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Comparison of TEMPO and its derivatives as mediators in laccase catalysed oxidation of alcohols

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Abstract—A series of TEMPO (2,2′,6,6′-tetramethylpiperidinyl-1-oxy) derivatives were studied as mediators of laccase (from *Trametes versicolor*) in the oxidation of benzyl alcohol and 1-phenylethyl alcohol. TEMPO (**1**), 4-hydroxy-TEMPO (**2**) and 4-acetylamino-TEMPO (**4**) turned out to be the most active mediators for laccase. In addition, 4-acetylamino-TEMPO and 4-hydroxy-TEMPO were more active in the oxidation of 1-phenylethanol compared to TEMPO. For these mediators kinetic isotope effects in the range of 2.1–3.2 were observed for α-monodeutero-*p*-methylbenzyl alcohol oxidation. These values are consistent with a mechanism involving oxoammonium intermediacy. Competition experiments between benzyl alcohol and 1-phenylethanol showed that TEMPO and its derivatives react faster with primary alcohols than with secondary alcohols, also in line with the proposed mechanism.

1. Introduction

The selective oxidation of alcohols is a pivotal reaction in organic synthesis. Recently, much attention has been focused on the development of green, catalytic methods employing dioxygen or hydrogen peroxide as the stoichiometric oxidant.[1] Many examples of aerobic oxidation of alcohols catalysed by transition metals—notably palladium,[2] platinum[3] and ruthenium[4]—have been described in the last few years. Copper compounds are also known to catalyse the aerobic oxidation of alcohols, both in vitro[5,6] and in vivo.[7] A well-known example of the latter is provided by galactose oxidase (EC 1.1.3.9).[8] Another group of copper-dependant oxidases that has attracted much attention recently comprises the laccases (EC 1.10.3.2).[9] These enzymes contain four copper centres per protein molecule and catalyse the oxidation of electron rich aromatic substrates, usually phenols or aromatic amines, via four single electron oxidation steps concomitant with the four electron reduction of O_2 to H_2O.[10–12] Recently few laccases were crystallised and their three-dimensional crystal structures are now available.[13–16] Its good thermal stability coupled with its lack of substrate inhibition and high rates of oxidation (10–100 fold higher than those of lignin peroxidase or manganese peroxidase) make laccase an ideal candidate for the development of enzymatic oxidation processes.[9] Envisaged applications[17] are in biobleaching of pulp as a replacement for chlorine, removal of (poly)phenolic compounds and decolourisation of dyes in wastewater, amperometric biosensors for phenol detection, and many uses in the processing of foods and beverages.

An important role for laccases lies in the delignification of lignocellulose, the major constituent of wood.[18] To that purpose they are secreted by white rot fungi as extracellular glycosylated enzymes. In lignin degradation as well as in most applications, laccase alone, however, is ineffective, since it is too large for a molecule to penetrate the cell walls of wood and react with the lignin. Consequently, so-called mediators, low molecular weight electron transfer agents, are employed to shuttle electrons from the lignin to the enzyme. For example, 3-hydroxy anthranilic acid is produced by the white rot fungus *Pycnoporus cinnabarinus* and is believed to play the role of an electron mediator.[19] 2,2′-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ABTS was the first compound found to be capable of mediating the laccase catalysed oxidation of nonphenolic model compounds, such as the oxidation of veratryl alcohol to the corresponding aldehyde.[18] Subsequently, 1-hydroxybenzotriazole[20] and other *N*-hydroxy compounds such as *N*-hydroxyacetanilide, violuric acid and *N*-hydroxyphthalimide were shown to act as mediators.[21] A common feature of these mediators appears to be the propensity to form *N*-oxy(nitroxyl) radicals (see Fig. 1).

