetramethylpiperidine-*N*-oxyl (TEMPO);[4] we report here on a mild and environmental friendly oxidation of alcohols, as well as of a few other compounds (Eq. (1)), obtained with laccase from a strain of *Trametes villosa* under mediation by TEMPO.

$$R{-}CH_2OH \xrightarrow[\text{TEMPO, r.t.}]{\text{Laccase, } O_2} R{-}CHO \qquad (1)$$

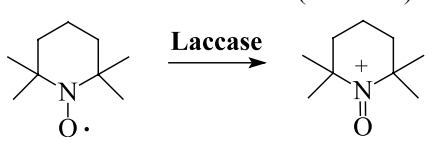
TEMPO is already known to catalyse efficiently the oxidation of alcohols to carbonyl products by a number of oxidants,[5] including copper salts.[6] In our particular case, the catalytic oxidant of TEMPO would be the copper-enzyme laccase.

The oxidation reactions were run at room temperature in a 3 mL water solution, buffered at pH 5 (0.1 M citrate) and preliminarily purged for 30 min with O_2; a 24 h reaction time was adopted. The initial concentrations were: [subst.] 20 mM, [TEMPO] 6 mM, with 9 Units of laccase.[4] The results are reported in Table 1, and the yields were determined by GC by the internal standard method (with respect to 4-methoxyacetophenone).

No overoxidation products were in general observed, while no conversion to oxidised products was obtained in the absence of laccase, nor in the absence of TEMPO.

Inspection of Table 1 shows that the primary benzyl and allyl alcohols (entries 1, 2, 3, 7 and 8) were converted into the corresponding aldehydes in high yields. Secondary benzyl alcohols (**1** and **3**, entries 4 and 5) were also efficiently oxidised to ketones (**2** and **4**), while the tertiary benzyl alcohol (**5**) was recovered unchanged, thus underlying the need for a C–H bond α to the alcohol group for a successful oxidation. Primary and secondary alkyl alcohols were oxidised in lower yields (entries 9, 10, 12–14), but doubling the reaction time increased the yield significantly (entry 11). In these cases, substantial amounts of the precursor were also recovered. Benzyl ethers were converted to aldehydes, albeit in low yields (entries 15 and 16). A quantitative conversion into benzaldehyde was finally obtained with primary and tertiary benzylamines (entries 17 and 18), but surprisingly no oxidation to benzaldehyde took place with benzyl mercaptan (entry 19), in spite of its structural analogy with benzyl alcohol (entry 1), nor any disulfide product was detected from it.

These results support our previous suggestion about an oxidation mechanism that proceeds through the oxoammonium ion of TEMPO,[4] as it is proposed for the oxidation of alcohols by other oxidants and TEMPO.[5,6] Laccase, having a redox potential in the range of 0.7–0.9 V,[2] can easily oxidise the stable oxyl-radical form of TEMPO to the oxoammonium ion ($E°$ 0.2 V)[7] (Eq. (2)).



(2)

Keywords: laccase; TEMPO; oxidation; alcohols; mediation.

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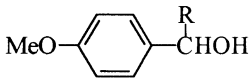
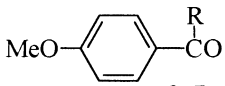
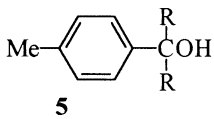
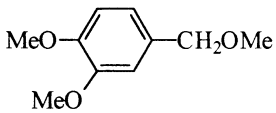
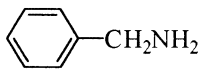
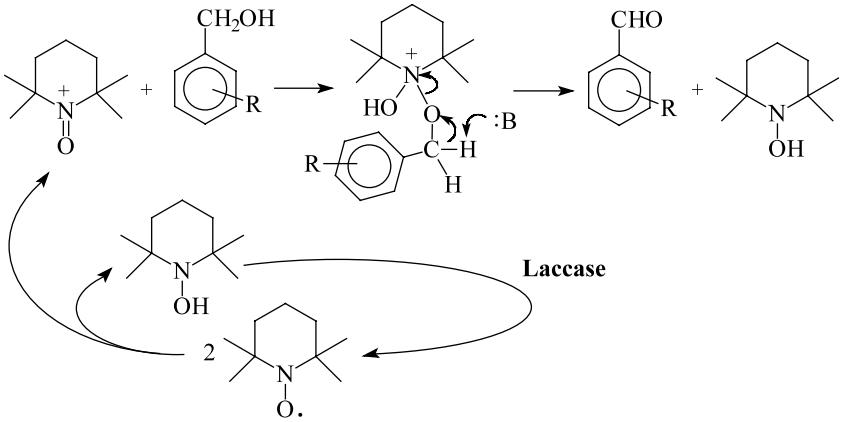
Table 1. Oxidations with oxygen by the laccase/TEMPO system[a]

