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Tetrahedron: Asymmetry
Tetrahedron: *Asymmetry* 16 (2005) 3077–3083

Oxidations catalyzed by phenylacetone monooxygenase from *Thermobifida fusca*

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Received 14 July 2005; accepted 1 August 2005
Available online 9 September 2005

Abstract—Several organic sulfides, ketones and other organic systems have been tested as substrates in oxidation reactions catalyzed by the recently discovered phenylacetone monooxygenase from *Thermobifida fusca*. The biocatalytic properties of this Baeyer–Villiger monooxygenase have been studied, revealing reactivity with a large range of sulfides and ketones. Oxidations of several sulfoxides, an amine and an organoboron compound were also observed. The enzyme is able to oxidize a number of sulfides with excellent enantioselectivity, demonstrating the catalytic potential of this novel biocatalyst.

1. Introduction

In recent years, the use of Baeyer–Villiger monooxygenases (BVMOs) has been shown to be an excellent methodology in Baeyer–Villiger reactions, sulfoxidations, amine oxidations and epoxidations.[1] In many of these reactions, conversions occur with high enantio- and/or regioselectivity, while using environmentally friendly conditions. In general, the observed selectivities are difficult to achieve by chemical means.

Products that can be obtained using BVMOs are of great value in organic chemistry. Recently, mono- and polycyclic lactones have received considerable attention as therapeutic agents and as intermediates in pharmaceutical synthesis.[2] Enantiomerically pure sulfoxides have significant commercial value as chiral synthons, particularly in the synthesis of biologically active molecules, while they can also serve as versatile chiral auxiliaries in asymmetric transformations.[3]

Only a limited number of BVMOs are available in recombinant form. In fact, until a few years ago, only cyclohexanone monooxygenase (CHMO; EC 1.14.13.22) had been extensively studied and applied in Baeyer–Villiger reactions and other selective oxidation processes.[4]

The recent recognition of a protein sequence motif that can be used to identify BVMOs has enabled mining of the genome database. Using this sequence motif, a large number of putative BVMO genes can be annotated.[5] Via this approach of enzyme discovery, a novel BVMO has been obtained from *Thermobifida fusca*. The initial characterization of this biocatalyst has shown that it represents a thermostable and monomeric enzyme, containing FAD as a cofactor and being NADPH dependent.[6] Previous studies reported that the best substrate was phenylacetone and therefore the enzyme was named phenylacetone monooxygenase (PAMO; EC 1.14.13.92). It was also demonstrated that the enzyme is able to oxidize other aromatic and aliphatic ketones and organic sulfides. PAMO represents the first BVMO of which the X-ray structure has been solved.[7] The availability of this structure will facilitate future mechanistic and enzyme redesign studies.

The main goal of this work was to explore the synthetic repertoire of phenylacetone monooxygenase, focusing on the biocatalytic asymmetric oxidation of aromatic sulfides and other systems in order to obtain the corresponding products with high enantiomeric excesses.

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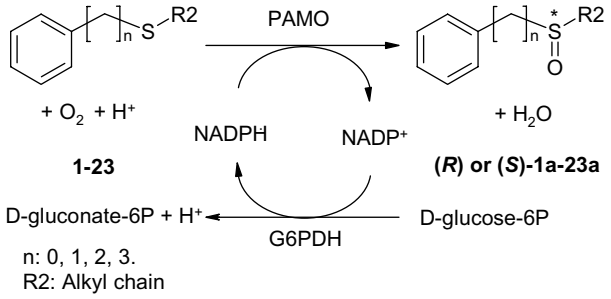
doi:10.1016/j.tetasy.2005.08.004

Furthermore, to increase the knowledge on the biocatalytic potential of this novel oxidative enzyme, the kinetic parameters of a set of ketones, sulfides, sulfoxides and tertiary amines in PAMO catalyzed oxidations were also determined.

2. Results and discussion

2.1. Synthetic applications of PAMO

The oxidation of a set of organic sulfides **1**–**23** to the corresponding sulfoxides **1a**–**23a** by recombinant histidine-tagged phenylacetone monooxygenase[6] was coupled to an ancillary enzymatic reaction in order to regenerate NADPH (Scheme 1). As a NADPH regeneration system, glucose-6-phosphate and glucose-6-phosphate dehydrogenase (G6PDH) were employed. All oxidations were carried out in a Tris/HCl buffer at pH 9. The results obtained in the PAMO catalyzed oxidation of different aromatic sulfides are summarized in Table 1.



Scheme 1. General procedure for the enzymatic oxidation of organic sulfides.

With alkyl phenyl sulfides **1**–**4**, low to moderate enantiomeric excesses were obtained. Oxidation of thioanisole led to the formation of (*R*)-**1a** with almost complete conversion after 24 h, but with moderate enantiomeric excess (ee = 44%). Sulfides with a longer alkyl chain **2**–**3** yielded the (*S*)-sulfoxides instead of the (*R*) ones, with lower conversions and enantiomeric excess compared to substrate **1**. Conversion of cyclopropyl phenyl sulfide **4** led to the preferential formation of the (*R*)-enantiomer, with an enantiomeric excess of 48%, close to that obtained with methyl group. No oxidation was observed when a carboxylic acid group was present in the sulfide structure **5**.

