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Laccase-Mediator System for Alcohol Oxidation to Carbonyls or Carboxylic Acids: Toward a Sustainable Synthesis of Profens

Paola Galletti,* Matteo Pori, Federica Funiciello, Roberto Soldati, Alberto Ballardini, and Daria Giacomini*[a]

By combining two green and efficient catalysts, such as the commercially available enzyme laccase from *Trametes versicolor* and the stable free radical 2,2,6,6-tetramethylpiperidine-*N*-oxyl (TEMPO), the oxidation in water of some primary alcohols to the corresponding carboxylic acids or aldehydes and of selected secondary alcohols to ketones can be accomplished. The range of applicability of bio-oxidation is widened by applying the optimized protocol to the oxidation of enantiomerically pure 2-arylpropanols (profenols) into the corresponding 2-arylpropionic acids (profens), in high yields and with complete retention of configuration.

Introduction

Oxidations are pivotal reactions in organic synthesis and are widely used in industrial processes for the synthesis of bulk, fine, and specialty chemicals.[1] At the same time, oxidations are among the most polluting and hazardous processes, delivering toxic waste, as in the case of traditional stoichiometric oxidants based on Cr^{VI} or Mn^{VII} salts. To develop cleaner and eco-efficient catalytic oxidation processes, a number of greener catalytic methodologies have been developed by using less-toxic metal catalysts and molecular oxygen or air as the oxidant.[2,3] However, many of these systems still require harsh reaction conditions, give metal-containing wastes, and selectivity can be difficult to control.

Hence, to develop more benign and selective redox processes, biocatalysis is emerging as a valuable tool.[4,5] Bio-oxidations have the added value of high levels of selectivity (regio-, chemo-, and stereo-) that are reliable even for fine chemicals with complex structures and possessing oxidation-sensitive functional groups.[6]

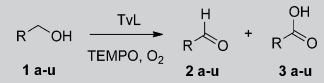
Considering the oxidation of alcohols, an important transformation in the synthesis of fine chemicals,[2a] more frequently biocatalysis made use of oxidoreductases (dehydrogenases and oxidases), whereas peroxidases and monooxygenases were employed to a lesser extent.[7] Some biocatalytic methods that used whole cells were also reported.[8] As representative examples, the enantioselective oxidation of 2-phenylpropanol by *Acetobacter aceti*[9] and the oxidation of primary alcohols to aldehyde by *Gluconobacter oxydans*[10] have been reported. In these cases, intact cells were used as the oxidizing agent. Laccases (EC 1.10.3.2) belong to the multi-copper family of oxidases. These enzymes contain four copper centers per protein molecule and catalyze the oxidation of electron-rich aromatic substrates, usually phenols or aromatic amines, by using oxygen as the electron acceptor.[11] Because water is the only byproduct formed, in principle, they are ideal catalysts for sustainable chemical and technological processes. Laccases are widely distributed in nature and perform a multiplicity of functions linked to either synthetic or degradation processes.[12] Fungal laccases, for instance, play a critical role in lignin and humus degradation,[13] and they are of particular interest because such enzymes are secreted extracellularly in response to simple inducers; this makes their production and purification relatively simple.[11] Laccases have broad industrial applications, for instance, in pulp and paper industry, biosensor technology, in the organic synthesis of useful compounds, and offer great interest in environmental biotechnology.[14,15]

Although the natural substrates of laccases are the phenolic residues of lignin, the inclusion of appropriate mediators in the laccase-mediator system (LMS) makes the oxidation of non-phenolic substrates accessible.[16] The application of the LMS in the oxidation of alcohols is well documented in the literature.[15c,17] Commonly, laccases oxidize secondary alcohols to ketones and primary alcohols to the corresponding aldehydes, whereas the overextended oxidation of primary alcohols to carboxylic acid was less reported.[18]

Herein, we report developments in chemoenzymatic oxidation by commercial laccase from *Trametes versicolor* (TvL) of some primary alcohols to the carboxylic acids or aldehydes and of selected secondary alcohols to ketones with a particular emphasis on stereoselectivity. Moreover, within an interdisciplinary project dedicated to improving the sustainable production of active pharmaceutical compounds by the use of chemoenzymatic processes,[19a,b] we report herein an important application of laccase TvL in the bio-oxidation of some (*S*)-profe-

[a] Dr. P. Galletti, Dr. M. Pori, F. Funiciello, R. Soldati, A. Ballardini, Prof. D. Giacomini
Department of Chemistry "G. Ciamician"
University of Bologna
Via Selmi 2, 40126 Bologna (Italy)
E-mail: paola.galletti@unibo.it
daria.giacomini@unibo.it

Table 1. Bio-oxidation of primary alcohols with laccase from TvL.



