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Research Paper

Stereoselective oxidation of bis-sulfides catalyzed by peroxygenase from oat

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ABSTRACT

The stereoselective oxidation of two classes of prochiral *bis*-sulfides, 1,3-dithianes and *bis*-(phenylthio)alkanes, was achieved by biocatalyzed reactions in the presence of a peroxygenase-containing enzymatic preparation from oat flour. In the oxidation of 2-substituted 1,3-dithianes the corresponding mono-sulfoxides were obtained as exclusive products and the reaction proceeded with preferential formation of *trans*-(1*S*,2*S*)-monosulfoxides in good enantiomeric and diastereoisomeric purity. The (1*S*,2*S*)-configuration of the obtained sulfoxides was assigned by correlation with known (1*S*,2*S*)-2-phenyl-1,3-dithiane 1-oxide through a combination of circular dichroism data, enantiomer elution orders in chiral HPLC and optical rotations. The enzymatic activity was monitored in different reaction media and in the presence of high concentration of acetonitrile or in organic solvent/aqueous buffer biphasic system a decrease in reaction rate was observed, however counterbalanced in some cases by increased stereoselectivity.

Unlike dithianes, *bis*-(phenylthio)alkanes were biocatalytically oxidized to the corresponding *bis*-sulfoxides, obtained as a mixture of chirally active C_2-symmetric and *meso*-isomers. Some over-oxidation products were detected in the reaction of *bis*-(phenylthio)methane while 1,2-*bis*-(phenylthio)ethane was cleanly converted into the corresponding *bis*-sulfoxide and the best stereoselectivity was obtained by performing the reaction in a biphasic water/*tert*-butyl methyl ether reaction medium.

1. Introduction

Biocatalysis is widely recognized as a powerful tool in organic synthesis for the generally high activity and intrinsic stereo-, regio- and chemoselectivity of the enzymes, resulting from a precise positioning of reactants in their active site [1]. An impressive variety of reactions is catalyzed by enzymes, whose available portfolio is continuously growing thanks to the modern protein-engineering techniques [2]. Many efforts have been devoted to increase productivity and biocatalysis has now become a mature technology for chemical and pharmaceutical synthesis [3], also meeting sustainability criteria for the use of mild reaction conditions and biocompatible catalysts as well as for the reduced generation of waste, resulting from avoided protection/deprotection steps and simplified purification procedures [4].

In this context, oxidative enzymes represent an important target since chemical oxidations often require harsh conditions and metal-based catalysts. Cytochrome P-450 and Bayer-Villiger monooxygenases have proven to be very effective in oxyfunctionalization reactions by using molecular oxygen as donor, but they require ancillary enzymatic systems for the regeneration of expensive NADPH cofactor [5–8]. Enzymes belonging to the peroxidase-peroxygenase superfamily [9] on the other hand, although containing a heme-binding domain that coordinates iron like cytochromes, show a specific ability to promote oxygen transfer from hydroperoxides to oxidizable substrates without the need of cofactors [10].

Unspecific peroxygenases (UPOs, E.C 1.11.2.1), whose sequences can be found in the genomes of many fungi, proved to be versatile catalysts in different reactions, also including the functionalization of non-activated carbons and heteroatoms [11–14].

Plant seed peroxygenases (EC 1.11.2.3), which play a key role in inducing plant response to abiotic stress and pathogen attacks through the production of phytooxylipins [15,16] deriving from the oxidative metabolism of fatty acids, have been less investigated. Synthetic applications of these plant enzymes have been mostly focused on peroxygenase from oat (*Avena sativa*) [17,18], which has been characterized at molecular [19] and genetic level [20,21]. Most of the reported applications of oat peroxygenase have been carried out by using the microsomal fraction of seeds, wherein the enzyme is localized [19], as biocatalyst. As an alternative, we have previously developed a simple protocol for obtaining a lyophilized crude preparation from oat seeds

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[22] that was shown equivalent, for that concerns the enzyme stereoselectivity, to the microsomal fraction extracted from it [23]. This crude peroxygenase preparation was found stable for up to 6 months at −20 °C [22], thus guaranteeing reproducibility within the same batch of enzymatic preparation, and revealed effective as biocatalyst in the selective epoxidation of polyunsaturated fatty acids (and the corresponding ethanolamides) [22,24] and both limonene enantiomers [25].