As described before,[6] the oxidation of **6** was not selective, leading to (*R*)-**6a** with low enantiomeric excess (ee = 10%) and 68% conversion after 24 h. The enzyme also showed very low selectivity with the other *p*-tolyl sulfides tested (compounds **7**–**9**; ee of the products ⩽17%).

The introduction of a methoxy group as a *para*-substituent (compound **10**) slightly decreased the (*R*)-selectivity (ee = 25%) compared to 44% for thioanisole **1**. Surprisingly, when a strong electron withdrawing group was located at the *para*-position **11**, the enantiomeric excess of the obtained sulfoxide (*R*)-**11a** was higher (ee = 76%) than the rest of phenyl sulfides previously examined. Despite its bulkiness, methyl 2-naphthyl sulfide **12** was also converted by PAMO to yield (*S*)-**12a** with a similar selectivity compared to thioanisole.

Table 1. Phenylacetone monooxygenase catalyzed oxidation of aromatic sulfides[a]

Compound	Structure	Time (h)	Conv.[b] (%)	ee[b] (%)	Configuration
1	C_6H_5–S–CH_3	24	94	44	*R*
2	C_6H_5–S–CH_2CH_3	24	79	33	*S*
3	C_6H_5–S–propyl	24	56	21	*S*
4	C_6H_5–S–cyclopropyl	24	67	48	*R*
5	C_6H_5–S–CH_2COOH	48	⩽3	—	—
6	*p*-CH_3–C_6H_4–S–CH_3	24	68	10	*R*
7	*p*-CH_3–C_6H_4–S–$(CH_2)_2$Cl	24	31	17	*S*
8	*p*-CH_3–C_6H_4–S–octyl	48	25	⩽3	—
9	*p*-CH_3–C_6H_4–S–CH_2COOEt	24	12	⩽3	—
10	*p*-CH_3O–C_6H_4–S–CH_3	24	47	25	*R*
11	*p*-O_2N–C_6H_4–S–CH_3	24	27	76	*R*
12	2-Naphthyl–S–CH_3	24	22	41	*S*
13	$C_6H_5CH_2$–S–S–$CH_2C_6H_5$	30	18	⩽3	—
14	$C_6H_5CH_2$–S–CH_3	6	29	94	*S*
15	C_6H_5–CH_2–S–CH_2–CH_3	6	36	98	*S*
16	$C_6H_5CH_2$–S–isopropyl	8	36	41	*R*
17	$C_6H_5CH_2$–S–butyl	18	32	28	*R*
18	$C_6H_5CH_2$–S–isopentyl	18	21	17	*R*
19	$C_6H_5CH_2$–S–CH_2COOMe	6	30	48	ND
20	$C_6H_5CH_2$–S–$(CH_2)_2$OAc	6	21	66	ND
21	$C_6H_5(CH_2)_2$–S–CH_3	8	27	80	*R*
22	$C_6H_5(CH_2)_2$–S–CH_2CH_3	6	23	83	ND
23	$C_6H_5(CH_2)_3$–S–CH_3	8	21	70	*R*

ND: not determined.

[a] For reaction details see Section 4.

[b] Conversion and enantiomeric excess determined by HPLC.

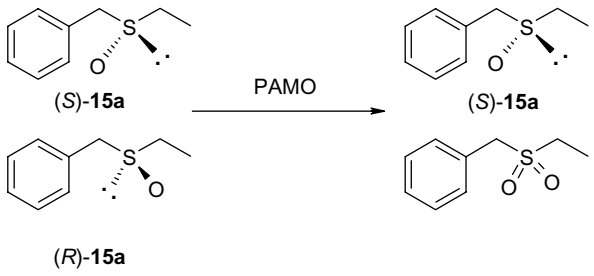
The alkyl benzyl sulfides possessing a small alkyl chain appeared to be very good PAMO substrates in terms of selectivity. The oxidations carried out with compounds **14** and **15** led to the formation of the (*S*)-sulfoxides in high enantiomeric excesses (ee = 94–98%) and good conversions (nearly 35% after 6 h). It is interesting to note that the structure of these sulfides is very similar to phenylacetone, the best PAMO substrate reported so far. By contrast, when the size of the alkyl chain for this kind of sulfide was larger than the ethyl group (isopropyl, butyl and isopentyl), the conversion and the enantiomeric excess of the sulfoxides decreased, as shown in Table 1 (**16**–**18**), lower values being obtained as the alkyl group increased. For chain lengths longer than ethyl, a change from (*S*)- to the (*R*)-configuration of the products was observed. Introduction of an ester group in the alkyl moiety of benzyl sulfides **19**–**20** gave sulfoxides **19a**–**20a** with moderate enantiomeric excesses. Conversions for these reactions were very similar to those obtained with methyl or ethyl groups.

Sulfides possessing the sulfur moiety further away from the aromatic ring were also converted with good selectivities. (*R*)-Methyl 2-phenylethyl sulfoxide (*R*)-**21a** was obtained with 80% ee and 27% conversion after 8 h using **21** as substrate. Furthermore, similar behavior was observed for the ethyl derivative **22** as shown in Table 1 (ee = 83%). When methyl 3-phenylpropyl sulfide **23** was used as PAMO substrate, moderate enantiomeric excess was achieved (ee = 70%) with 21% conversion after 8 h.

