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COMMUNICATION

One-pot chemoenzymatic synthesis of aldoximes from primary alcohols in water

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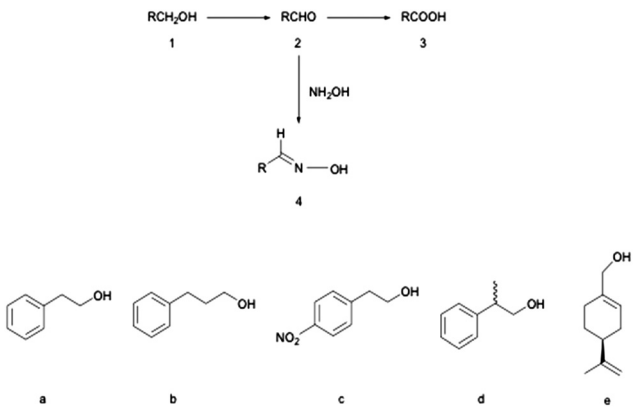
A new synthetic method for the one-pot preparation of aldoximes in water was developed; the method is based on the combination of the enzymatic oxidation of primary alcohols to aldehydes using different acetic acid bacteria and *in situ* condensation of the aldehydes with hydroxylamine.

Enzymatic oxidation of alcohols can be catalytically efficient and may offer distinct advantages over classical chemical synthetic methods, since it can be carried out under mild and ecologically compatible conditions, being often chemo-, regio- and enantioselective.[1] Oxidation of primary alcohols catalyzed by membrane-bound alcohol and aldehyde dehydrogenases of acetic acid bacteria generally leads to carboxylic acids or ketones.[2,3] Acetic acid bacteria, which are generally regarded as safe (GRAS) microorganisms, can also be employed to perform the partial oxidation of primary alcohols to aldehydes, using strains lacking aldehyde dehydrogenase activity or when systems for the *in situ* extraction of the aldehydes are applied.[4] Otherwise, enzymatic oxidation of primary alcohols to aldehydes can be obtained by using isolated enzymes.[3,5]

In this work, aldoximes were prepared using a one-pot chemoenzymatic reaction which combines the oxidation of primary alcohols by acetic acid bacteria with the subsequent condensation of the aldehyde with hydroxylamine; this protocol allows the preparation of the corresponding aldoximes (Scheme 1).

Aldoximes have wide application in medicine, industry and analytical chemistry.[6] Moreover, they are very useful and versatile intermediates in synthetic organic chemistry. Notably, they can be reduced to amines[7] or oxidized to nitrile oxides,[8] which are in turn precursors for the synthesis of isoxazoles. In addition, they can undergo Beckmann rearrangement leading to the corresponding amides.[9] Aldoximes can be chemically[10] or enzymatically[11] converted into nitriles through a dehydration reaction. Finally, it has also been reported that dehydrogenases can catalyse the reduction of phenylacetaldoxime to the corresponding alcohol.[12] One-pot preparation of aldoximes from primary alcohols deserves special interest since often aldehydes have low stability, making it difficult to prepare them in high yields.[13] Thus, a method where aldehydes are not isolated, but *in situ* directly converted into aldoximes seems advantageous.



Scheme 1 One-pot chemoenzymatic synthesis of aldoximes from primary alcohols.

Three strains of acetic acid bacteria (belonging to different genera) have been used in this study: *Acetobacter* sp. MIM 2000/61, *Gluconobacter oxydans* DSM 2343 and *Asaia bogorensis* SF2.1. *G. oxydans* DSM 2343 had been previously used for the preparation of aliphatic and aromatic aldehydes with isolated yields ranging from 43% to 82%.[4c] *Acetobacter* sp. MIM 2000/61 was recently isolated from vinegar and demonstrated good oxidative capacities. *Asaia bogorensis* SF2.1 showed some unusual properties for acetic acid bacteria such as rapid growth and low production of acetic acid and had never been used before for biotransformation of primary alcohols.[14]

Our initial studies were aimed at finding the most suitable conditions for the one-pot chemoenzymatic reaction; 2-phenyl-1-ethanol (**1a**) was initially chosen as a substrate, since it can be easily converted into phenylacetic acid (**3a**) by different strains of acetic acid bacteria[2e,15] and into phenylacetaldehyde (**2a**) by *G. oxydans* DSM 2343, as reported in previous work from our group.[4c] Biotransformations were performed starting from 10 mM of substrate with and without $NH_2OH{\cdot}HCl$, using the same amount of cells (dry weight = 10 g L^{-1}) at 28 °C in 0.1 M phosphate buffer, pH 7.5 (Table 1).