1 a-u 2 a-u 3 a-u

Entry	RCH_2OH (**1 a**–**u**)	Conditions[a]	*t* [d]	Yield[b] [%] RCH_2OH (**1 a**–**u**)	RCHO (**2 a**–**u**)	RCOOH (**3 a**–**u**)
1	benzyl alcohol (**1 a**)	H_2O, pH 4.5 0.1 M, RT	6	80	20	–
2	**1 a**	H_2O, RT	3	50	50	–
3	**1 a**	H_2O, RT	8	–	50	50
4	**1 a**	H_2O, 30 °C	6	–	51	49
5	**1 a**	H_2O, 30 °C, no enzyme	7	100	–	–
6	cinnamyl alcohol (**1 b**)	H_2O, RT	8	–	99	–
7	*p*-OMe benzyl alcohol (**1 c**)	H_2O, RT	5	65	35	–
8	**1 c**	H_2O, acetone 10%, RT	6	32	68	–
9	2,4-dimethoxybenzyl alcohol (**1 d**)	H_2O, RT	5	74	26	–
10	**1 d**	H_2O, acetone 10%, 30 °C	5	75	14	11
11	3,4-dimethoxybenzyl alcohol (**1 e**)	H_2O, RT	7	–	95	5
12	**1 e**	H_2O, acetone 10%, RT	7	–	87	13
13	4-nitrobenzyl alcohol (**1 f**)	H_2O, RT	5	61	33	6
14	2,4-dinitrobenzyl alcohol (**1 g**)	H_2O, RT	6	25		75
15	pentafluorobenzyl alcohol (**1 h**)	H_2O, RT	5	–	13[c]	67
16	3,5-ditrifluoromethylbenzyl alcohol (**1 i**)	H_2O, RT	5	25	48[c]	21
17	2-pyridinemethanol (**1 j**)	H_2O, RT	0.5	–	–	>99
18	3-pyridinemethanol (**1 k**)	H_2O, RT	2	–	–	>99
19	4-pyridinemethanol (**1 l**)	H_2O, RT	4	–	–	>99
20	furfurol (**1 m**)	H_2O, RT	6	98	traces	–
21	2-thienylmethanol (**1 n**)	H_2O, RT	7	–	74	26
22	octanol (**1 o**)	H_2O, RT	8	66	30	traces
23	cyclohexylmethanol (**1 p**)	H_2O, RT	6	99	–	–
24	trifluoroethanol (**1 q**)	H_2O, RT	13	85	7	8
25	**1 q**	H_2O, pH 4.5 0.5 M, RT	43	10	–	90
26	4-phenyl-1-butanol (**1 r**)	H_2O, RT	7	81	19	–
27	3-phenyl-1-propanol (**1 s**)	H_2O, RT	6	76	12	12
28	2-phenylethanol (**1 t**)	H_2O, RT	6	traces	traces[d]	75
29	2-phenylpropanol (**1 u**)	H_2O, pH 4.5 0.1 M, RT	6	–	traces[d]	60
30	**1 u**	H_2O, RT	6	–	traces[d]	74
31	**1 u**	H_2O, acetone 10%, RT	2	14	–	86
32	(2*S*)-2-phenylpropanol (**1 u**)	H_2O, RT[e]	5	–	–	>99 (>99 *ee*)
33	(2*R*)-2-phenylpropanol (**1 u**)	H_2O, RT[e]	5	–	–	>99 (>99 *ee*)