Besides, the compounds listed in Table 1, some disulfides with bulky chains, such as *p*-tolyl disulfide or thiantrene, were found not to be oxidized by PAMO, presumably because of steric hindrance. A nonaromatic disulfide, such as 1,3-dithiane, was also examined as an enzyme substrate. However, after 48 h only the racemic monosulfoxide was obtained with low conversion ($c = 18\%$).

Based on the obtained results, we can deduce, in terms of PAMO enantioselectivity for different sulfur positions in aromatic methyl sulfides, that the benzyl structure **14** is the one preferred by PAMO. The 2-phenylethyl group **21** also led to good enantiomeric excesses. The enantiomeric excess of the sulfoxide obtained decreased when the sulfur atom was still further away from the aromatic ring, as shown for compound **23**. Finally, the presence of the sulfur atom next to the aromatic ring **1** seemed to have a marked negative effect on PAMO enantioselectivity.

Previous studies carried out with CHMO revealed that the oxidation of the sulfoxide products to the corresponding sulfones was very slow and could not be exploited for kinetic resolution purposes.[4b] Instead, an increase in the enantiomeric excess of the sulfoxide products as a function of time was observed in some of the PAMO catalyzed oxidations. This indicated that the enzyme was not only able to catalyze the asymmetric oxidation from sulfide to sulfoxide, but also the kinetic resolution of the sulfoxide to the sulfone, as exemplified in Scheme 2 for (±)-**15a**.



Scheme 2. PAMO catalyzed kinetic resolution for (±)-**15a**.

Table 2 summarizes the results obtained in the kinetic resolution of a set of sulfoxides. Oxidation of both (±)-**1a** and (±)-**11a** occurred, but did not show any selectivity. More interesting results were obtained with the benzyl sulfoxides (±)-**14a** and (±)-**15a**, where the enantioselectivities (*E*),[8] especially in the case of the ethyl derivate ($E = 110$), were very high. This allowed the recovery of (*S*)-**15a** with high enantiomeric excess at conversions near 50% in short reaction times. Instead, when the isopropyl or methylcarboxymethyl benzyl derivates (**16a** and **19a**) were used, the reactions were slower and the selectivities very low. Finally, the (*S*)-enantiomer of sulfoxide (±)-**21a** was selectively oxidized ($E = 57$) to sulfone, leaving the (*R*)-enantiomer behind (ee = 95%). The high enantiomeric excess of compounds (*S*)-**14a**, (*S*)-**15a** and (*R*)-**21a** (Table 1) was the result of a combination of the asymmetric oxidation of the starting sulfides and, in part, of the resolution process of the sulfoxides formed.

Table 2. Kinetic resolution of racemic sulfoxides catalyzed by PAMO at 25 °C[a]

Compound	Structure	Time (h)	Conv.[b] (%)	ee[b] (%)	*E*[c]	Configuration
(±)-**1a**	C_6H_5–SO–CH_3	24	52	⩽3	≈1	—
(±)-**11a**	*p*-O_2N–C_6H_4–SO–CH_3	24	21	⩽3	≈1	—
(±)-**14a**	$C_6H_5CH_2$–SO–CH_3	4	39	60	53	*S*
(±)-**15a**	$C_6H_5CH_2$–SO–CH_2CH_3	4	49	93	110	*S*
(±)-**16a**	$C_6H_5CH_2$–SO–isopropyl	6	25	30	17	*R*
(±)-**19a**	$C_6H_5CH_2$–SO–CH_2COOMe	4	30	24	4.3	ND
(±)-**21a**	$C_6H_5(CH_2)_2$–SO–CH_3	8	51	95	57	*R*

ND: not determined.

[a] Reactions details described in Section 4.

[b] Conversion and optical purity determined by HPLC.

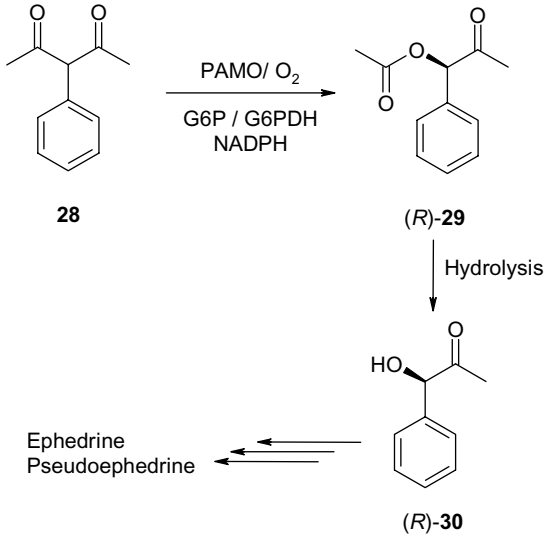
[c] Enantiomeric ratio, $E = \ln[(1-c)(1-ee_s)]/\ln[(1-c)(1+ee_s)]$.

The kinetic resolution of racemic sulfoxides by bacterial dimethyl sulfoxide reductases has recently been described with moderate to good enantioselectivities being found depending on the sulfoxide.[9] The resolution for these biocatalysts was based on the selective reduction of the starting sulfoxide to sulfides, not through their oxidation, as we described herein using PAMO.

