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Aerobic oxidation of isosorbide and isomannide employing TEMPO/laccase†

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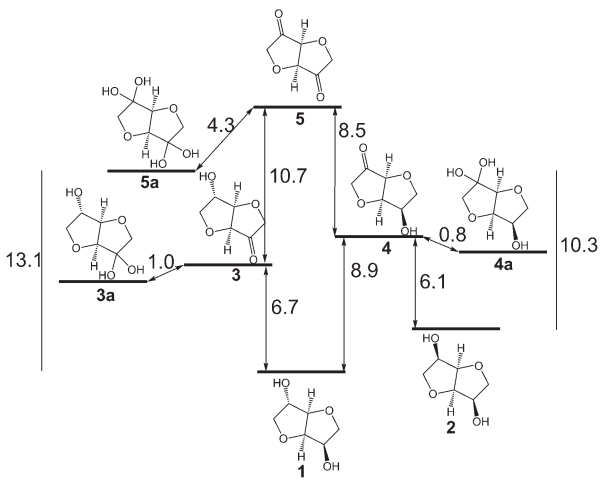
The oxidation of the renewable diols isosorbide and isomannide was successfully achieved using a TEMPO/laccase system. Furthermore, various TEMPO-derivatives were tested leading to conversions of up to >99% for the oxidation of isosorbide, isomannide, indanol and a halohydrin to the corresponding ketone.

The diols isosorbide **1** and isomannide **2** (Scheme 1) are both gained from renewable resources like cellulose[1] and starch.[2] They represent platform chemicals for the potential replacement of oil-based products.[3] Consequently, they are in focus of interest as starting materials for pharmaceutical applications as well as for organic solvents or fuels and for biopolymers.[3,4] For instance, recently, **1** was derivatised to the corresponding diamine as a building block for polymers,[5] or oxidised by chemical means.[6] Amination of **1** by a ADH/transaminase biocascade led to only a low amount of mono-amination.[7] Especially the transformation of isosorbide to valuable derivatives by functionalization or substitution of both hydroxyl groups is difficult due to steric hindrance and different reactivities of the two hydroxyl groups.



Scheme 1 Relative Gibbs free energies (kcal mol^{-1}) for the oxidation and hydration of isosorbide **1** and isomannide **2**. Energies for oxidations were calculated for the reaction of the alcohol with acetone yielding the ketone plus 2-propanol. The calculations were performed using the MP2/cc-pVTZ//MP2/cc-pVDZ procedure in aqueous solution (IEFPCM solvation model).

Results and discussion

Alcohol oxidation employing alcohol dehydrogenases (ADHs)[7,8] represents one possible common biocatalytic method to prepare ketones or aldehydes from the corresponding alcohols.[9] Consequently, various ADHs were investigated to oxidise isosorbide **1** as well as isomannide **2** to the corresponding diketone **5**. However, the levels of conversion were unsatisfactory. A maximum of 4% conversion was reached at 50 mM substrate concentration over 24 hours. Calculating the required Gibbs free energy for the oxidation revealed that the energy needed for a mono-oxidation to the hydroxy ketone followed by hydration to the favoured hydrate (**3a**, **4a**) is rather high (Scheme 1), even higher than for the disfavoured biocatalytic oxidation of halohydrins by ADHs (4–6 kcal mol^{-1}).[10] For instance, for the oxidative hydrogen transfer of isosorbide **1** to the *exo*-hydroxy ketone **3** 6.7 kcal mol^{-1} are required while 8.9 kcal mol^{-1} are needed to get the *endo*-hydroxy ketone **4**. Considering the hydrated forms (**3a**, **4a**), the energy decreases by approximately 1 kcal mol^{-1}, still representing a significant burden for the hydrogen transfer to yield high amounts of ketone. The higher energy needed to get the *endo*-hydroxy ketone **4** can be explained by the higher steric hindrance of the hydride to be removed at the *exo*-hydroxy of **1**. Also for the second oxidation (transformation of **3** and **4**, respectively, to diketone **5** and its hydrate **5a**) a significant energy barrier has to be overcome. To address the