[a] Procedure A: substrate (0.5 mmol), enzyme (5 mg (68U)), mediator TEMPO (20 mol%), solvent (6 mL), oxygen bubbled through a closed vial. [b] Ratio between starting alcohol, aldehyde, and acid was evaluated by ^{1}H NMR spectroscopy, or ^{19}F NMR spectroscopy in the case of entries 24 and 25. [c] Formation of acetal between aldehyde and alcohol was observed. [d] Formation of acetophenone, which is a known byproduct of 2-phenylpropanal (**2 u**), was observed, see Ref. [19b]. [e] Procedure B was used, see the Experimental Section for details.

nols to (*S*)-profens, which are important benchmark drugs in the class of non-steroidal anti-inflammatory drugs (NSAIDs).[19c,d] To the best of our knowledge, laccase-mediated oxidation of this important class of substrate has not yet been reported; the only example reported is the bio-oxidation of 2-flurbiprofenal by using an engineered alcohol dehydrogenase (ADH).[19c]

Results and Discussion

The use of isolated laccases for alcohol oxidation to obtain aldehydes was already reported.[15c,17] As a first attempt, we used the commercially available fungal laccase from TvL (Sigma–Aldrich) and 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO; free radical) as a mediator. Reactions were conducted on benzyl alcohol (**1 a**; 0.5 mmol) as a model compound, in NaOAc buffer (pH 4.5, 0.1 M), and TEMPO (20 mol%) in open vials to ambient air. As expected, the reaction gave benzaldehyde (**2 a**) and better yields were obtained in the absence of acetate buffer (Table 1, entries 1 and 2). However, for prolonged reaction times with closed vials under an oxygen atmosphere, a considerable amount of benzoic acid was obtained (Table 1, entries 3 and 4). Thus, to increase the efficiency of the process leading to carboxylic acids, further experiments were conducted and the most effective reaction conditions in terms of enzyme amount, solvent and cosolvent, pH, and oxygen source were found (Table 1).

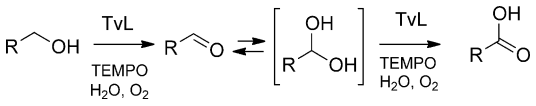
The best experimental conditions were found to be as follows: 1) the use of TEMPO in 20% molar ratio, other mediators, such as 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 1-hydroxybenzotriazole (HOBT), and *p*-OH- and *p*-amino-TEMPO, were used without positive results; 2) several cosolvents and additives were tested, such as THF, acetone, DMSO, CH_3CN, *t*BuOMe, CH_2Cl_2, *t*BuOH, Triton X, two ionic liquids, NaCl, and NBu_4HSO_4, but better results were obtained in pure water or with little amounts of acetone; 3) different pH conditions were explored and better results were obtained with buffers at pH 4.5 or no buffer (the pH becomes acidic

during the reaction course); and 4) better results were obtained by bubbling oxygen into the reaction balloon or vial, then closing it with a cap.

As far as the reaction time is concerned, reactions were monitored by TLC, HPLC, or NMR spectroscopy generally every 24 h and stopped at complete alcohol conversion or when substrate conversion did not proceed further.

Representative results on several primary alcohols are reported in Table 1. For benzylic alcohols **1a** and **1c–i**, the efficiency in the oxidation to acids strongly depends on the nature of the substituents on the aromatic ring. Donor-substituted benzylic alcohols **1c–e** provided null or modest results, whereas acceptor-substituted benzylic alcohols gave the corresponding benzoic acids in modest to good yields, as in the case of **1g** and **1h** (Table 1, entries 14 and 15). The best results were obtained with heteroaromatic primary alcohols, in particular, pyridylmethanols **1j–l** gave the corresponding pyridyl–carboxylic acids quantitatively (Table 1, entries 17–19). Aliphatic alcohols **1o** and **1p** reacted poorly, but activated **1q** gave the trifluoroacetic acid, even if extended reaction times were required (Table 1, entry 25); for substrate **1q**, a buffered solution (pH 4.5) was necessary because of the incoming strong acidity of trifluoroacetic acid, as a matter of fact, in H_2O alone the oxidation to acid was poor (Table 1, entry 24).