In 1996 it was shown that by using ABTS as a mediator, laccase from *Trametes versicolor* was able to catalyse the aerobic oxidation of a series of benzylic alcohols to their corresponding benzaldehydes.[22] Subsequently, Galli and

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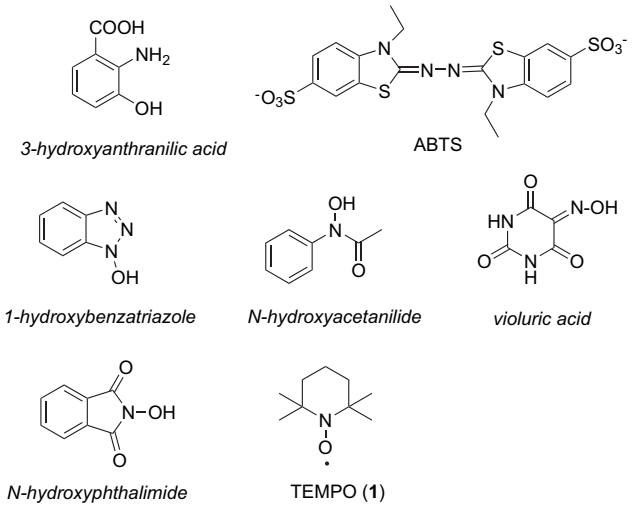

Figure 1. Laccase mediators.

co-workers found that the stable *N*-oxy radical, 2,2′,6,6′-tetramethylpiperidinyl-1-oxy (TEMPO, **1**, Fig. 1) in combination with laccase from *Trametes villosa*, catalyses the aerobic oxidation of primary benzylic alcohols.[23] The selective oxidation of the primary alcohol moiety in carbohydrates has been previously reported in two patents.[24] In a recent publication laccase from *Trametes pubescens* was used to oxidize a water-soluble cellulose sample.[25] In a subsequent comparison of various mediators, in the laccase catalysed aerobic oxidation of benzylic alcohols, TEMPO proved to be the most effective.[26]

The mechanistic details of these laccase/mediator catalysed aerobic oxidations are still a matter of conjecture.[27–29] However, they are generally believed to involve one electron oxidation of the mediator by the oxidised (cupric) form of the laccase followed by the reaction of oxidised mediator with the substrate, either via electron transfer (ET), e.g., with ABTS, or via hydrogen atom transfer (HAT), e.g., with *N*-hydroxy compounds, which forms *N*-oxy radicals.[30] TEMPO and its derivatives form a unique case: one electron oxidation of TEMPO affords the oxoammonium cation (**1a**), which oxidises the alcohol via a heterolytic pathway, giving the carbonyl product and the hydroxylamine **1b** (Fig. 2). The T1 copper centre in fungal laccases has a redox potential of ca. 0.8 V versus NHE (in contrast plant laccases exhibit redox potentials between 0.3 and 0.5 V). Consequently, fungal laccases are able to oxidise TEMPO to the corresponding oxoammonium cation (oxidation of **1a** to **1b**) since the oxidation potential of the latter, which was first measured by Golubev and co-workers, is 0.75 V.[31] This value was confirmed recently.[32]

There are various alternatives for reoxidising the hydroxylamine (**1b**) back to TEMPO (**1**) to complete the catalytic cycle. It can be oxidised by O_2, O_2/laccase or the oxoammonium cation (**1a**). Since it is known that (uncatalysed) reoxidation of **1b** is slow under acidic conditions,[33] we suppose that regeneration of **1** takes place via the laccase/O_2 route.

The active oxidant (**1a**) is the same as that in the TEMPO catalysed oxidation of alcohols with hypochlorite (or other single oxygen donors), a method that is widely used in the oxidation of a broad range of alcohols using low catalyst loadings (1 mol % or less).[34] In contrast, the laccase/TEMPO catalysed aerobic oxidations of alcohols require high loadings of TEMPO (typically 30 mol % on substrate), which militates against commercial viability.[23] The aim of this study was to obtain mechanistic information regarding the laccase/TEMPO catalysed aerobic oxidations of alcohols, by studying the isotope effect in water as well as in biphasic mixtures. Furthermore, we wanted to investigate whether the use of other, including more water-soluble, TEMPO-derivatives could increase the performance of the catalyst. Previously, hydroxy-TEMPO and acetylamino-TEMPO were also shown to be active in the laccase/TEMPO catalysed oxidation of benzylic alcohol. However, in this case the reaction was pursued up to 100% conversion in all cases, so that no differences in reactivity could be observed.[26]