Biotransformations carried out without NH_2OH gave mostly the corresponding aldehyde **2a** with *G. oxydans*, as previously

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Table 1 Oxidation of 2-phenyl-1-ethanol (**1a**), 3-phenyl-1-propanol (**1b**), *p*-nitro-2-phenyl-1-ethanol (**1c**) and 2-phenyl-1-propanol (**1d**) with acetic acid bacteria with or without addition of hydroxylamine. Starting concentration of the alcohols was 10 mM

Entry	Strain	Substrate	Equivalents of NH_2OH	**2** (%)	**3** (%)	ee (%)	**4** (%)	ee (%)	Time (h)
1	*Acetobacter* sp.	**1a**	0	0	100		0		1
2	*Acetobacter* sp.	**1a**	1	0	0		94		48
3	*Gluconobacter oxydans*	**1a**	0	75	21		0		5
4	*Gluconobacter oxydans*	**1a**	1	0	0		96		48
5	*Asaia bogorensis*	**1a**	0	0	48		0		48
6	*Asaia bogorensis*	**1a**	1	0	0		68		48
7	*Acetobacter* sp.	**1b**	0	0	100		0		1
8	*Acetobacter* sp.	**1b**	1	0	0		97		5
9	*Gluconobacter oxydans*	**1b**	0	52	37		0		5
10	*Gluconobacter oxydans*	**1b**	1	0	0		86		24
11	*Asaia bogorensis*	**1b**	0	0	38		0		48
12	*Asaia bogorensis*	**1b**	1	0	0		80		24
13	*Acetobacter* sp.	**1c**	0	0	93		0		5
14	*Acetobacter* sp.	**1c**	1	0	0		65		48
15	*Gluconobacter oxydans*	**1c**	0	0	17		0		48
16	*Gluconobacter oxydans*	**1c**	1	0	0		32		48
17	*Asaia bogorensis*	**1c**	0	0	91		0		24
18	*Asaia bogorensis*	**1c**	1	0	0		12		24
19	*Acetobacter* sp.	**1d**	0	0	50	92 (*R*)	0		24
20	*Acetobacter* sp.	**1d**	1	0	0		45	92 (*R*)	48
21	*Gluconobacter oxydans*	**1d**	0	8	0		0		48
22	*Gluconobacter oxydans*	**1d**	1	0	0		34	38 (*R*)	48
23	*Asaia bogorensis*	**1d**	0	0	94	<5	0		24
24	*Asaia bogorensis*	**1d**	1	0	0		59	<5	48

reported, while *Ac. aceti* and *As. bogorensis* gave the carboxylic acid **3a** as the only detectable product. All reactions in the presence of NH_2OH gave the aldoxime **4a** as product, with yields ranging from 87 to 96%, depending on the strain used. The high conversions into aldoxime suggest that condensation of intermediate **2a** with hydroxylamine is faster than further oxidation to acid. It is noteworthy that no acid or aldehyde was detected in the biotransformations performed in the presence of NH_2OH. In addition, for *Gluconobacter* and *Asaia*, with both substrates (entries 4 and 6), the yield of the aldoxime was higher than the yields of aldehyde or acid in the absence of hydroxylamine, showing that removal of the formed aldehyde is beneficial for the overall yield of the biotransformation.

The good results obtained in the preparation of **4a** were confirmed in the case of the preparation of 3-phenylpropionaldehyde oxime **4b** (entries 7–12, Table 1), which was obtained with satisfactory yields (80–97%). When *p*-nitro-2-phenylethanol **1c** was used as a substrate, higher yields were observed in the oxidation without NH_2OH; indeed, *Acetobacter* and *Asaia*, in the absence of NH_2OH, furnished the carboxylic acid **3c** with 93 and 91% yield, respectively, while working in the presence of NH_2OH, **4c** was prepared with moderate yields (12–65%) from **1c** (Table 1, entries 14, 16, and 18).

The transformation was also evaluated on the chiral substrate **1d**. Oxidations performed without NH_2OH showed that *Acetobacter* and *Asaia* (Table 1, entries 19 and 23) oxidized **1d** to **3d** as the only product; *Acetobacter* gave **3d** with good enantioselectivity (92%, *R*-enantiomer at 50% molar conversion), while no kinetic resolution could be observed with *Asaia* (Table 1, entry 23). *Gluconobacter* (Table 1, entry 21) gave only traces of aldehyde **3d**. When working in the presence of NH_2OH, aldoxime **4d** was obtained with 34–59% yields (Table 1, entries 20, 22, and 24); good enantioselectivity (92%, *R*-enantiomer, 45% molar conversion) was observed only with *Acetobacter* (Table 1, entry 20).