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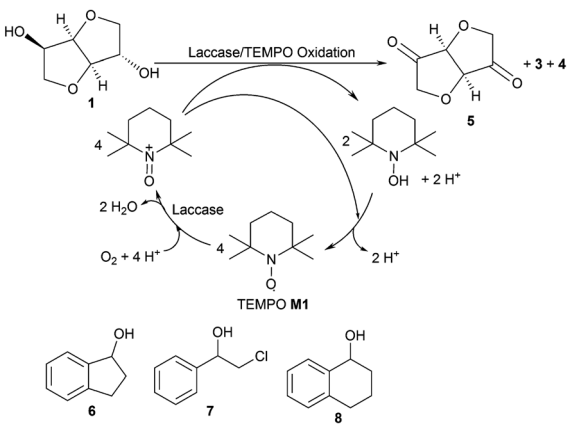
problem of steric hindrance for the oxidation of the *exo*-hydroxy moiety as well as to provide sufficient energy, an oxidation method was needed involving a small mediator as an oxidizing agent and a high energy terminal oxidant like molecular oxygen. In case of success, the same approach could be applied for the oxidation of compounds **6–8**, which are also difficult susbtrates for ADHs.

Consequently, the combination of laccase and TEMPO **M1** as a mediator was tested employing O_2 as an oxidant (Scheme 2).[11] Laccases[12] can be produced in large quantities,[13] and the oxidation system can be employed at ambient temperature (20–30 °C) and in aqueous medium. Although TEMPO has been employed for the oxidation of various *sec*-alcohols using various chemical reoxidation reagents,[14] *sec*-alcohols have only been sparsely oxidised with the TEMPO/laccase system.[15] TEMPO enables an aerobic green oxidation.[16]

In a first attempt, commercial laccases from *Trametes versicolor* (TvLac) and *Agaricus bisporus* (AbLac) were applied using sodium acetate buffer at varied pHs or in deionised water (Table 1). Best results were achieved with TvLac while AbLac led to very low conversions. Based on the published sequences of laccases from these fungi, they are similar in size (TvLac: 519aa, AbLac: 520aa), but have only 47.5% sequence identity.[17] A redox potential is only published for TvLac ($E^{0\prime}$ = 785 mV *vs.* normal hydrogen electrode).[18] TEMPO/TvLac oxidised the two equal hydroxy groups of isomannide **2** as well as the two energetically different hydroxy groups of isosorbide **1** to the diketone **5** at pH 5.5 and 6.5. For **1** both possible intermediates – the *endo*- and the *exo*-hydroxyyketone – were observed at pH 4.5, indicating that in the first oxidation step no absolute discrimination between the two hydroxy moieties occurred; for **2** obviously only the *endo*-hydroxyketone can be formed due to the stereochemistry of the substrate. Blank experiments showed that in the absence of an enzyme or a mediator no product formation was observed. Interestingly, just using water without buffer salts (last entry, pH 6.5) led to perfect conversion, offering another opportunity to minimize waste.



Scheme 2 Oxidation of isosorbide **1** employing TEMPO/laccase and further substrates investigated.

Table 1 Oxidation of isosorbide **1** and isomannide **2** applying TEMPO/laccase[a]

Substr.	Buffer	pH	Laccase[b]	*endo*-OH-ketone **4** [%]	*exo*-OH-ketone **3** [%]	Diketone **5** [%]
1	NaOAc	5.5	None	<0.1	<0.1	<0.1
1	NaOAc	4.5	AbLac	<0.1	<0.1	<0.1
1	NaOAc	5.5	AbLac	1	<0.1	<0.1
1	H_2O dist.	6.5	AbLac	2	<0.1	<0.1
1	NaOAc	4.5	TvLac	8	13	79
1	NaOAc	5.5	TvLac	<0.1	<0.1	>99
1	NaOAc	6.5	TvLac	<0.1	<0.1	>99
1	NaOAc	5.5	TvLac[c]	<0.1	<0.1	<0.1
2	NaOAc	4.5	AbLac	4	<0.1	<0.1
2	NaOAc	4.5	TvLac	23	<0.1	77
2	NaOAc	5.5	TvLac	<0.1	<0.1	>99
2	H_2O dist.	6.5	TvLac	<0.1	<0.1	>99

[a] Relative amounts as peak areas measured by GC-FID. Substrate (50 mM), laccase (10 U), TEMPO (30 mol%), 24 h at 30 °C, 120 rpm. [b] TvLac = Laccase from *Trametes versicolor*; AbLac = *Agaricus bisporus*. [c] In the absence of TEMPO.