2. Results and discussion

For the sake of convenience, TEMPO-like nitroxyl radicals are referred to as mediators (Med) and alcohols as substrates (Sub), although strictly speaking the nitroxyl is the substrate of the laccase according to the proposed mechanism (Fig. 2). The stable nitroxyl radicals shown in Figure 3 were tested as mediators. They are easily synthesised and/or commercially available.[35,36]

First the performance of these TEMPO-derivatives was tested under typical reaction conditions. These conditions were largely similar to those previously used,[23] except for the amount of TEMPO, which was only 10 mol % relative to substrate in the present case. In all experiments 0.1 M acetate buffer (AB) at pH 4.5 was used and the reactions were carried out at room temperature under an oxygen atmosphere. The conversion of benzyl alcohol in the presence of laccase and various mediators obtained after 24 h is given in Table 1. In all cases, selectivity to the aldehyde is >95%.

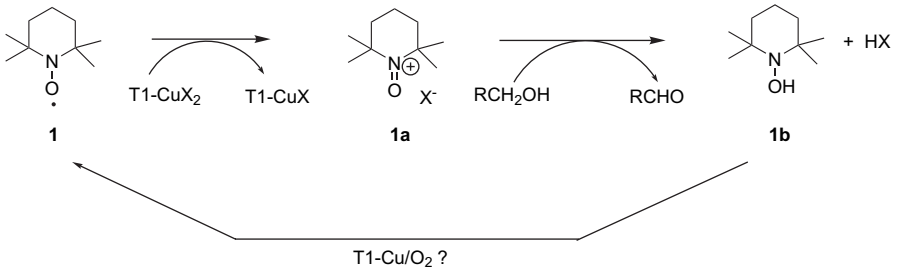

Figure 2. Pathway of laccase/TEMPO catalysed oxidation. T1 refers to Type I copper site.

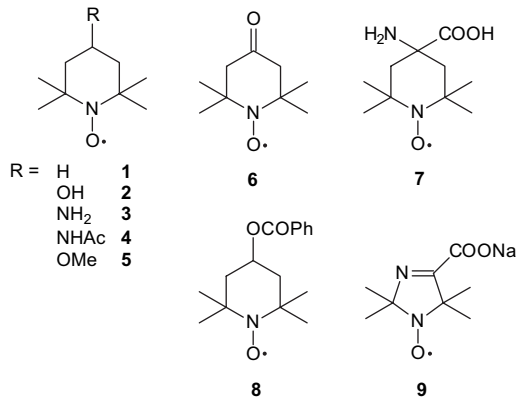

Figure 3. TEMPO and its derivatives used in this study.

Table 1. Aerobic oxidation of benzyl alcohol catalysed by laccase and nitroxyl radicals

Nitroxyl radical	Conv (%)
1	100
2	100
4	95.7
5	26
7	20.4
3	6.5
6	2.7
8	<1
9	<1

Conditions: $[Sub]_0$: 140 mM, Lac: 3.5 U/ml, [Med]: 14 mM, 24 h, rt, 1 atm O_2.

Among the nitroxyl radicals tested, oxo-TEMPO (**6**), amino-TEMPO (**3**), 4-hydroxy-TEMPO benzoate (**8**) and 2,2,5,5-tetramethyl-3-imidazoline-1-oxy-carboxylic acid sodium salt (**9**) showed very low activity. Methoxy-TEMPO (**5**) and 4-amino-4-carboxy-TEMPO (**7**) were moderately active. The conversion of benzyl alcohol was complete after 24 h in the systems mediated by TEMPO (**1**), hydroxy-TEMPO (**2**) and acetylamino-TEMPO (**4**). It was surprising that **8** showed no activity. This could be due to a high K_m of this mediator with the laccase.