PAMO has also been tested in the enzymatic oxidation of organic compounds possessing heteroatoms different from sulfur. Tertiary amine *N*-oxides play an important role in chiral catalysis and in biological processes.[10] These compounds are prepared by the oxidation of the corresponding amines and have been synthesized by biocatalytic methods using CHMO.[11] Herein, *N*,*N*-dimethylbenzylamine **24** was oxidized by PAMO to the corresponding *N*-oxide **24a** with 73% conversion after 48 h. When a bulky tertiary amine such as (*S*)-(−)-nicotine (*S*)-**25** was subjected to PAMO oxidation, the unaltered starting material was fully recovered after 2 days reaction.

From the literature, it is known that organoboron compounds can also be oxidized by BVMOs.[12a] We have tested one organoboron compound, phenylboronic acid **26**, as a PAMO substrate which led to the formation of phenol **27** ($c = 11\%$ after 24 h). The same product can be formed by chemical oxidation.[12b] This adds another type of oxidative reactivity to the broad catalytic repertoire of PAMO.

Finally, PAMO was used as a biocatalyst in the oxidation of 3-phenylpenta-2,4-dione **28**. Conversion of this diketone can lead to the formation of an interesting pharmaceutical intermediate, (*R*)-phenylacetylcarbinol (*R*)-**30** (Scheme 3), a well-known precursor in the synthesis of ephedrine and pseudoephedrine.[13] Currently, this compound is produced in an enzymatic process on industrial scale, using pyruvate and benzaldehyde as starting compounds.[14] As shown in Scheme 3, diketone **28** is indeed converted by PAMO with a good enantioselectivity (ee = 82%) into (*R*)-1-acetoxy-phenylacetone (*R*)-**29** in a relatively fast process ($c = 88\%$ after 1.5 h reaction). Hydrolysis of this ester would yield (*R*)-1-hydroxy-1-phenylacetone (*R*)-**30**. The production of (*R*)-**29** from **28** by an enzymatic Baeyer–Villiger oxidation shows the potential of PAMO to convert prochiral phenyldiketones.



Scheme 3. Biooxidation of 3-phenylpenta-2,4-dione **28**.

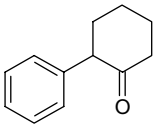
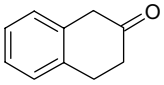
2.2. Determination of the kinetic parameters of PAMO

To obtain a better understanding on the catalytic efficiency of PAMO, the steady-state kinetic parameters of ketones **28**, **31**–**39**, sulfides **1**, **14**, **15**, **21** and their corresponding (±)-sulfoxides **1a**, **14a**, **15a** and **21a** were determined using isolated PAMO (Table 3).

The maximal catalytic rate (k_{cat}) was found to be remarkably similar for all substrates (1.2–3.6 s^{-1}). Only for some substrates, the exact value for k_{cat} could not be obtained due to solubility problems. More variation was found for the K_M values suggesting differences in substrate affinity, while the rate of catalysis is probably restricted by a common substrate-independent kinetic step. The oxidation of the sulfur atom at the α-position from the phenyl ring **1** occurred with a low catalytic efficiency (k_{cat}/K_M) due to a high K_M. A shift of the sulfur atom to the β- or γ-position **14**, **15** and **21** resulted in a 30- to 50-fold increase in catalytic efficiency. This increase coincided with an increased enantioselectivity. The racemic sulfoxides turned out to be rather poor substrates due to relatively high K_M values. It is interesting to note that sulfoxide **15a** not only displayed a relatively high catalytic efficiency but also showed the highest enantioselectivity. The presence of the sulfoxide moiety also seemed to negatively influence the apparent affinity of the enzyme for sulfoxides in comparison with the corresponding sulfides, while the catalytic rate hardly altered. Surprisingly, the sulfoxides showed a higher conversion in shorter times than the corresponding sulfides, while the catalytic efficiency of the sulfoxides was significant lower. A reason for this observation might be that the produced sulfoxides inhibited the conversion of the sulfides in a greater extent than the sulfones inhibiting the oxidation of the sulfoxides. This latter statement could however not be true for thioanisole **1**, which showed a higher conversion than **1a** with a lower catalytic efficiency.

Comparison of the steady-state kinetic parameters of ketones **31** and **33** indicated that the presence of an electron-withdrawing fluorine group at the *para*-position of phenylacetone **33** enhanced the catalytic efficiency of the enzyme by increasing the rate of catalysis. When fluorine atoms were positioned next to the carbonylic function (**34**), a 50-fold lower catalytic efficiency was observed when compared with phenylacetone, resulting from an increase in K_M. As shown above, PAMO also converted the prochiral diketone **28**. Steady-state analysis showed that this substrate was oxidized with a reasonable catalytic rate while displaying a relatively large K_M, suggesting a low affinity. The apparent affinity decreased even further when larger and bulkier groups **35**–**37** were introduced, resulting in a very poor catalytic efficiency.