Isomannide **2** was in general transformed faster than **1**. Although these first tests gave promising results, optimisation studies were required to decrease the concentration of the mediator and the enzyme.

In the next experiments the TEMPO concentration was lowered to 5 mol%. In two buffer systems (acetate and phosphate) the highest conversion was achieved at low buffer concentration (Fig. 1). In general, best results were obtained at pH 6 and low buffer concentrations (5–10 mM).

Interestingly, for bis-tris buffer and sodium citrate buffer conversion did not exceed 3%, although oxidation of glycols and various alcohols is reported using sodium citrate buffer at a pH of 4.5 and high buffer concentrations.[15c] Obviously these conditions are not favourable for isosorbide or isomannide as a substrate. Further decrease of the buffer concentration to 0.5–1.5 mM improved the conversion and led to full diketone formation as was observed in the deionised water-system.

To minimize the concentration of the mediator, the TEMPO concentration was decreased successively. Reducing the TEMPO concentration from 30 mol% to 4 mol% (mol mediator/mol substrate) did not lead to a significant decrease of the diketone formation employing isosorbide **1** as the substrate over 24 hours under the conditions employed. However, as soon as the mediator concentration was below 4 mol%, conversion decreased rapidly (Fig. 2).

To identify the most suitable mediator,[15a,19] a selection of different mediators **M2–M15** were tested (Fig. 3).

However, for isosorbide **1** as a substrate, TEMPO **M1** was superior to all other TEMPO-derivatives **M2–M15**.

Investigating the substrate scope of the oxidation method, also 1-indanol **6** was tested. Using **6** as a substrate, TEMPO **M1** led to 45% conversion at 50 mM substrate concentration within 4 hours. For this substrate, a number of mediators proved to be superior compared to **M1** (Fig. 4): for instance, **M3** led to 99% conversion, **M4** gave 65% conversion, **M8** 76%,

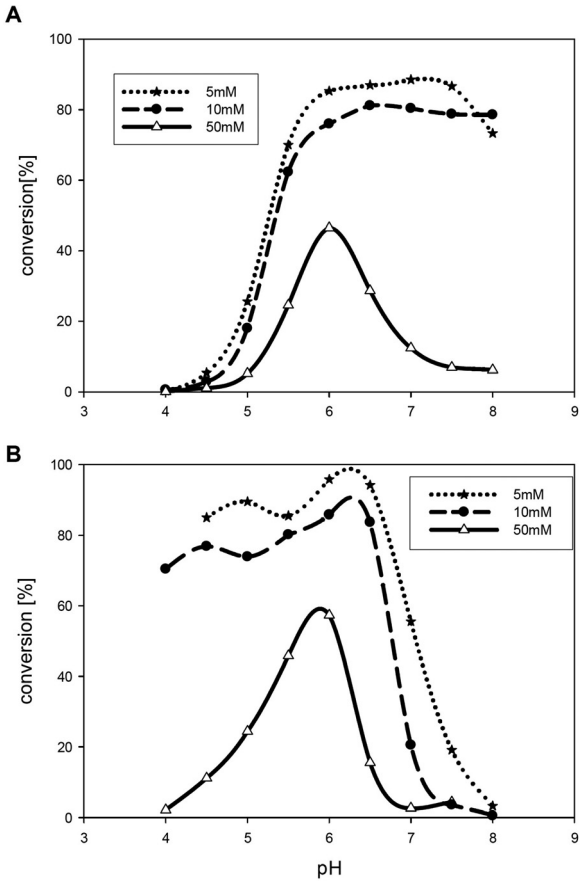


Fig. 1 Conversions at varied pH in acetate buffer (A) and phosphate buffer (B) (5 mM, 10 mM and 50 mM) using **1** as a substrate. Conversions were measured by GC-FID. Reaction conditions: TEMPO (5 mol%), isosorbide (50 mM), TvLac (0.5 mg ml^{-1}); 24 h at 30 °C at 120 rpm. Lines between the measured points are only drawn as a guide for the eye to ease reading and not to interpolate values.