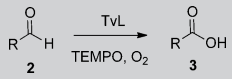
The oxidation of primary alcohols to the corresponding carboxylic acids can be considered a two-step oxidation: first to aldehyde and then to carboxylic acid (Scheme 1). Oxidation in water of aldehydes to acids would proceed through a geminal diol intermediate.[20]



Scheme 1. The two-step oxidation of primary alcohols to the corresponding carboxylic acids.

To ascertain effective catalysis on both oxidative steps, we tested some aldehydes as substrates (Table 2). As a first test, we tried **2a** as a substrate under standard conditions and benzoic acid was successfully obtained (Table 2, entry 2). With no enzyme, the oxidation was poor; thus giving evidence for a low efficiency of spontaneous aldehyde oxidation (Table 2, entry 1). Substituted pyridine–carbaldehyde **2j** gave the corresponding acid in excellent yield, whereas **2g** gave a poorer result probably because of the hydrophobicity of the substrate and poor solubility in water (Table 2 entry 3).

Table 2. Bio-oxidation of aldehydes with laccase from TvL.



Entry	RCHO (**2**)	Conditions[a]	*t* [d]	Yield of acid (**3**)[b] [%]
1	Benzaldehyde (**2a**)	H_2O, 30 °C, no enzyme	6	17
2	**2a**	H_2O, 30 °C	6	77
3	2,4-dinitrobenzaldehyde (**2g**)	H_2O, RT	6	30
4	2-pyridincarboxaldehyde (**2j**)	H_2O, 30 °C	1	>99
5	**2j**	H_2O, pH 4.5k, 2 M, 30 °C	1	>99
6	**2j**	H_2O, RT	0.5	>99
7	3-pyridincarboxaldehyde (**2k**)	H_2O, 30 °C	1	>99
8	**2k**	H_2O, pH 4.5, 2 M, 30 °C	1	>99
9	2-phenylpropanal (**2u**)	H_2O, RT, no enzyme	7	–
10	**2u**	H_2O, 30 °C	7	>99

[a] Procedure A: substrate (0.5 mmol), enzyme (5 mg), mediator TEMPO (20 mol%), solvent (6 mL), oxygen bubbled through a closed vial. [b] Yields of products isolated after acid–base workup.

Concerning the mechanism of the LMS oxidation, Baiocco et al. proposed two possible routes by which the mediator could oxidize the substrate: the electron transfer (ET) and hydrogen abstraction transfer (HAT) routes.[21] Kinetic studies on TEMPO strongly supported an ionic hydrogen abstraction route[22, 16, 17b] that has precedents in the efficient oxidation procedures of alcohols by TEMPO with chemical oxidants.[23] The effectiveness of the catalytic activity of TEMPO in alcohol oxidation by O_2 is due to the intermediate formation of the oxammonium salt, which is the actual oxidant species[24] and continuously restored by laccase. A tentative ionic route is proposed in Figure 1. The efficiency of the process could depend on the

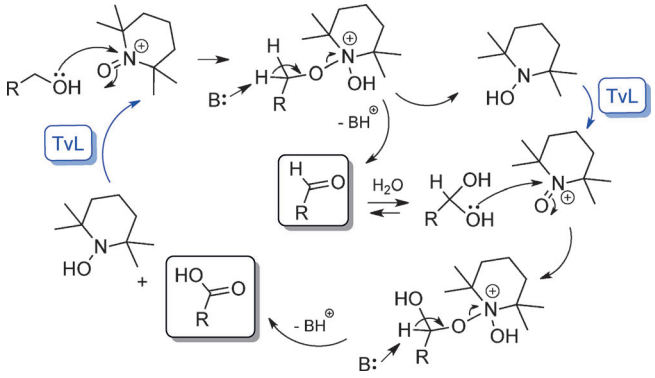
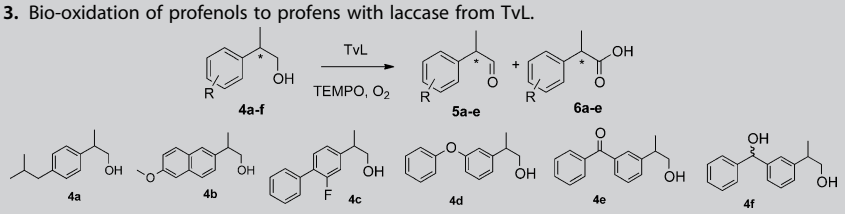