The differences observed in Table 1 cannot be explained simply on the basis of redox potential (E^0). In Table 2 some published values of E^0 of TEMPO-derivatives are given.[31,37–39] The data in Table 2 were taken from different sources, and obtained under different conditions. Therefore, these values cannot be compared directly. We believe that the E^0 of TEMPO-derivatives generally lies around 0.75 V, which was the value obtained by Golubev and co-workers (see above) and thus all the E^0 values of the nitroxyl radicals (Table 2) are comparable to the E^0 of TvL. Only amino-TEMPO (**3**) exhibits a value, which is somewhat higher (0.89 V), but strongly pH-dependent.[37] We can state, however, that TvL has sufficient oxidation potential to oxidise the nitroxyl radicals and forms the corresponding oxoammonium ions as proposed in Figure 2.

Table 2. Redox potential of some nitroxyl radicals and laccase

Nitroxyl radical	E^0 (V) versus NHE	Ref.[a]
1	0.75, 0.74	31,37
2	0.80	37
3	0.89	37
6	0.78	38
TvL	0.78	11,39

[a] Values in Ref. 37 measured by CV at pH 5 in citrate buffer. Values at pH 7.4 for **1**, **2** and **3**, amount to 0.74, 0.80 and 0.82, respectively.

The oxoammonium salt of **6** was previously shown to be unstable in weakly acidic and basic medium.[40] Cyclic voltammetry revealed that it decomposed irreversibly into nonradical species at pH above 3.5. The low stability of **6**, in the presence of acids such as trichloroacetic acid, was also observed by Abakunov and Tikhonov.[41] A mixture of the corresponding hydroxylamine and a ring-opened nitroso compound was formed (Fig. 4). Presumably this involves initial disproportionation of the nitroxyl radical to the hydroxylamine and oxoammonium cation. The latter then undergoes ring opening to form the nitroso compound.

In our system, **6** is presumably oxidised by laccase to form its oxoammonium ion, and the latter undergoes ring cleavage to form a –N═O compound (Fig. 5), thus losing its oxidising ability. In acetate buffer, the oxoammonium cation can be coupled with the acetate anion leading to decomposition via the mechanism shown in Figure 5. Decomposition of **1a** under basic aqueous conditions was previously described by Golubev and co-workers[31] leading to similar nitroso compounds. In the end as a result of intra- and intermolecular processes, about 2/3 of the cation **1a** was reduced under basic conditions to the radical and the rest was transformed into a mixture of nitrones and their condensation products.

In this context, the higher reactivity of **1** and **2** is probably a result of the higher stability of their oxoammonium ions. In the case of **3**, the situation is more complicated. Under acidic conditions, first **3** will be converted into the corresponding oxoammonium cation either by disproportionation (compare Fig. 4) or by laccase oxidation. The amino group, either in the nitroxyl radical structure or in the oxoammonium cation, can then be oxidised by the oxoammonium cation. In addition, it is reported that amines can react easily with nitroxyl radicals in an acid medium.[42,43] This is confirmed by the results of Nakatsuji co-workers,[44] who reported that **3** is more readily oxidised than **1** and **2**. They found by cyclic voltammetry that **1** and **2** showed only one oxidation potential corresponding to nitroxyl radical, while **3** showed two oxidation potentials related to the oxidation of the amino group and the nitroxyl radical, respectively.

We studied the stability of **3** spectrometrically in acidic medium. When **3** was incubated in acetate buffer at pH 4.5, the absorbance maximum at 430 nm increased (Fig. 6a). This could be due to the formation of the –N═O group (unfortunately we could not isolate the product). On the other hand, there was no absorbance change observed for **1** under the same conditions (Fig. 6b). Another possibility is oxidation of the amine to the imine. In this case, a similar

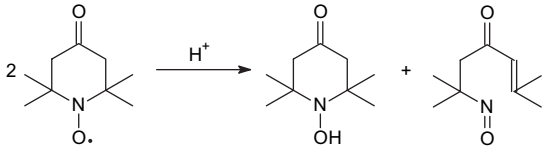

Figure 4. Interaction of **6** with acid.