Entry	Substrate	Product	Yield (%)[b]
1	benzyl alcohol	benzaldehyde	92
2	4-MeO-benzyl alcohol	4-MeO-benzaldehyde	99
3	3,4-dimethoxybenzyl alcohol	3,4-dimethoxybenzaldehyde	99
4	 **1**, R=Me	 **2**, R=Me	85
5	**3**, R=Et	**4**, R=Et	95
6	 **5**	-	0
7	cinnamyl alcohol	cinnamaldehyde	94
8	geraniol	geranial	96
9	5-hexen-1-ol	5-hexen-1-al	19
10	1-decanol	decanal	15
11	1-decanol	decanal	58[c]
12	cyclohexanol	cyclohexanone	35
13	cyclohexylmethanol	cyclohexanecarboxaldehyde	18
14	2-cyclohexylethanol	cyclohexylacetaldehyde	48
15		3,4-dimethoxybenzaldehyde	18
16	$(PhCH_2)_2O$	benzaldehyde	16[d]
17		benzaldehyde	99
18	N,N-diethylbenzylamine	benzaldehyde	99
19	benzyl mercaptan	-	0
20	3,4-dimethoxybenzyl alcohol	3,4-dimethoxybenzaldehyde	79[e]
21	3,4-dimethoxybenzyl alcohol	3,4-dimethoxybenzaldehyde	99[f]
22	3,4-dimethoxybenzyl alcohol	3,4-dimethoxybenzaldehyde	75[g]
23	3,4-dimethoxybenzyl alcohol	3,4-dimethoxybenzaldehyde	72[h]
24	3,4-dimethoxybenzyl alcohol	3,4-dimethoxybenzaldehyde	36[i]

[a] Typical conditions: 24 h reaction time, rt; [subst.] 20 mM, [TEMPO] 6 mM, 3 U/mL of laccase, in 3 mL water solution at pH 4.5 (0.1 M citrate). Oxygen had been initially purged for 30 min in the solvent.
[b] Determined by GC analysis.
[c] Longer reaction time (48 h).
[d] Benzyl benzoate (3%) was additionally detected.
[e] Shorter reaction time (7 h).
[f] Without purging with O_2.
[g] With a lower [TEMPO], i.e. 2 mM.
[h] With [TEMPO] 2 mM and only 1 U/mL of laccase.
[i] As in h, but without purging with O_2.

This ion is the actual oxidant (Scheme 1), while laccase would regenerate TEMPO from the generated hydroxylamine. Then, either acid-induced disproportionation of TEMPO, or further oxidation of it by laccase, would form the oxoammonium ion once again. Since laccase is in turn oxidised by oxygen, this synthetic procedure represents an oxidation of alcohols by air, catalysed by the laccase/TEMPO system.

The last experiments of Table 1 show that a shorter reaction time can be adopted with the more reactive substrates, or a higher substrate/mediator ratio or even a higher mediator/laccase ratio can be used, without a major compromise in oxidation efficiency. Finally, the need for initial purging with O_2 becomes relevant only under the more demanding conditions, where a high substrate/laccase ratio is used.



Scheme 1. Possible mechanism of oxidation by the laccase/TEMPO system.

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