Table 3. Kinetics parameters in PAMO oxidation for sulfides, sulfoxides and ketones

Num.	Compound	K_M (mM)	k_{cat} (s^{-1})	k_{cat}/K_M (M^{-1} s^{-1})
1	C_6H_5–S–CH_3	$\geqslant$2.5[a]	$\geqslant$0.12	~47
14	$C_6H_5CH_2$–S–CH_3	1.6	1.8	1100
15	$C_6H_5CH_2$–S–CH_2CH_3	1.3	2.3	1800
21	$C_6H_5(CH_2)_2$–S–CH_3	0.75	1.5	2000
1a	C_6H_5–SO–CH_3	19.5	1.9	96
14a	$C_6H_5CH_2$–SO–CH_3	23.7	1.2	51
15a	$C_6H_5CH_2$–SO–CH_2CH_3	8.7	2.7	310
21a	$C_6H_5(CH_2)_2$–SO–CH_3	19	1.3	68
28	CH_3COCH–C_6H_5–$COCH_3$	6.9	1.4	200
31	$C_6H_5CH_2COCH_3$[b]	0.059	1.9	32,000
32	$C_6H_5CH_2CH_2COCH_3$[b]	0.36	1.8	5000
33	*p*-F–$C_6H_4CH_2COCH_3$	0.056	3.6	65,000
34	$C_6H_5CH_2COCF_3$	4.0	2.3	580
35	$C_6H_5CH_2COCH_2CH_2CH_2CH_3$	$\geqslant$2.5[a]	$\geqslant$0.03	~10
36		$\geqslant$5.0[a]	$\geqslant$0.06	~10
37		$\geqslant$10.0[a]	$\geqslant$0.08	~8
38	*p*-HO–$C_6H_4CH_2CH_2COCH_3$	8.9	3.6	410

[a] Due to limited solubilities of the compounds, the substrate concentration could not be increased beyond the indicated values.
[b] Values taken from Ref. 6.

Other substrates such as 2-phenylcycloheptanone and 2-indanone also showed a low catalytic efficiency (28 and 18 M^{-1} s^{-1}, respectively) indicating that PAMO has difficulties accepting bulky aromatic ketones. Besides phenylacetone derivatives, benzylacetone **38** was also included in this study in order to compare with benzylacetone **32**. The electron donating hydroxyl group at *para*-position resulted in a ~10-fold lower catalytic efficiency. When also taking into account the above mentioned effect of a fluorine substituent, this indicates that the enzyme prefers electron withdrawing *para*-substituents. However, the effect of the hydroxyl group could also reflect steric hindrance.

Finally, steady-state kinetic studies were also performed with *N*,*N*-benzyldimethylamine **24** as substrate, which was previously shown to be converted to its corresponding *N*-oxide. However, by monitoring the consumption of NADPH in time, a catalytic rate of only 0.03 s^{-1} was measured.

3. Conclusion

Herein, the substrate acceptance and enatioselectivity of PAMO was explored. This has revealed that the enzyme is able to oxidize a wide range of sulfides and sulfoxides with varying degrees of selectivity, depending on the substrate structure. Also, the enantiopreference of the enzyme is substrate dependent, ranging from 98% ee for the (*S*)-configuration for benzyl ethyl sulfoxide **15a** to 80% ee for the (*R*)-configuration for methyl 2-phenylethyl sulfoxide **21a**. Furthermore, the enzyme can selectively oxidize benzyl- and 2-phenylethyl sulfoxides ($E > 50$). PAMO is also able to convert the prochiral substrate 3-phenylpenta-2,4-dione **28**, yielding mainly (*R*)-1-acetoxy-phenylacetone (*R*)-**29** (ee = 82%). In addition, it was also found that nitrogen and boron oxidations can be catalyzed by PAMO. The broad substrate specificity and reactivity makes this newly discovered biocatalyst a valuable tool for performing selective oxidation reactions. The kinetic analysis revealed efficient oxidation of either the sulfur atom or the carbon atom from the carbonyl group at the β- or γ-position from the phenyl ring, whereas oxidation at the α-position was either rather poor or did not occur at all.[6] The presence of the oxygen atom in the sulfoxides seemed to negatively influence the apparent affinity of the enzyme for these compounds in comparison with their corresponding sulfides. Apart from this, the introduction of bulky groups in phenylacetone derivatives **35**–**37** resulted in a large decrease of catalytic efficiency, indicating that PAMO has difficulties accepting bulky aromatic ketones. It was also established that PAMO prefers electron withdrawing *para*-substituents in the aromatic moiety in order to obtain high catalytic efficiencies. As described by Bocola et al., the substrate acceptance of PAMO can be increased even further by the creation of various enzyme mutants.[6b]

4. Experimental

4.1. General

Recombinant histidine-tagged phenylacetone monooxygenase was overexpressed and purified according to previously described methods.[6] Oxidation reactions were performed using the purified enzyme. One unit of

phenylacetone monooxygenase oxidizes 1.0 μmol of thioanisole **1** to **1a** per minute at pH 9 and 25 °C in the presence of NADPH. Glucose-6-phosphate dehydrogenase from *Leuconostoc mesenteroides* was obtained from Fluka-BioChemika. Glucose-6-phosphate and NADPH were purchased by Sigma–Aldrich.