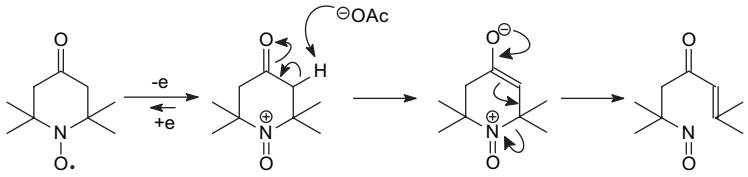

Figure 5. Postulated route for decomposition of **6** in acidic medium.

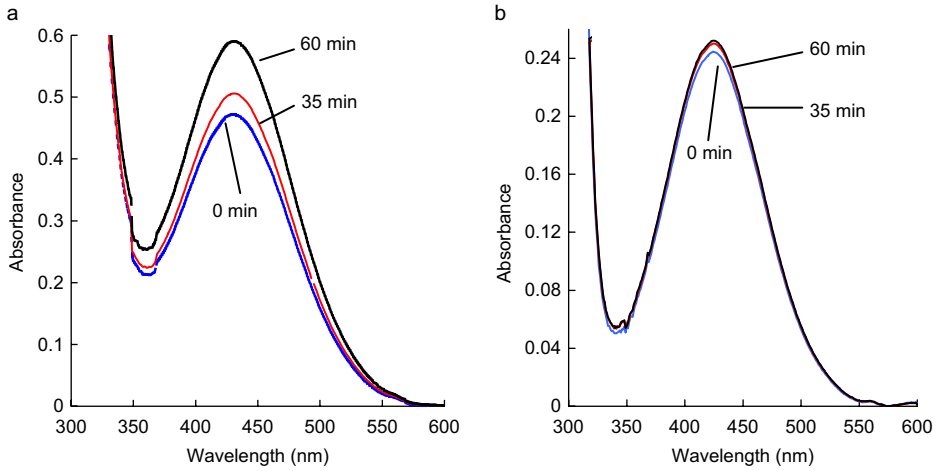

Figure 6. Absorption as a function of time of **3** (a) and **1** (b) in acetate buffer at pH 4.5.

instability as observed for oxo-TEMPO (see above) can be anticipated.

When the amino group in **3** is protected with an acetyl group, the resulting amide moiety is stable towards oxidation. Hence, **4** was an efficient mediator. It is not clear why **5** and **7** display intermediate activities. Especially the cation of **5** is known to be a good oxidant.[45,46] We suggest that decomposition of the cation of **5**, is probably faster than that of **1a**, leading to lower activities.

For a better comparison of mediators **1**, **2** and **4**, the reaction was followed in time. Figure 7 shows that **2** and **4** display similar activity in the oxidation of benzyl alcohol, whereas **1** is more active. In case of **1**, already 87% conversion was observed after 7 h, while for **2** and **4**, the conversion only amounted to 56 and 44% at this point. In all cases the reaction was completed after 24 h.

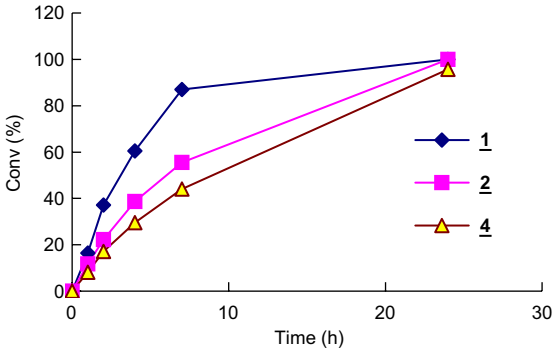

Figure 7. Comparison of nitroxyl radicals in the laccase catalysed aerobic oxidation of benzyl alcohol. $[Sub]_0$: 125 mM, Lac/Sub: 5.9 U/ml, [Med] 19 mM, rt, 1 atm O_2.