Our peroxygenase-containing preparation was also found active in catalyzing the oxidation of some aromatic sulfides to give the corresponding chiral sulfoxides and changes in the substituent on the sulfur atom affected the enzyme selectivity. In the same study it was also evidenced that the resolution of racemic thioanisole sulfoxide *via* overoxidation to sulfone was not applicable due to the low enantiodiscrimination ability of the enzyme. [23]

As a part of a study aimed to better explore the potential of oat peroxygenase in organic synthesis, we planned to extend the biocatalyzed sulfur oxidation reaction to two different classes of symmetric *bis*-sulfides, namely 1,3-dithianes **1** and *bis*-(phenylthio)alkanes **3** (Scheme 1), that could represent useful probes for a better evaluation of the stereochemical recognition features of the enzyme. The expected products, chiral mono-sulfoxides and/or C_2-symmetric *bis*-sulfoxides, have gained interest in asymmetric synthesis for their use as organocatalysts or ligands for catalytically active metal-complexes [26–28]. Specific methods have been developed for the asymmetric oxidation of *bis*-sulfides [29] with a large focus on 2-substituted 1,3-dithianes [30–32], whose *S*-oxides are known as efficient chiral auxiliaries in organic synthesis [33,34].

While biocatalyzed oxidation of monosulfides has been largely explored [35,36], the oxidation of *bis*-sulfides has been less investigated and mostly relies on the use of bacterial or fungal whole cell cultures [37–39], with few examples on the use of purified monooxygenases or chloroperoxidase [40–42]. The use of a peroxygenase-containing raw preparation from oat seeds could offer a cheap alternative in avoiding specialized microbiology laboratories as well as expensive procedures for enzyme purification and here we report the obtained results in peroxygenase-catalyzed oxidation of **1** and **3** to give the corresponding chiral sulfoxides.

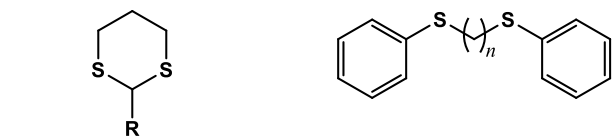
2. Material and methods

2.1. General

1,3-Dithiane **1**, *bis*-(phenylthio)methane, **3** and 1,2-*bis*-(phenylthio) ethane **3a** were obtained from Alfa Aesar and used without further purification. All the other reagents were purchased from Sigma-Aldrich and used as received. Aqueous *tert*-butyl hydroperoxide (TBHP, 70 wt % in H_2O, 7.3 M) was obtained from Santa Cruz Biotechnology. Potassium phosphate buffer (50 mM) was prepared from K_2HPO_4 adjusting pH to 7.5 with H_3PO_4.

TLC analyses were performed on Merck silica gel 60 F254 aluminum plates revealing the compounds by UV at 254 nm. Column chromatography was performed on silica gel (LiChroprep Si 60, 25–40 μm, Merck,) using the specified eluents. ^{1}H NMR and ^{13}C NMR spectra were recorded with a Bruker AvanceTM 400 spectrometer at 400.13 and 100.62 MHz, respectively. Chemical shifts (δ) are given as ppm relative to the residual solvent peak and coupling constants (*J*) are expressed in Hz. Optical rotations were measured at 23 °C on a Jasco P-2000 polarimeter using a 10 cm length cell. Circular dichroism spectra were acquired at 20 °C in methanol (1 cm cell length) on a Jasco J-810 spectropolarimeter equipped with the PFD-452S Peltier cell holder.

High resolution mass spectra (HR-MS) were acquired by a Thermofisher Orbitrap Exploris 120 instrument with ESI ionization mode, set with 3.1 kV source voltage and 275 °C capillary temperature.



R = H **1** R = Et **1a** R = Ph **1b** R = 4-MePh **1c** R = 4-OMePh **1d** R = 4-BrPh **1e** R = 4-NO_2Ph **1f**

n = 1 **3** *n* = 2 **3a**

Scheme 1. Chemical structure of bis-sulfides investigated in this study.