Table 2 Optimization of the preparation of *p*-nitro-1-phenylethanol oxime **4c** with *Acetobacter* sp. 2000/61 MIM

Entry	[Biocatalyst] $g_{dry\ weight}\ L^{-1}$	**1c** (mM)	Equivalents of NH_2OH	**4c** (%)	Time (h)
1	10	5	1.0	85	24
2	10	5	1.5	24	24
3	10	10	1.0	65	48
4	10	10	1.5	6	48
5	10	20	1.0	11	48
6	10	20	1.5	<5	48
7	15	5	1.0	85	24
8	15	5	1.5	95	24
9	15	10	1.0	75	24
10	15	10	1.5	90	24
11	15	20	1.0	24	48
12	15	20	1.5	30	48

The preparation of *p*-nitro-2-phenylethanol oxime **4c**, which gave the lowest yields under the conditions employed for the experiments described in Table 1, was optimized using *Acetobacter* sp. 2000/61 MIM by changing substrate and biocatalyst concentration (Table 2). Under optimized conditions (12 g per cell of biocatalyst, 10 mM of both substrates, entry 10), a 90% yield was obtained after 24 h. Higher concentrations of substrates resulted in lower yields; this is likely due to the known toxic effect of hydroxylamine towards many enzymatic systems.[16]

Finally, the one-pot transformation was applied to perillyl alcohol [**1e**, (4-isopropenyl-cyclohexen-1-enyl)-methanol]. Two products of the oxidation of perillyl alcohol have great interest in the food industry: perillyl aldehyde **2e** (or perillaldehyde used as a flavor component)[17] and perillyl aldehyde oxime **4e**

Table 3 Oxidation of perillyl alcohol **1e** (15 mM) with acetic acid bacteria with or without addition of hydroxylamine

Entry	Strain	Equivalents of NH_2OH	**2e** (%)	**3e** (%)	**4e** (%)	Time (h)
1	*Acetobacter*	0	25	70	0	2
2	*Acetobacter*	1.5	0	0	96	48
3	*G. oxydans*	0	16	0	0	24
4	*G. oxydans*	1.5	0	0	25	48
5	*As. bogorensis*	0	12	0	0	24
6	*As. bogorensis*	1.5	0	0	70	48

(or perillartine used as a sweetener).[18] Table 3 summarizes the results obtained using perillyl alcohol as a substrate. The reaction conditions were those optimized for the conversion of **1d** into **4d**. For the first time, aldehyde **2e** was accumulated (16–25%) as the only product at the end of the reaction in the absence of NH_2OH with *Gluconobacter* and *Asaia*. In the presence of NH_2OH, *Acetobacter* was able to give 96% yield of **4e** after 48 h. Here again, it should be stressed that generally much higher yields were obtained for the preparation of **4e**, than for **2e**.

The one-pot chemoenzymatic synthesis of perillartine **4e** with *Acetobacter* sp. MIM 2000/61 was also carried out on a preparative scale (2 L of total volume, under the conditions of entry 2). The oxime **4e** was recovered with 94% yield and no detectable formation of by-products was observed.

Conclusions

In conclusion, we have established an easy and efficient alternative to heavy metal-based solutions for the one-pot preparation of aldoximes directly from alcohols. Satisfactory yields can be obtained for different substrates by choosing suitable microorganisms and reaction conditions. Oxidation of alcohols to aldehydes is catalyzed by acetic acid bacteria dehydrogenases: a high turnover of dehydrogenases under medium–high dissolved oxygen tension is observed with consequent shift of the equilibrium towards oxidation of primary alcohols. The produced aldehydes are immediately converted into aldoximes by nearly stoichiometric amounts of hydroxylamine, preventing further oxidation to carboxylic acids. The yields and productivity of the batch transformations described in this communication can be increased by using continuous systems in membrane reactors, as previously described for oxidations catalyzed by acetic acid bacteria.[19]

Experimental

Microorganisms and growth conditions

Strains from an official collection (*Gluconobacter oxydans* DSM 2343; DSM, Deutsche Sammlung von Mikroorganismen) and from our collection (*Acetobacter* sp. MIM 2000/61 and *Asaia bogorensis*) were used. Acetic acid bacteria were routinely maintained on GYC slants (glucose 50 g L^{-1}, yeast extract 10 g L^{-1}, $CaCO_3$ 30 g L^{-1}, agar 15 g L^{-1}, pH 6.3) at 28 °C. The strains, grown on GYC slants for 24 h at 28 °C, were inoculated into 500 mL Erlenmeyer flasks containing 50 mL of the liquid medium containing yeast extract (10 g L^{-1}) and glycerol (25 g L^{-1}) at pH 5 in distilled water and incubated on a reciprocal shaker (100 spm). The dry weights were determined after centrifugation of 100 mL of cultures; cells were washed with distilled water and dried at 110 °C for 24 h.