Figure 1. The ionic route for the two-step oxidation of alcohols to carboxylic acids by TvL laccase/TEMPO.

facility of hydrogen abstraction[25] on the starting alcohol and the *gem*-diol intermediate, together with the hydration equilibrium of the aldehyde.[20] If the hydrogen acidity were too low, oxidation would be difficult. Moreover, if the solubility of the aldehyde in H_2O were too low and/or its hydration equilibrium unfavorable, the reaction could stop at the aldehyde level. From this perspective, the results obtained, for instance, with **1o**, **1p**, or cynnamyl alcohol (**1b**) could be interpreted.

Bio-oxidations by laccases are usually conducted in acetate buffer at pH 4.5–4.8.[17a] However, we observed improved yields with unbuffered solutions in water. This result could derive from a salting-out effect in buffered solutions with a lowered solubility of reagents or intermediates. Moreover, it is known that the stability of the TEMPO nitroxyl radical in acidic medium, such as in acetate buffer, is low, and for longer reaction times the mediator decomposed to a greater extent.[26] A better result with unbuffered aqueous solutions could be thus consistent with the pH-dependent stability of the mediator. However, in the case of **1q** (Table 1, entries 24 and 25), acetate buffer was necessary because during the reaction progress, the incoming trifluoroacetic acid strongly decreases pH and could decompose the mediator and/or denature enzyme over long reaction times.

Table 3. Bio-oxidation of profenols to profens with laccase from TvL.



Entry	Alcohol (**4a–f**)	Conditions[a]	*t* [d]	Yield[b] [%] aldehyde	acid
1	ibuprofenol (**4a**)	H_2O, pH 4.5, 0.1 M, RT	6	traces	–
2	(*S*)-ibuprofenol (**4a**)	H_2O, RT	6	–	79 (*S*/*R* > 99:1)
3	ibuprofenol (**4a**)	H_2O, acetone 10%, RT	7	–	72
4	naproxenol (**4b**)	H_2O, pH 4.5, 0.1 M, RT	6	–	–
5	naproxenol **4b**	H_2O, DMSO 10%, RT	6	traces	–
6	(*S*)-naproxenol (**4b**)	H_2O, acetone 10%, RT	7	–	92 (*S*/*R* > 99:1)
7	flurbiprofenol (**4c**)	H_2O, pH 4.5 0.1 M, RT	6	traces	40
8	flurbiprofenol (**4c**)	H_2O, RT	4	traces	35
9	(*S*)-flurbiprofenol (**4c**)	H_2O, DMSO 10%, RT	7	–	99 (*S*/*R* > 99:1)
10	(*S*)-fenoprofenol (**4d**)	H_2O, RT	7	–	91 (*S*/*R* > 99:1)
11	fenoprofenol (**4d**)	H_2O, acetone 10%, RT	7	10	13
12	(*S*)-ketoprofenol (**4e**)	H_2O, RT	7	–	95 (*S*/*R* > 99:1)
13	ketoprofenol (**4e**)	H_2O, acetone 10%, RT	7	traces	13
14	(*S*)-ketoprofendiol[c] (**4f**)	H_2O, RT	7	–	82 *S*/*R* > 99:1)[d]
15	ketoprofendiol (**4f**)	H_2O, acetone 10%, RT	7	traces	24[d]

[a] Procedure B. Substrate (1 mmol), enzyme (20 mg mmol^{-1}), mediator TEMPO (20 mol%), solvent (20 mL), oxygen bubbled in a closed vial. [b] Yields of products isolated after acid–base workup. [c] *S* refers to the marked stereogenic center. [d] Yield refers to ketoprofen **6e**.

Following our interest in the oxidation of arylpropanols,[27] we then tried the optimized protocol on **1u**, which successfully gave the 2-phenylpropanoic acid in good yields (Table 1, entries 29–33). The LMS oxidation worked well: conversion was always complete, the selectivity to the carboxylic acid was good, and only traces of the corresponding aldehyde **2u** were detected in the crude reaction mixture. The efficiency and selectivity strongly depended on the relative positions (span) of the aromatic ring with the hydroxy group: **1t** and **1u** were efficiently converted into the carboxylic acids, on the contrary **1r** and **1s** gave none or only traces of the corresponding acids (Table 1, entries 26–29).