In Table 3, an overview of the reactivities of various TEMPO-derivatives with the secondary alcohol 1-phenylethanol is given. Surprisingly, in this case **2** and **4** displayed higher activity than **1**. The difference was more pronounced in water (pH 4.5)/toluene (W/T=1/1) mixtures than in aqueous buffer. Again, no activity was observed for **3**. It was expected that by adding more organic solvent to the system (the alcohols themselves already form a separate phase) the whole oxoammonium reaction could be moved from the aqueous layer to the organic layer and the reaction rate could be improved. However, it transpired that by adding toluene to the system, the reaction rate is retarded considerably. This could be due to a lower activity of the enzyme at the interphase layer.

Table 3. Conversion of 1-phenylethanol by laccase-nitroxyl radicals catalysed aerobic oxidation

Mediator	Acetate buffer[a]	W/T (1/1)[b,c]
1	66.8	27.6 (44.0)
2	76.8	60.7 (80.8)
4	78.5	81.7
5	2.4	—
3	2.4	—
9	—	3.9 (4.2)

Conditions: $[Sub]_0$: 125 mM, Lac: 5.9 U/ml, [Med]: 19 mM, rt, 1 atm O_2.
[a] Reaction time: 5.5 h.
[b] Reaction time: 24 h.
[c] Data in parentheses were obtained after 48 h.

To confirm the difference in overall reactivities between **1**, **2** and **4** for benzyl alcohol and 1-phenylethanol, respectively, competition experiments were performed in which both substrates were present. In Table 4, the conversions of benzylic alcohol and 1-phenylethanol as well as the ratio of the overall rate constants are denoted. The overall rates were denoted

Table 4. Competition reaction of benzyl alcohol and 1-phenylethanol

Mediator	AB (5 h)		W/T (1/1) (23 h)	
	$Conv_1/Conv_2$	k_1/k_2	$Conv_1/Conv_2$	k_1/k_2
1	76.8/35.2	3.4	96.5/21.9	13.6
2	40.6/32.1	1.3	98.9/68.9	3.9
4	51.6/31.8	1.9	97.4/50.5	5.2

Conditions: equimolar amounts of benzyl alcohol and 1-phenylethanol were applied, $[Sub]_0$ 62.5 mM, Lac: 2.9 U/ml, [Med]: 9.3 mM. The ratio k_1/k_2 was calculated according to $k_1/k_2=\ln(1-Conv_1)/\ln(1-Conv_2)$.

as k_1 and k_2 for benzylic alcohol and 1-phenylethanol, respectively.

The observations made in separate experiments were confirmed. In all cases benzyl alcohol was converted 1–3 times faster than 1-phenylethanol. When toluene was added to the medium, this difference was even larger.

2.1. Kinetic isotope effects

Further evidence for the alcohol oxidation mechanism was sought in kinetic isotope effects. Therefore, the primary kinetic isotope effects (k_H/k_D) were measured in the laccase-nitroxyl radical catalysed oxidation of α-monodeutero-*p*-methylbenzyl alcohol. We used this technique previously to unravel the mechanism of Ru/TEMPO[47] and Cu/TEMPO[48] catalysed oxidation of alcohols. A k_H/k_D value for the room temperature oxidation of α-monodeutero-*p*-methylbenzyl alcohol is 2.05, which was measured in acetate buffer at pH 4.5 (Table 5). Under biphasic conditions, using a mixture of toluene and water as solvent the k_H/k_D value was slightly higher and amounted to 2.32. These values are much lower than those obtained for laccase/violuric acid, laccase/1-hydroxybenzotriazole and laccase/*N*-hydroxyphathalimide reactions. In these cases k_H/k_D values of 6.2–6.4 were observed, which is in line with a radical hydrogen atom transfer route of oxidation.[49]