2.2. Enzyme preparation from oat seeds

Crude enzymatic preparation from oat seeds flour was obtained as previously reported [25]. Briefly, commercial whole seeds (60 g) of air-dried oat (*Avena sativa*) from organic crops were ground in a domestic blender and the obtained flour was defatted by washing with diethyl ether (3 × 150 mL), then the suspension was centrifuged at 4000 RPM (2930 *g*) and the supernatant discarded. The final residue was dried at room temperature overnight to give defatted flour (56 g).

Defatted oat flour was suspended in water (160 mL) and stirred for 10 min. The slurry was centrifuged at 4000 RPM for 7 min and the suspension was collected. The pellet was washed with water and the suspension centrifuged again. The pooled supernatant fractions were freeze-dried to give 5.2 g of a white light powder (lyophilized enzymatic preparation).

For the enzyme activity assay, oat preparation (50 mg) was suspended in phosphate buffer pH 7.5 (5 mL) and methyl oleate (13 μL, 38 μmol) was added. After addition of TBHP (6 μL, 44 mmol) the mixture was stirred at 25 °C and the reaction progress monitored by GC (Supelco SPB-5 capillary column, 15m×0.1 mm ID×0.1 μm film thickness, oven temperature from 100 °C to 280 °C at 10 °C/min). The enzyme activity was expressed as enzyme unit (U), defined as the amount of enzyme that catalyzes the conversion of 1 μmol of methyl oleate per minute to the corresponding epoxide in the assay conditions. An activity of 0.0075 ±0.001 U/mg was determined for the raw enzymatic preparation used in this work.

2.3. Chemical synthesis of 2-substituted 1,3-dithiane derivatives *1a* and *1c-1f*

The chemical synthesis of title compounds was carried out following a reported procedure [43]. Briefly, the suitable aldehyde (3.5 mmol) and I_2 (104 mg, 0.4 mmol) were dissolved in $CHCl_3$ (15 mL) at room temperature and 1,2-propanedithiol (0.44 mL, 4.3 mmol) was added. The reaction course was followed by TLC analysis and when the aldehyde had been consumed (5–30 min) 0.1 M sodium thiosulfate pentahydrate (15 mL) and 1 M NaOH (2 mL) were added. The mixture was then extracted with CH_2Cl_2 (3 × 10 mL) and the organic phase was separated, dried on Na_2SO_4 and taken to dryness. The obtained solid was washed with *n*-hexane and isolated by crystallization from a mixture of CH_2Cl_2/hexane in 85–95 % yield. Compound **1b,** which was a pale oil, was instead purified by chromatography on silica gel by eluting with *n*-hexane/CH_2Cl_2 8:2 (v/v) mixture. The ^{1}H and ^{13}C NMR analysis confirmed the structure of all isolated compounds and the obtained spectra were in agreement with data reported in the literature [43].

2.4. HPLC analyses

HPLC analyses were performed with a Dionex instrument equipped with a Ultimate 3000 high-pressure binary pump, an ASI-100 autosampler, a TCC-100 thermostated column compartment and a UVD-100 multiple wavelength detector set at 220, 230, 240 and 260 nm. Chromeleon software (version 6.7) was used for instrument control, data acquisition and data handling.

Chiral HPLC analyses were carried out at 23 °C by using Lux cellulose-1 (4.6 mm × 150 mm, Phenomenex) or Lux amylose-1 (4.6 mm x 150 mm, Phenomenex) column, eluting with *n*-hexane/2-PrOH mixtures. Reference samples of the racemic mono- and disulfoxides were obtained by standard oxidation of the disulfides with glacial AcOH and 30 % H_2O_2 mixture in CH_2Cl_2.

The separation of *cis*-sulfoxide and *trans*-sulfoxides isomers (and enantiomers) was achieved in most cases on Lux-Amylose-1 column. For the comparison of enantiomer elution orders with literature data pure dithiane 1-oxides were analyzed on Lux cellulose-1 column, which is equivalent to the reported Chiralcel OD column.