Biotransformations

Biotransformations were carried out with cells centrifuged and suspended in phosphate buffer (0.1 M, pH 7.5). Neat substrates were directly added to the suspensions and flasks were shaken on a reciprocal shaker (100 spm). The results were expressed as molar conversion, defined as the number of converted moles per number of starting moles. Products were recovered after centrifugation and extraction with EtOAc; the organic extracts were dried over Na_2SO_4, the solvent evaporated under reduced pressure and the products purified by flash chromatography.

^{1}H NMR spectra of oximes (**4a–e**) were recorded with a Varian Mercury 300 (300 MHz) spectrometer. Chemical shifts (δ) are expressed in parts per million and coupling constants (J) in hertz.

4a: a 1 : 1 mixture of geometric isomers **a** and **b**. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.57 (t, J = 6.3, 1H, isomer **b**), 7.45–7.15 (m, 5H, isomer **a** and 5H, isomer **b**), 6.93 (t, J = 5.3 Hz, 1H, isomer **a**), 3.75 (d, J = 5.3 Hz, 2H, isomer **a**), 3.57 (d, J = 6.3, 2H, isomer **b**).

4b: a 3 : 2 mixture of geometric isomers **a** and **b**. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.45 (t, J = 5.8 Hz, 1H, isomer **b**), 7.35–7.18 (m, 5H, isomer **a** and 5H, isomer **b**), 6.78 (t, J = 5.8 Hz, 1H, isomer **a**), 2.90–2–80 (m, 2H, isomer **a** and 2H, isomer **b**), 2.78–2.68 (m, 2H, isomer **a**), 2.58–2.50 (m, 2H, isomer **b**).

4c: a 1 : 1 mixture of geometric isomers **a** and **b**. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.19 (m, 2H, isomer **a** and 2H, isomer **b**), 7.55 (t, J = 6.1, 1H, isomer **b**), 7.42–7.38 (m, 2H, isomer **a** and 2H, isomer **b**), 6.90 (t, J = 5.5 Hz, 1H, isomer **a**), 3.84 (d, J = 5.5 Hz, 2H, isomer **a**), 3.65 (d, J = 6.1, 2H, isomer **b**).

4d: a 2 : 1 mixture of geometric isomers **a** and **b**. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.55 (d, J = 7.0 Hz, 1H, isomer **a**), 7.25–7.42 (m, 5H, isomer **a** and 5H, isomer **b**), 6.82 (d, J = 7.2 Hz, 1H, isomer **b**), 4.42 (dq, J = 7.2, 7.2 Hz, 1H, isomer **b**), 3.68 (dq, J = 7.0, 7.0 Hz, 1H, isomer **a**), 1.45 (d, J = 7.0 Hz, 3H, isomer **a**), 1.43 (d, J = 7.2 Hz, 3H, isomer **b**).

4e: ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.70 (s, 1H), 6.05–6.00 (m, 1H), 4.80–4.65 (m, 2H), 2.45–2.00 (m, 4H), 1.95–1.80 (m, 2H), 1.70 (s, 3H), 1.60–1.40 (m, 1H).

Analytical methods

Samples (0.5 mL) were taken at intervals, brought to pH 1 by addition of 0.5 M HCl, extracted with an equal volume of EtOAc and the organic extracts were dried over Na_2SO_4. The biotransformations of 2-phenyl-1-ethanol (**1a**), 3-phenyl-1-propanol (**1b**), *p*-nitro-2-phenyl-1-ethanol (**1c**) and 2-phenyl-1-propanol (**1d**) were routinely analyzed by HPLC. Analysis was performed on an HPLC Merck Hitachi 655A, with a UV detector (254 nm) Merck Hitachi L-4000 using a Purospher® STAR

RP-18e (5 μm) column. The solvent system consisted of a solution of water and acetonitrile (1 : 1) containing 0.1% trifluoroacetic acid. The flow-rate was 0.8 mL min^{-1}; injection volume was 20 μL. The biotransformation of perillyl alcohol (**1e**) was followed by GLC analysis using a chiral capillary column (diameter 0.25 mm, length 25 m, thickness 0.25 μm, Megadex DET-beta, MEGA, Legnano, Italia). The analysis was performed after conversion of the acid to the corresponding methyl ester by treatment with CH_2N_2; the organic extracts were then dried and dissolved in EtOAc. The enantiomeric composition of the products **3d** and **4d** was determined using the methods described before;[20,21] the absolute configuration of the products was determined by comparison with samples of the optically pure enantiomers obtained by a chemical route.

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