Sulfides **1**, **2**, **4**, **6**, thiantrene, *p*-tolyldisulfide, 1,3-dithiano; racemic sulfoxides (±)-**1a**, (±)-**6a**, (−)-nicotine **25**, phenylboronic acid **26** and ketones **31**–**35**, **37** and **38** were purchased from Sigma–Aldrich–Fluka. Phenyl sulfides **11**–**12**, benzyl sulfides **14**–**15** and amine **24** were products from Lancaster. Sulfides **5**, **10** and ketone **36** were from Acros-Organics. Diketone **28** was purchased from TCI Europe. (*R*)-1-Hydroxy-1-phenylacetone **30** was a kind gift from Dr. M. Breuer (BASF). Compounds **3**,[15] **7**,[16] **8**,[17] **9**,[18] **13**,[15] **16**,[15] **17**,[15] **18**,[19] **19**,[20] **20**,[21] **21**,[19] **22**,[22] **23**,[19] (±)-**29**[23] and (±)-**30**[23] were prepared according to the literature. Sulfoxides were prepared by chemical oxidation from the corresponding sulfides and exhibit physical and spectral properties in accord with those reported.[15,17–21,24] *N*,*N*-dimethyl benzylamine *N*-oxide **24a** and *cis*-(*S*)-(−)-nicotine *N*-1′-oxide **25a** were obtained by chemical oxidation with 30% H_2O_2.[11,25] All other reagents and solvents were of the highest quality grade available, supplied by Sigma–Aldrich–Fluka.

IR spectra were recorded on a Jasco FTIR 610. Optical rotations were determined on a Perkin–Elmer 141 polarimeter. Chemical reactions were monitored by analytical TLC, performed on Merck silica gel 60 F_{254} plates and visualized by UV irradiation. Flash chromatography was carried with silica gel 60 (70–230 mesh, Merck). ^1H and ^{13}C NMR spectra at 300 and 72.5 MHz were recorded on a Bruker AC-300. Mass spectra were performed on a GC-MS-EI (Finnigan-Thermo). Chiral HPLC analyses were performed on a Jasco HPLC instrument (model 880-PU pump, model 870-UV/vis detector) equipped with a Chiralcel OD (Daicel) or a Chiralcel OB (Daicel) chiral column. Acetanilide was used as internal standard to determine the conversion of the oxidation processes. Retention times of the chiral samples were in accordance with those of the purified racemic ones. Chiral and achiral GC analyses were performed on a Shimadzu GC17 instrument equipped with a FID-detector and a Chiraldex G-TA column (Alltech, 30 m × 0.25 mm × 0.125 mm) or a HP1 column (Agilent, 30 m × 0.25 mm × 0.25 mm), respectively. The kinetic measurements were carried out with a PerkinElmer Lambda Bio40 spectrophotometer.

Unless otherwise stated, the absolute configurations of chiral sulfoxides were established by comparison of the HPLC chromatograms with the patterns described in previous experiments for the known configurations. For sulfoxides **3a**,[19] **4a**,[23a] **11a**[23e] and **16a**,[15] the absolute configuration was established by comparison of the specific rotation measured with the ones reported. The configuration of (*R*)-**29** was established by a comparison with an authentic sample prepared from chemical acetylation of (*R*)-**30**.

4.2. Typical procedure for the enzymatic oxidation of substrates

Substrates (20 mM, except for substrate **28**, 2.5 mM) were dissolved in a Tris/HCl buffer (50 mM, pH 9.0, pH 7.5 for **28**, 1.0 mL), containing glucose-6-phosphate (40 mM, 2 equiv), glucose-6-phosphate dehydrogenase (10.0 units), NADPH (0.02 mM), acetanilide (0.02 mg) as internal standard and 1.0 unit of phenylacetone monooxygenase. The mixture was shaken at 250 rpm and 25 °C in a rotatory shaker for the times established. The reactions were then stopped, worked up by extraction with dichloromethane (3 × 0.5 mL), dried over Na_2SO_4 and analyzed by chiral HPLC in order to determine the conversion and the enantiomeric excesses of the sulfoxides. The conversion and enantiomeric excess for compound (*R*)-**29** were determined by means of GC. Control experiments in the absence of enzyme were performed for all substrates tested, not observing reaction after long times.

4.3. General procedure for the enzymatic oxidations at multimilligram scale of sulfides 3, 4, 11 and 16

The sulfides (0.33 mmol for **3** and **4**; 0.29 mmol for **11** and 0.30 mmol for **16**) were dissolved in a Tris/HCl buffer (50 mM, pH 9.0, 25 mL) containing glucose-6-phosphate (1.5 equiv), glucose-6-phosphate dehydrogenase (2.5 units), NADPH (0.02 mM), acetanilide (0.5 mg) and phenylacetone monooxygenase (0.25 units). The mixtures were shaken at 25 °C and 250 rpm for 48 h for substrates **4** and **16**, 60 h for sulfide **3** and 72 h for compound **11**. Once finished, the reactions were extracted with dichloromethane (3 × 25 mL) and the combined organic layers dried over Na_2SO_4, filtered and evaporated under reduced pressure. The residues were purified by flash chromatography on silica gel with petroleum ether–ethyl acetate (9:1) to afford the corresponding sulfoxides: (*S*)-**3a** (colorless oil, 23.2 mg, 42% yield) (*S*)-**4a** (colorless oil, 17.6 mg, 32% yield), (*R*)-**11a** (yellow pale solid, 26.2 mg, 49% yield) and (*S*)-**16a** (yellow pale oil, 25.8 mg, 48% yield).