2.5. Preliminary experiments of biocatalyzed oxidation of dithianes

To a suspension of the lyophilized enzymatic preparation (30 mg) in phosphate buffer:acetone 8:2 (v/v) (8 mL) the chosen dithiane (**1, 1a** or **1b**) was added (0.1 mmol) and the resulting mixture was vigorously stirred at 30 °C. The oxidant TBHP (27 μl, 0.2 mmol) was added in three portions over 1 h and the reaction course monitored by chiral HPLC. At suitable times, aliquots (0.3 mL) of the reaction mixtures were withdrawn and extracted with diethyl ether (0.6 mL). The organic phase was separated, dried over anhydrous Na_2SO_4 and diluted with mobile phase before HPLC injection. Chiral HPLC data: t_R 11.86 and 15.19 for **2** (Amylose-1, *n*-hexane:2-PrOH 8:2, flow 1.0/mL); t_R 14.95 and 16.57 for *cis*-**2a** and t_R 17.46 and 22.59 for *trans*-**2a** (Lux-1, *n*-hexane:2-PrOH 75:25, flow 0.5 mL/min); t_R 9.20 and 9.71 for *cis*-**2b** and t_R 10.39 and 18.22 for *trans*-**2b** (Amylose-1, *n*-hexane:2-PrOH 8:2, flow 1.0 mL/min).

2.6. Biocatalyzed oxidation of 2-phenyl-1,3-dithiane **1b** in different reaction media

For the reactions carried out in the presence of water miscible organic solvent (acetone or acetonitrile) the peroxygenase lyophilized preparation (30 mg) was suspended in phosphate buffer containing the desired percentage of cosolvent (8 mL total volume). The reaction was started by adding substrate **1b** (19.6 mg, 0.1 mmol) and TBHP (16 μl, 0.12 mmol) in two aliquots over 30 min. For reactions carried out in a two-phase system the peroxygenase lyophilized preparation (30 mg) was suspended in phosphate buffer (6 mL) and the immiscible organic solvent (TBME or EtOAc, 2 mL) was added. The reaction was started as above and maintained under vigorous magnetic stirring at 30 °C for 2 h. Aliquots (0.4 mL) of the reaction mixture were withdrawn and extracted with Et_2O; the organic layer was then separated, dried on Na_2SO_4 and taken to dryness. The residue was dissolved in 2-PrOH and analyzed by chiral HPLC (Lux amylose-1 column, eluent: *n*-hexane/2-PrOH 70:30 v/v at flow rate of 0.7 mL/min).

2.7. Pre-incubation experiments

In these experiments the enzyme was exposed to the reaction medium for some time intervals before starting the reaction by addition of the thioanisole as substrate. The enzymatic preparation (30 mg) was suspended in selected solvent/buffer mixture (8 mL) and stirred at 30 °C for the chosen time (1, 2, 3, 6, 17, 24 h). At the suitable time, thioanisole (44 mg, 0.35 mmol) and TBHP (38 μL, 0.28 mmol) were added and the reaction mixture was analyzed by HPLC after 15 min for the substrate conversion in comparison with a parallel reaction carried out without pre-incubation.

2.8. Biocatalytic oxidation of 1,3-dithiane derivatives

The substrates (0.1 mmol) were suspended in phosphate buffer/CH_3CN 8:2 v/v (8 mL) and peroxygenase-containing preparation (30 mg) was added. The reactions were started by adding TBHP (16 μl, 0.12 mmol) in two aliquots over 30 min and maintained under vigorous magnetic stirring at 30 °C. After 2 h the reactions were extracted with Et_2O, the organic phase was dried over Na_2SO_4 and analyzed by chiral HPLC using the appropriate stationary phases and eluents.