To rule out the possibility that any racemization of the stereogenic center occurred during bio-oxidation, we tested (2*S*)- or (2*R*)-phenylpropanol (Table 1, entries 32 and 33). The enantiomeric purity of the starting alcohols was completely retained in the final acids. For a five-day reaction in simple water, enantiomerically pure (2*R*)- or (2*S*)-phenylpropionic acids were thus quantitatively obtained.

The good result obtained with **1u** prompted us to explore the laccase-catalyzed oxidation of a series of 2-arylpropanols (Table 3). Our attention was addressed to some profenols, **4a–f**, as industrially relevant substrates. All profenols could be oxidized in good to excellent yields with a total retention of configuration when (*S*)-2-arylpropanols were used (Table 3, entries 2, 6, 9, 10, 12, and 14). The enantiomerically pure 2-arylpropanols were obtained through enzymatic reduction of the corresponding 2-arylpropanals following the dynamic kinetic resolution (DKR) protocol we developed.[19b]

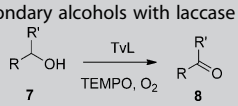
The use of a 10% organic cosolvent, such as acetone or DMSO, gave improved results, depending on the substrate, for instance, compound **4b** gave a better result with acetone 10%, whereas **4c** gave a better result with DMSO (Table 3, entries 6 and 9). In case of **4e** and **4f**, the use of 10% acetone was detrimental (Table 3, entries 13 and 15) and resulted in poor yields of ketoprofen; a significant amount of byproducts, such as methylarylketones, and partially oxidized products were obtained.

To expand the scope, some secondary alcohols were tested under the optimized reaction conditions. Cyclohexanol (**7a**), 1-phenylethanol (**7b**), and 1-phenylpropanol (**7c**) gave good results in the production of the corresponding ketones (Table 4, entries 1–4). 2-Substituted cyclohexanols **7h–i** gave worse yields (Table 4, entries 10–13), but they showed stringent stereospecific behavior: only the 1,2-*cis*-cyclohexanols were oxidized. This is consistent with previously reported results on the oxidation of 2-methylcyclohexanols by cytochrome P-450, which showed higher reactivity for *cis*-2-methylcyclohexanol than that of the *trans* isomer due to steric hindrance.[28] Natural menthol did not work at all (Table 4, entry 14). Interesting and promising results were obtained with α-hydroxyacids or α-hydroxyesters. Mandelic acid (**7e**) gave the corresponding oxoacid in satisfactory yields (Table 4, entries 6 and 7), whereas its methyl ester (**7d**) gave a better result with quantitative yield in a very short reaction time (Table 4, entry 5)

Conclusions

There is a great need for sustainable oxidation of fine chemicals by employing clean primary oxidants, such as oxygen and

Table 4. Bio-oxidation of secondary alcohols with laccase from TvL.



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Entry	RR′CHOH	Conditions[a]	*t* [d]	Yield of RCOR′[b] [%]
1	cyclohexanol (**7a**)	H_2O, RT	6	80
2	1-phenylethanol (**7b**)	H_2O, RT	6	>99
3	**7b**	H_2O, pH 4.5, 0.1 M, RT	7	77
4	1-phenylpropanol (**7c**)	H_2O, RT	7	>99
5	mandelic acid methyl ester (**7d**)	H_2O, RT	0.1	>99
6	mandelic acid (**7e**)	H_2O, pH 4.5, 0.1 M, RT	8	66
7	**7e**	H_2O, RT	8	60
8	diphenylmethanol (**7f**)	H_2O, acetone 10%	7	61
9	lactic acid ethyl ester (**7g**)	H_2O, RT	8	50
10	*cis*-2-methoxycyclohexanol (**7h**)	H_2O, RT	7	12
11	*trans*-2-methoxycyclohexanol (**7h**)	H_2O, RT	7	0
12	*cis*-2-methylcyclohexanol (**7i**)	H_2O, RT	7	42
13	*trans*-2-methylcyclohexanol (**7i**)	H_2O, RT	7	0
14	(−)-menthol (**7j**)	H_2O, RT	7	0