4.3.1. (*S*)-Phenyl propyl sulfoxide. Determination of ee by HPLC analysis: Chiralcel OB, petroleum ether–*i*-propanol (85:15), 1.0 mL/min, 254 nm, t_R 13.4 (*S*) and 29.3 (*R*) min, $[\alpha]_D^{25} = -51.8$ (*c* 0.88, $CHCl_3$), ee 21%.

4.3.2. (*S*)-Cyclopropyl phenyl sulfoxide. Determination of ee by HPLC analysis: Chiralcel OD, petroleum ether–*i*-propanol (95:5), 1.0 mL/min, 254 nm, t_R 13.1 (*R*) and 17.3 (*S*) min, $[\alpha]_D^{25} = +56.6$ (*c* 0.71, acetone), ee 48%.

4.3.3. (*R*)-Methyl *p*-nitrophenyl sulfoxide. Determination of ee by HPLC analysis: Chiralcel OB petroleum ether–*i*-propanol (75:25), 1.0 mL/min, 254 nm, t_R 37.9 (*S*) and 51.0 (*R*) min, $[\alpha]_D^{25} = +84.1$ (*c* 1.30, $CHCl_3$), ee 76%.

4.3.4. (*R*)-Benzyl isopropyl sulfoxide. Determination of ee by HPLC analysis: Chiralcel OD, petroleum ether–*i*-propanol (95:5), 1.0 mL/min, 254 nm, t_R 26.8 (*S*) and 28.9 (*R*) min, $[\alpha]_D^{25} = +53.7$ (*c* 1.29, EtOH), ee 41%.

4.4. Procedure for the determination of the kinetic parameters

For the determination of the steady-state kinetic parameters of phenylacetone monooxygenase with various ketones, sulfides and amines, the enzyme activity was determined by monitoring the decrease in NADPH concentration at 340 nm ($\varepsilon_{340} = 6.22\ mM^{-1}\ cm^{-1}$) or 370 nm ($\varepsilon_{370} = 2.7\ mM^{-1}\ cm^{-1}$). A reaction mixture of 1.0 mL usually contained 50 mM Tris/HCl, pH 7.5, 100 μM NADPH, 1% (v/v) DMSO and 0.5 μM PAMO. The presence of 1% DMSO resulted in only a slight decrease in PAMO activity (<1%), while a higher solubility of certain compounds could be obtained. The steady-state kinetic parameters were determined at 30 °C using air-saturated buffers.

Acknowledgements

We thank CERC3 for funding and Dr. Ivan Lavandera for his technical assistance. COST D25/0005/03 is gratefully acknowledged.