2.9. General procedure for the synthesis of trans-sulfoxides **2b-2f**

To a suspension of peroxygenase-containing preparation (500 mg) and dithiane (1 mmol) in phosphate buffer/CH_3CN 8:2 v/v (30 mL), TBHP (1.2 mmol, 165 μl) was added in three aliquots over 2 h. The mixture was stirred at 30 °C for 3–24 h and the reaction course was monitored by TLC analysis. At the suitable time, the reaction was quenched with MeOH (1 mL) and extracted with EtOAc (3 × 10 mL). The organic phase was dried over Na_2SO_4 and the solvent evaporated under reduced pressure. Substrate conversion was determined by HPLC analyses of the reaction mixture (>99 % for **1b** and **1c**; 80 % for **1e**; 65 % for **1d** and 31 % for **1f**). The residue of the reaction mixture was purified by column chromatography (EtOAc:2-PrOH 94:6). Fractions containing single compounds were pooled and taken to dryness.

*(1S,2S)-2-phenyl-1,3-dithiane 1-oxide, trans-**2b***: 46 % yield (after crystallization from EtOAc), >99% *ee*, $[\alpha]_D^{23}$ + 124.2 (*c* 0.8, $CHCl_3$) (lit. [44] $[\alpha]_D$ + 94 (*c* 1.2, $CHCl_3$); lit. [30] $[\alpha]_D^{25}$ + 117.4 (*c* 0.63, $CHCl_3$); HPLC (Lux-1, *n*-hexane:2-PrOH 7:3, flow 0.7 mL/min t_R 13.9 min [(1*R*, 2*R*)-*trans*-**2b**] and 34.4 min [(1*S*,2*S*)-*trans*-**2b**]; CD: λ/nm 214 (Δε –4.74), 227 (Δε 20.06), 247 (Δε 5.06).^{1}H NMR δ 2.31–2.44 (1H, m, H-5a), 2.49–2.58 (1H, m, H-5b), 2.65–2.73 (1H, m, H-4a), 2.75–2.85 (1H, m, H-6a), 2.89 (1H, dt, *J* = 13.2 and 2.1, H-4b), 3.53–3.62 (1H, m, H-6b), 4.59 (1H, s, H-2), 7.26–7.41 (5H, m, Ar-H); ^{13}C NMR δ 29.49, 31.38, 54.69, 69.60, 128.70, 129.08, 129.32, 133.26. HR-ESI-MS: 235.0202 $[M + Na]^+$; theor. for $C_{10}H_{12}S_2O + Na^+$ 235.0228.

*(1S,2R)−2-phenyl-1,3-dithiane 1-oxide, cis-**2b***: 13 % isolated yield, >99% *ee*, 97:3 *dr*; $[\alpha]_D^{23}$ + 35.08 (*c* 0.29, $CHCl_3$); HPLC (Lux-1, *n*-hexane:2-PrOH 7:3, flow 0.7 mL/min t_R 14.7 min [(1*R*,2*S*)-*cis*-**2b**] and 15.6 min [(1*S*,2*R*)-*cis*-**2b**]; CD: λ/nm 214 (Δε –5.20), 227 (Δε 10.92).^{1}H NMR δ 1.85–1.87 (1H, m, H-5a), 2.64–2.77 (3H, m, H-5b, H-4a and H-6a), 3.01–3.08 (1H, m, H-4b), 3.21–3.24 (1H, m, H-6b), 4.78 (1H, s, H-2), 7.36–7.42 (3H, m, Ar-H), 7.43–7.44 (2H, m, Ar-H); ^{13}C NMR δ 13.91, 30.02, 47.36, 64.62, 128.38, 128.92, 129.15, 135.46. HR-ESI-MS: 235.0202 $[M + Na]^+$; theor. for $C_{10}H_{12}S_2O + Na^+$ 235.0228.