[a] Procedure C. Substrate (0.5 mmol), enzyme (5 mg), mediator TEMPO (20 mol%), solvent (6 mL), oxygen bubbled in a closed vial. [b] Yield calculated from NMR spectroscopic analysis of the crude reaction mixture.

greener catalysts; thus avoiding the use of harsh organic and inorganic oxidants, even in catalytic amounts. From this point of view, the combination of two green and efficient catalysts, such as the commercially available enzyme laccase TvL and the stable free radical TEMPO, offers great opportunities. We widened the range of applicability by exploring the oxidation, in water, of some primary alcohols to the corresponding carboxylic acids or aldehydes and of selected secondary alcohols to ketones.

Moreover, we succeeded in an important application: the development of the laccase-mediator system (LMS) oxidation of 2-arylpropanols (profenols) to the corresponding 2-arylpropionic acids (profens), in high yields and with complete retention of configuration.

Thus, the chemoenzymatic reduction of arylpropanals we already successfully developed through the DKR process,[19a,b] coupled with the chemoenzymatic oxidation reported herein, depict a more environmentally friendly alternative route to the synthesis of enantiomerically pure profens and contributes to improved sustainability in the synthesis of this important class of drugs (Figure 2).

Experimental Section

General: Commercial reagents were used as received without additional purification. ^1H and ^{19}F NMR spectra were recorded with an INOVA 400 instrument with a 5 mm probe. TLC: Merck 60 F254 plates. HPLC-MS: Agilent Technologies HP1100 instrument, equipped with a ZOBRAX-Eclipse XDB-C8 Agilent Technologies column; mobile phase: H_2O/CH_3CN, 0.4 mL min^{-1}, gradient from 30 to 80% of CH_3CN in 8 min, 80% of CH_3CN until 25 min, coupled with an Agilent Technologies MSD1100 single-quadrupole mass spectrometer, full-scan mode from *m/z* 50 to 2600, scan time of 0.1 s in positive ion mode, ESI spray voltage of 4500 V, nitrogen gas of 35 psi (1 psi = 6894.7 Pa), drying gas flow of 11.5 mL min^{-1}, fragmentor voltage of 20 V. Starting materials: alcohols and aldehydes used as starting materials in Tables 1, 2, and 4 were commercially available or known compounds. Primary alcohols **1c**, **1d**, **1f**, **1j**, **1k**, **1l**, and **1p** were obtained by reduction of the corresponding commercial aldehyde with $NaBH_4$ in MeOH; compound **1g** was obtained by reduction of the corresponding aldehyde with BH_3·THF (5 equiv) in THF; **1h** and **1p** were obtained from the corresponding carboxylic acids by reduction with borane–dimethylsulfide (BH_3· Me_2S) in Et_2O; **1s** was obtained by reduction from **1b** with H_2 on Pd/C. Racemic 2-arylpropanols **4a–f** in Table 3 were obtained by BH_3·Me_2S reduction of racemic commercial acids, enantiomerically pure (*S*)-**4a–f** were obtained by enantioselective biocatalysis starting from the corresponding racemic aldehydes.[19] Secondary alcohols **7a**, **7b**, **7f**, **7i**, and **7j** were obtained by $LiAlH_4$ reduction from the corresponding ketone in Et_2O; **7d** was obtained by acid-catalyzed esterification of **7e** and MeOH.

Procedure A: Experimental oxidation procedure for compounds reported in Tables 1 and 2. TEMPO (0.1 mmol) and the enzyme (5 mg) were added to a stirred solution of the alcohol or aldehyde (0.5 mmol) in the appropriate solvent (6 mL) in a 10 mL vial with a screw cap, and then O_2 was bubbled for 30 s. The solution was stirred on an orbital shaker at 150 rpm, retained, and the reaction was monitored by TLC. When the reaction was complete, the aqueous solution was kept at 0 °C and adjusted to pH 2 by slow addition of aqueous HCl (1 N). The acid aqueous phase was then extracted with CH_2Cl_2 (3×5 mL). The collected organic phases were dried over Na_2SO_4, filtered, concentrated in vacuo, and analyzed by HPLC and ^1H and ^{19}F NMR for alcohols **1h** and **1i**; ^{19}F NMR for **1q**. In the case of compounds **1j**, **1k**, **1l**, **2j**, and **2k**, the crude aqueous phase was directly lyophilized and analyzed by ^1H NMR spectroscopy. Spectroscopic data were consistent with those reported in the literature and in the NMR spectroscopy database (Reaxys and AIST SDBS).