References

1. (a) Alphand, V.; Furstoss, R.. *Enzyme Catalysis in Organic Synthesis* In *Baeyer–Villiger Oxidations*; Drauz, K., Waldmann, H., Eds.; VCH Publishers: Weinheim, 1995; pp 745–772; (b) Willets, A. *Trends. Biotechnol.* **1997**, *15*, 55–62; (c) Mihovilovic, M. D.; Müller, B.; Stanetty, P. *Eur. J. Org. Chem.* **2002**, 3711–3730; (d) Alphand, V.; Carrea, G.; Wohlgemuth, R.; Furstoss, R.; Carrea, G. *Trends Biotechnol.* **2003**, *21*, 318–321; (e) Kamerbeek, N. M.; Janssen, D. B.; van Berkel, J. H.; Fraaije, M. W. *Adv. Synth. Catal.* **2003**, *345*, 667–678.
2. (a) Roberts, S. M.; Wan, P. W. H. *J. Mol. Catal. B: Enzym.* **1998**, *4*, 111–136; (b) Ottolina, G.; de Gonzalo, G.; Carrea, G.; Danieli, B. *Adv. Synth. Catal.* **2005**, *347*, 1035–1040.
3. (a) Fernández, I.; Khiar, N. *Chem. Rev.* **2003**, 3651–3705; (b) Legros, J.; Dehli, J. R.; Bolm, C. *Adv. Synth. Catal.* **2005**, *347*, 19–31.
4. (a) Ottolina, G.; Carrea, G.; Colonna, S.; Rückemann, A. *Tetrahedron: Asymmetry* **1996**, *7*, 1123–1136; (b) Colonna, S.; Gaggero, N.; Pasta, P.; Ottolina, G. *Chem. Commun.* **1996**, 2303–2307; (c) Mihovilovic, M. D.; Chen, G.; Wang, S.; Kyte, B.; Rochon, F.; Kayser, M. M.; Stewart, J. D. *J. Org. Chem.* **2001**, *66*, 733–738; (d) Sheng, D.; Ballou, D. P.; Massey, V. *Biochemistry* **2001**, *40*, 1156–1167; (e) Zambianchi, F.; Pasta, P.; Carrea, G.; Colonna, S.; Gaggero, N.; Woodley, J. M. *Biotechnol. Bioeng.* **2002**, *78*, 489–496; (f) Hilker, I.; Alphand, V.; Wohlgemuth, R.; Furstoss, R. *Adv. Synth. Catal.* **2004**, *346*, 203–214.
5. (a) Kamerbeek, N. M.; Moonen, M. J. H.; van der Ven, J. G. M.; van Berkel, W. J. H.; Fraaije, M. W.; Janssen, D. B. *Eur. J. Biochem.* **2001**, *268*, 2547–2557; (b) Kamerbeek, N. M.; Olsthoorn, A. J. J.; Fraaije, M. W.; Janssen, D. B. *Appl. Environ. Microbiol.* **2003**, 419–426; (c) Fraaije, M. W.; Kamerbeek, M. N.; Heidekamp, A. J.; Fortin; Janssen, D. B. *J. Biol. Chem.* **2004**, *279*, 3354–3360; (d) Mihovilovic, M. D.; Kapitan, P.; Rydz, J.; Rudroff, F.; Ogink, F. H.; Fraaije, M. W. *J. Mol. Catal. B: Enzym.* **2005**, *32*, 135–140.
6. (a) Fraaije, M. W.; Wu, J.; Heuts, D. P. H. M.; van Hellemond, E. W.; Lutje Spelberg, J. H.; Janssen, D. B. *Appl. Microbiol. Biotechnol.* **2005**, *66*, 393–400; (b) Bocola, M.; Schulz, F.; Leca, F.; Vogel, A.; Fraaije, M. W.; Reetz, M. T. *Adv. Synth. Catal.* **2005**, *347*, 979–986.
7. Malito, E.; Alfieri, A.; Fraaije, M. W.; Mattevi, A. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 13157–13162.
8. The enantiomeric ratio *E* is generally accepted as an adequate parameter to quantify the enantioselectivity in biochemical kinetic resolutions. See: Chen, C.-S.; Fujimoto, Y.; Girdauskas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294–7299.
9. Luckarift, H. R.; Dalton, H.; Sharma, N. D.; Boyd, D. R.; Holt, R. A. *Appl. Microb. Biotechnol.* **2004**, *65*, 678–685.
10. (a) Lindigkeit, R.; Biller, A.; Buch, M.; Schiebel, H. M.; Boppré, M.; Hartmann, T. *Eur. J. Biochem.* **1997**, *245*, 626–636; (b) Nakajiama, M.; Saito, M.; Shiro; Hashimoto, S.-I. *J. Am. Chem. Soc.* **1998**, *120*, 2511–2512.
11. Ottolina, G.; Bianchi, S.; Belloni, B.; Carrea, G.; Danieli, B. *Tetrahedron Lett.* **1999**, *40*, 8343–8346.
12. (a) Walsh, C. T.; Chen, Y.-C. J. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 333–343; (b) Kuivila, H. G.; Armour, A. G. *J. Am. Chem. Soc.* **1957**, *79*, 5659–5662.
13. Shin, H. S.; Rogers, P. L. *Biotechnol. Bioeng.* **1996**, *49*, 52–62.
14. (a) Leksawasdi, N.; Chow, Y. Y. S.; Breuer, M.; Hauer, B.; Rosche, B.; Rogers, P. L. *J. Biotechnol.* **2004**, *111*, 179–189; (b) Rosche, B.; Breuer, M.; Hauer, B.; Rogers, P. L. *J. Biotechnol.* **2005**, *115*, 91–99.
15. Holland, H. L.; Rand, C. G.; Viski, P.; Brown, F. M. *Can. J. Chem.* **1991**, *69*, 1989–1993.
16. Bird, R.; Stirling, J. M. *J. Chem. Soc., Perkin Trans. 2* **1973**, 1221–1226.
17. Colonna, S.; Gaggero, N.; Manfredi, A. *Biochemistry* **1990**, *29*, 10465–10468.
18. Truce, W. E.; Steltenkamp, R. J. *J. Org. Chem.* **1962**, *27*, 2816–2820.
19. Holland, H. L.; Brown, F. M.; Larsen, B. G. *Bioorg. Med. Chem.* **1994**, *2*, 647–652.
20. Jouen, C.; Lasne, M. C.; Pommelet, J. C. *Tetrahedron Lett.* **1996**, *14*, 2413–2416.
21. Gasparrini, F.; Giovannoli, M.; Misiti, D.; Natile, G.; Palmieri, G. *Tetrahedron* **1983**, *39*, 3181–3184.
22. Fehnel, E. A.; Carmack, M. *J. Am. Chem. Soc.* **1949**, *71*, 84–93.
23. Kanemoto, S.; Makoto, S.; Yoshioka, H. *Tetrahedron Lett.* **1987**, *28*, 6313–6316.
24. (a) Davis, F. A.; Reddy, R. T.; Weismiller, M. C. *J. Am. Chem. Soc.* **1989**, *111*, 5964–5965; (b) Colonna, S.; Gaggero, N.; Casella, L.; Carrea, G.; Pasta, P. *Tetrahedron: Asymmetry* **1992**, *3*, 95–106; (c) Secundo, F.; Carrea, G.; Dallavalle, S.; Franzosi, G. *Tetrahedron: Asymmetry* **1993**, *4*, 1981–1982; (d) Pasta, P.; Carrea, G.; Holland, H. L.; Dallavalle, S. *Tetrahedron: Asymmetry* **1995**, *6*, 933–936; (e) Brunel, J.-M.; Diter, P.; Duetsch, M.; Kagan, H. B. *J. Org. Chem.* **1995**, *60*, 8086–8088; (f) Yabuuchi, T.; Kusumi, T. *J. Am. Chem. Soc.* **1999**, *121*, 10646–10647.
25. Crooks, P. A.; Pool, W. F.; Damani, L. A.; Winkle, S. A. *Chem. Ind.* **1988**, 95–96.