*(1S,2S)-2-(4′-methylphenyl)-1,3-dithiane 1-oxide, trans-**2c***: 50 % yield (after crystallization from EtOAc), >99% *ee*, $[\alpha]_D^{23}$ + 127.1 (*c* 0.5, $CHCl_3$) (lit [32]. $[\alpha]_D^{26}$ + 88.6 (*c* 1.0, $CHCl_3$) for a sample with 62 % *ee*); HPLC (Lux-1, *n*-hexane:2-PrOH 7:3, flow 1.0 mL/min t_R 9.5 min [(1*R*, 2*R*)-*trans*-**2c**] and 21.8 min [(1*S*,1*S*)-*trans*-**2c**]; CD: λ/nm 216 (Δε –6.38), 231 (Δε 20.80). ^{1}H NMR δ 2.29–2.42 (4H, s and m, –CH_3 and H-5a), 2.48–2.56 (1H, m, H-5b), 2.64–2.70 (1H, m, H-4a), 2.75 (1H, dt, *J* = 13.2 and 2.6, H-6a), 2.88 (1H, dt, *J* = 13.2 and 2.2, H-4b), 3.51–3.60 (1H, m, H-6b), 4.53 (1H, s, H-2), 7.20 (2H, d, *J* = 8.0, Ar-H), 7.30 (2H, d, *J* = 8.0, Ar-H); ^{13}C NMR δ 21.22, 29.54, 31.46, 54.67, 69.43, 128.56, 129.83, 130.18, 139.39. HR-ESI-MS: 249.0358 $[M + Na]^+$; theor. for $C_{11}H_{14}S_2O + Na^+$ 249.0384.

*(1S,2S)-2-(4′-methoxyphenyl)-1,3-dithiane 1-oxide, trans-**2d***: 34 % yield (after crystallization from EtOAc), 97% *ee*, $[\alpha]_D^{23}$ + 156.2 (*c* 0.5, $CHCl_3$); HPLC (Lux-1, *n*-hexane:2-PrOH 7:3, flow 0.7 mL/min t_R 19.1 min [(1*R*,2*R*)-*trans*-**2d**] and 36.8 min [(1*S*,1*S*)-*trans*-**2d**]; CD: λ/nm 221 (Δε –11.97), 243 (Δε 18.45), 275 (Δε 1.68).^{1}H NMR δ 2.27–2.41 (1H, m, H-5a), 2.46–2.55 (1H, m, H-5b), 2.62–2.69 (1H, m, H-4a), 2.73 (1H, dt, *J* = 13.2 and 2.7, H-6a), 2.86 (1H, dt, *J* = 13.2 and 2.6, H-4b), 3.50–3.59 (1H, m, H-6b), 3.79 (3H, s, –OMe), 4.51 (1H, s, H-2), 6.91 (2H, d, *J* = 8.8, Ar-H), 7.33 (2H, d, *J* = 8.8, Ar-H); ^{13}C NMR δ 29.49, 31.46, 54.63, 55.25, 69.03, 114.53, 125.12, 129.87,160.29. HR-ESI-MS: 265.0294 $[M + Na]^+$; theor. for $C_{11}H_{14}S_2O_2 + Na^+$ 265.0333.

*(1S,2S)-2-(4′-bromophenyl)-1,3-dithiane 1-oxide, trans-**2e***: 68 % isolated yield, 66 % *ee*, $[\alpha]_D^{23}$ + 80.0 (*c* 0.5, $CHCl_3$) (lit.[32] $[\alpha]_D^{26}$ + 87.6 (*c* 1.2, $CHCl_3$) for a sample with 70 % *ee*); HPLC (Lux-1, *n*-hexane:2-PrOH 7:3, flow 1.0 mL/min t_R 10.9 min [(1*R*,2*R*)-*trans*-**2e**] and 35.3 min [(1*S*, 1*S*)-*trans*-**2e**]; CD: λ/nm 218 (Δε –5.08), 237 (Δε 7.62).^{1}H NMR δ 2.29–2.43 (1H, m, H-5a), 2.48–2.57 (1H, m, H-5b), 2.65–2.72 (1H, m,

H-4a), 2.77 (1H, dt, $J = 13.2$ and 2.4, H-6a), 2.88 (1H, dt, $J = 14.4$ and 2.4, H-4b), 3.55–3.58 (1H, m, H-6b), 4.53 (1H, s, H-2), 7.29 (2H, d, $J = 8.0$, Ar-H), 7.52 (2H, d, $J = 8.0$, Ar-H); ^{13}C NMR δ 29.46, 31.33, 54.66, 68.91, 123.61, 130.30, 132.26, 132.31. HR-ESI-MS 312.9284 and 314.9260 $[M + Na]^+$; theor. for $C_{10}H_{11}S_2OBr^{79} + Na^+$ 312.9332.