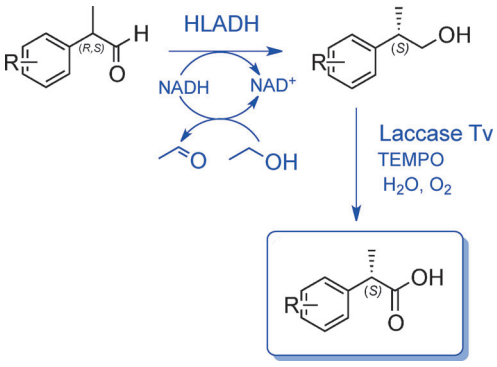

Figure 2. Chemoenzymatic route to enantiomerically pure arylpropanoic acids. HLADH = horse liver alcohol dehydrogenase, NADH/NAD^+ = nicotinamide adenine dinucleotide redox couple.

Procedure B: Experimental procedure for the synthesis of 2-arylpropionic acids (profens; Table 3): TEMPO (0.2 mmol) and the enzyme (20 mg) were added to a stirred solution of the alcohol **4a–f** (1 mmol) in the appropriate solvent (20 mL) in a 50 mL balloon. O_2 was bubbled for 30 s and then the balloon was closed with a cap. The solution was stirred on an orbital shaker at 150 rpm and kept at room temperature. The reaction course was monitored by TLC.

When the starting material disappeared, an aqueous saturated solution of $NaHCO_3$ at 0 °C was added to the flask followed by CH_2Cl_2 (20 mL). The organic layer was separated and discharged, then the aqueous solution was kept at 0 °C and adjusted to pH 2 by slow addition of aqueous HCl (1 N). The acid aqueous phase was then extracted with CH_2Cl_2 (3×15 mL). The collected organic phases were dried over Na_2SO_4, filtered, and concentrated in vacuo to afford 2-arylpropionic acids. NMR spectroscopy and HPLC data of obtained products were consistent with those previously reported. Enantiomeric excess values were obtained on HPLC chiral columns.[27]

Procedure C: Experimental oxidation procedure for compounds reported in Table 4. TEMPO (0.1 mmol) and the enzyme (5 mg) were added to a stirred solution of the alcohol (0.5 mmol) in the appropriate solvent (6 mL) in a 10 mL vial with a screw cap, and then O_2 was bubbled for 30 s. The solution was stirred on an orbital shaker at 150 rpm, maintained at room temperature, and the reaction was monitored by TLC. In case of compounds **7b**, **7c**, **7d**, and **7f**, when the reaction was complete, the aqueous solution was extracted with AcOEt (3×5 mL). The collected organic phases were dried over Na_2SO_4, filtered, concentrated in vacuo, and analyzed by HPLC and ^1H and ^{13}C NMR spectroscopy. In case of compound **7e**, when the reaction was complete, the aqueous solution was kept at 0 °C and adjusted to pH 2 by slow addition of aqueous HCl (1 N). The acid aqueous phase was then extracted with CH_2Cl_2 (3×5 mL). The collected organic phases were dried over Na_2SO_4, filtered, concentrated in vacuo, and analyzed by HPLC and ^1H and ^{13}C NMR spectroscopy. In the case of compounds **7a**, **7g**, **7h**, **7i**, and **7j**, when the reaction was complete, the aqueous solution was extracted with Et_2O (3×5 mL). The collected organic phases were dried over Na_2SO_4, filtered, concentrated in vacuo, and analyzed by HPLC and ^1H and ^{13}C NMR spectroscopy. Spectroscopic data were consistent with those reported in the literature and in the NMR spectroscopy database.

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