Table 5. Kinetic isotope effect (k_H/k_D) in the laccase/TEMPO/O_2 catalysed oxidation of α-monodeutero-*p*-methylbenzyl alcohol

Entry	Catalyst	AB (pH 4.5)	W/T (1/1)
1	Laccase/TEMPO	2.05	2.32
2	$TEMPO^+$–ClO_4^-	3.58	2.97
3	Laccase/4-hydroxy-TEMPO	2.54	2.61
4	Laccase/4-acetylamino-TEMPO	2.51	3.19

Substrate: 125 mM, [Med]: 18.8 mM, Lac: 5.9 U/ml, $TEMPO^+$–ClO_4^-: 1 equiv, 24 h; AB: acetate buffer pH 4.5; W/T: water/toluene (1/1).

We thus observed that the k_H/k_D ratio of benzylic alcohol oxidation by TEMPO and laccase is in the range expected for oxoammonium ions. Usually these values lie between 1.7 and 2.3[50] under basic conditions, and 3.1[51] under acidic conditions. This low kinetic isotope effect is in accordance with a transition state of the process in which first oxoammonium forms a complex with the alcohol, after which intramolecular transfer of hydrogen takes place, as illustrated in Figure 8.[34] The exact value for k_H/k_D is dependent on solvent, pH and the counterion. For that reason we also measured the k_H/k_D value for α-monodeutero-*p*-methylbenzyl alcohol, using separately prepared $TEMPO^+$–ClO_4^- under identical conditions (see entry 2, Table 5). In acetic buffer, as well as under biphasic conditions using a mixture of toluene and water, slightly higher values were obtained using $TEMPO^+$–ClO_4^- (3.6 and 3.0, respectively). We think that the nature of the counterion is essential in determining the k_H/k_D value. The counterion in the case of our laccase/TEMPO system has not been established, but it seems most likely that acetate ions function as counterion. For the sake of completeness, data using other TEMPO-derivatives were also measured. The use of 4-hydroxy-TEMPO and 4-acylamino-TEMPO leads to kinetic isotope effects, which are identical in acetate buffer (2.54 and 2.51, respectively) and even higher under biphasic conditions, 2.61 and 3.19, respectively.

3. Conclusions

A number of stable TEMPO-like nitroxyl radicals were tested as mediators in the laccase catalysed aerobic oxidation of alcohols. Among them TEMPO (**1**) itself, as well as its hydroxy (**2**) and acetylamino (**4**) derivative are the most effective mediators, both in pH 4.5 buffered media as well as in buffer/toluene mixtures. The stability of the *N*-oxy radical and/or its resulting oxoammonium ions at pH 4.5 is limited for derivatives such as oxo-TEMPO and amino-TEMPO, resulting in very low conversion of alcohols. Kinetic isotope effect studies for **1**, **2** and **4** were performed for a primary benzylic alcohol, and values range from 2.1–3.2 in various media. These k_H/k_D values are characteristic of the ionic mechanism known in literature for alcohol oxidations with oxoammonium ions, and were verified in separate experiments under the current reaction conditions.

Competition studies indicate that, for all mediators, the primary alcohol reacts faster than the secondary alcohol. Also this observation is in line with literature data.[34] The overall reaction kinetics cannot be fully understood at this point because the concentration of the oxoammonium ions will depend on both the interaction with laccase as well as on its rate of regeneration by laccase (or otherwise). Future studies will be directed towards determining the rate constants of the individual steps.