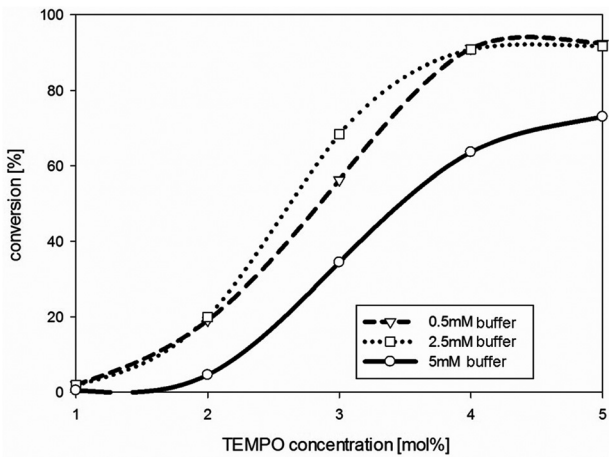


Fig. 2 Variation of TEMPO concentration in phosphate buffer (0.5, 2.5 and 5 mM, pH 6) using isosorbide **1** as a substrate. Conversions measured by GC-FID. Reaction conditions: substrate (50 mM); TvLac (0.5 mg ml^{-1}), 24 h at 30 °C, 120 rpm. Lines between the measured points are only drawn as a guide for the eye to ease reading and not to interpolate values.

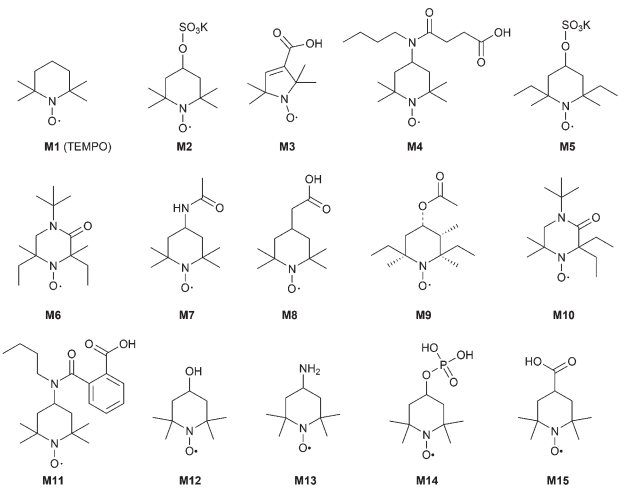


Fig. 3 Selection of mediators: TEMPO and derivatives.

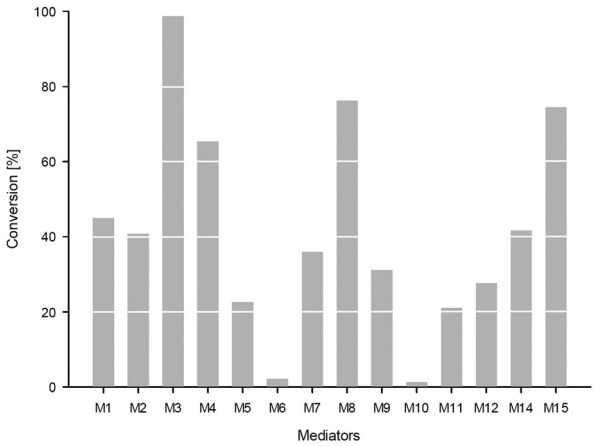


Fig. 4 Oxidation of 1-indanol **6** (50 mM) employing different mediators (5 mol%) in phosphate buffer (pH 6, 5 mM) employing TvLac (0.5 mg ml^{-1}); 4 h at 30 °C, 120 rpm. Conversions were measured by GC-FID.

and mediator **M15** 75%. It is noticeable that all these mediators possess a carboxylic acid moiety. Mediator **M2** (41%) and mediator **M14** (42%) led to results comparable to TEMPO **M1**.

Increasing the mediator concentration of TEMPO **M1** to 10 mol%, 1-indanol **6** was quantitatively oxidised within 24 hours (Fig. 5). In contrast, the halohydrin 2-chloro-1-phenyl ethanol 7 was oxidised only efficiently in the presence of elevated TEMPO concentration (45 mol%) giving 87% conversion after 24 hours.

While indanol (50 mM) could be oxidised using **M3** as a mediator at only 2.5 mol% with full conversion to the corresponding ketone within 24 hours, substrate 7 led with **M3** only to 4% conversion under the same conditions. On the other hand, 1,2,3,4-tetrahydronaphthalen-1-ol **8** was oxidised with 88% with 2.5 mol% **M3**. The results leading to highest conversions are summarized in Table 2.

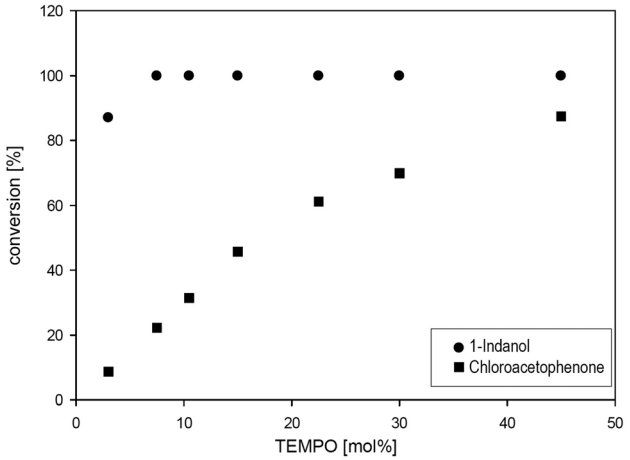


Fig. 5 Variation of the concentration of **M1** in phosphate buffer (pH 6, 5 mM) for the oxidation of 1-indanol **6** and chloroacetophenone **7** (50 mM). Conversions were measured by GC-FID. Conditions: TvLac (0.5 mg ml^{-1}), 24 h at 30 °C, 120 rpm.

Table 2 Optimised conditions for oxidation[a]

Substr.	Mediator	Mediator conc. [mol%]	Conv. [%]
1	TEMPO **M1**	5	>99[b]
2	TEMPO **M1**	5	>99[b]
6	**M3**	2.5	>99
7	**M3**	45	87
8	**M3**	2.5	88

[a] Conditions: 50 mM substrate, laccase (10 U), phosphate buffer (5 mM, pH 6) 24 h, 30 °C, 120 rpm. [b] >99% of diketone **5** were obtained.

Conclusion

Full conversions were achieved for the di-oxidation of the renewable platform compounds isosorbide **1** and isomannide **2** to the corresponding diketone **5** using a TEMPO/laccase system. Best results were obtained for instance in phosphate buffer (2.5 mM, pH 6.0) with 5 mol% of TEMPO. Alternative mediators were not as efficient as TEMPO for diols **1** and **2**, but mediator **M3** proved to be superior to TEMPO for the oxidation of indanol **6** and 1,2,3,4-tetrahydronaphthalen-1-ol **8**. Additionally, employing a buffer with a very low salt content (2.5 mM) minimizes the amount of resources required.

Experimental

Reaction conditions for isosorbide 1/isomannide 2

TEMPO/acetone-solution (15 µl, mediator concentration in the reaction: 2.5 mM) was transferred into Sarstedt tubes (15 ml) and acetone was evaporated. Then phosphate buffer (980 µl, 2.5 mM, pH 6 containing 50 mM isosorbide **1** or isomannide **2**) was added and the oxidation was started by addition of the enzyme solution (20 µl, end concentration 0.5 mg ml^{-1}). Reactions took place at 30 °C and 120 rpm on a rotatory shaker. For work-up the samples were lyophilised, extracted with acetone (1 ml) and centrifuged (13 000 rpm, 3 min at room temperature). The organic layer was dried (Na_2SO_4) and analysed on GC-FID. Products were identified by comparison with authentic reference material.

Reaction conditions for alcohols 6–8

TEMPO/acetone-solution (15 µl, mediator concentration in the reaction: 2.5 mM) was transferred into Sarstedt tubes (15 ml) and acetone was evaporated. Then phosphate buffer (980 µl, 2.5 mM, pH 6) as well as the substrate (50 mM) was added and the oxidation was started by addition of the enzyme solution (20 µl, end concentration 0.5 mg ml^{-1}). Reactions took place at 30 °C and 120 rpm on a rotatory shaker. For work-up the samples were extracted with ethyl acetate (2 × 500 µl) and centrifuged (13 000 rpm, 3 min at room temperature). The organic layer was dried (Na_2SO_4) and analysed on GC-MS. Products were identified by comparison with authentic reference material.

Acknowledgements

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