4. Experimental

4.1. Enzymes and chemicals

Laccase from *T. versicolor* (TvL) was purchased from Juelich Fine Chemicals as a lyophilised powder. The content

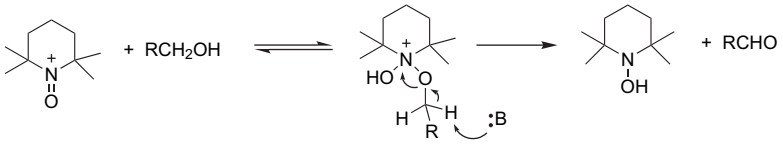

Figure 8. Mechanism for oxoammonium catalysed oxidation of alcohols under acidic conditions.

of the protein is 8.8 mg/g solid (data from the enzyme supplier). TEMPO was obtained from BASF, Germany. The other nitroxyl radicals were purchased from Acros. 2,2′-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) was purchased from Fluka. 1-Phenylethanol and benzyl alcohol were distilled before use. Other chemicals were purchased from Aldrich or Acros and used as received. Acetate buffer (0.1 M) at pH 4.5 was used for preparing solutions for the activity assay and as oxidation reaction medium unless otherwise specified.

4.2. Preparation of $TEMPO^+$–ClO_4^- [52]

To a suspension of TEMPO (3.95 g, 25.3 mmol) in water (20 ml) was added dropwise a solution of 70% $HClO_4$ (3.66 g, 25.6 mmol) in water (5 ml) under ice-cooled conditions in 15 min, followed by addition of 15% NaOCl solution (6.4 g, 12.8 mmol) with ice-cooling in 10 min. The slurry of the reaction mixture was stirred for further 1 h at below 4 °C, and then the yellow precipitate was filtered and washed with 5% $NaHCO_3$ solution, ice-water and ether. The solid was dried under vacuum overnight to give the product $TEMPO^+$–ClO_4^- 3.0 g (yield 46.4%). Mp 148–149 °C.

4.3. Laccase activity assay

The laccase activity was determined spectroscopically at 25 °C using ABTS as substrate. To an UV cuvette, a certain amount of ABTS (4.3–9.6 mg) was added to 2.0 ml of 0.1 M acetate buffer (pH 4.5) containing 2.0–7.0 μg of laccase. The absorbance change at 420 nm was recorded for 5 min (ε_{420}=36,000 M^{-1} cm^{-1}).[53] One unit (U) of the enzyme was defined as 1 μmol ABTS oxidised per minute under the stated assay conditions.

4.4. Typical procedure for alcohol oxidation by laccase-nitroxyl radical

A mixture of benzyl alcohol (54 mg, 0.5 mmol), dodecane (34 mg, internal standard), laccase (24.5 mg, 47 U) and TEMPO (11.7 mg, 0.075 mmol) in acetate buffer (pH 4.5, 4 ml) was placed in a 10 ml glass vial. The vial was connected with an oxygen source to keep the system under atmospheric pressure of oxygen. The mixture was stirred at room temperature for a certain time. After reaction, the mixture was washed with diethyl ether (2×4 ml). The organic solution was dried with anhydrous sodium sulfate, centrifuged and analysed with GC.

4.5. Kinetic isotope effect studies

Intramolecular kinetic isotope effect (k_H/k_D): α-monodeutero-*p*-methylbenzyl alcohol (125 mM) was used as the substrate in the above procedure. TEMPO 18.8 mM; laccase 47 U/mmol. After complete conversion of alcohol to aldehyde (monitored with TLC after 24 h), the reaction mixture was quenched with MTBE and dried over Na_2SO_4. Removal of the solvent under vacuum gave a mixture of TEMPO and *p*-methylbenzaldehyde. Both labelled and unlabelled aldehydes were isolated and purified by column chromatography using petroleum/CH_2Cl_2 (5/5). The k_H/k_D was determined by ^1H NMR by measuring the intensity of the α-proton.

4.6. Analysis methods

Alcohol conversion was analysed by GC with column WAX 52 CB (on Varian 3400 CX) or Sil 5 CB (on Varian STAR 3400). FID detector and temperature program (70 °C for 9 min, then increased at a rate of 10 °C/min to 250 °C for 6 min) were used on either column. Dodecane or hexadecane was used as an internal standard. The products were characterised by GCMS.

References and notes

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