(1S,2S)-2-(4′-nitrophenyl)-1,3-dithiane 1-oxide, trans-**2f**: 12 % yield (after crystallization from EtOAc), 74 % *ee*, $[\alpha]_D^{23} + 124.3$ (*c* 0.5, $CHCl_3$) (lit.[32] $[\alpha]_D^{26} + 121.4$ (*c* 0.92, $CHCl_3$) for a sample with 66 % *ee*); HPLC (Lux-1, *n*-hexane:2-PrOH 7:3, flow 1.0 mL/min t_R 19.9 min [(1*R*, 2*R*)-*trans*-**2f**] and 59.1 min [(1*S*,1*S*)-*trans*-**2f**]; CD: λ/nm 213 (Δε 1.98), 232 (Δε –7.46), 274 (Δε 6.09).^{1}H NMR δ 2.35–2.50 (1H, m, H-5a), 2.54–2.64 (1H, m, H-5b), 2.71–2.88 (2H, m, H-4a and H-6a), 2.94 (1H, dt, $J = 13.2$ and 1.5, H-4b), 3.57–3.69 (1H, m, H-6b), 4.66 (1H, s, H-2), 7.60 (2H, d, $J = 8.4$, Ar-H), 8.26 (2H, d, $J = 8.4$, Ar-H); ^{13}C NMR δ 29.48, 31.29, 54.89, 68.90, 124.21, 129.88, 140.46, 148.39. HR-ESI-MS: 280.0048 $[M + Na]^+$; theor. for $C_{10}H_{11}NS_2O_3 + Na^+$ 280.0078.

2.10. Analytical scale oxidation of bis-(phenylthio)alkanes *3* and *3a*

To a suspension of peroxygenase preparation (100 mg) and **3** or **3a** (0.1 mmol) in phosphate buffer/CH_3CN 80:20 v/v (9 mL), TBHP (0.20 mmol) was added in two aliquots over 1 h and the mixture maintained under vigorous magnetic stirring at 30 °C. The reactions were monitored by chiral HPLC analysis using a Lux cellulose-1 (*n*-hexane/2-PrOH 7:3, flow 0.5 mL/min) for oxidation of **3** and Amylose-1 (*n*-hexane/2-PrOH 8:2, flow 1.0 mL/min) for oxidation of **3a**.

2.11. Biocatalytic oxidation of bis-(phenylthio)methane, *3*

Peroxygenase preparation (400 mg) was added to a suspension of **3** (230 mg, 1.0 mmol) in phosphate buffer/CH_3CN 80:20 v/v (18 mL). The reaction was started by adding TBHP (300 μl, 2.2 mmol) in four aliquots over 2 h and maintained under vigorous magnetic stirring at 30 °C. After 4 h, the reaction was extracted with EtOAc (3 × 10 mL) and the organic phase dried over anhydrous Na_2SO_4. After removal of the solvent under reduced pressure, the residue was purified by column chromatography (Si gel, *n*-hexane:EtOAc from 7:3 to 1:1) and fractions containing pure compounds were pooled and taken to dryness. The following compounds were isolated:

Phenyl Phenylthiomethyl Sulfoxide, **5**: 20 % yield, 40 % *ee*; HPLC (Lux-1, *n*-hexane:2-PrOH 7:3, flow 0.5 mL/min) t_R 14.8 min (major) and 17.4 (minor); ^{1}H NMR δ AB-system: 4.09 (1H, d, $J = 13.6$, $–CH_{2a}$) and 4.21 (1H, d, $J = 13.6$, $–CH_{2b}$); 7.24–7.35 (3H, m, Ar-H), 7.42–7.55 (5H, m, Ar-H), 7.67–7.7.76 (2H, m, Ar-H); ^{13}C NMR δ 60.94, 124.89, 127.76, 129.12, 129.29, 130.98, 131.66, 133.55, 142.69. HR-ESI-MS: 271.0199 $[M + Na]^+$; theor. for $C_{13}H_{12}S_2O + Na^+$ 271.0227.

Phenyl Phenylthiomethyl Sulfone, **6**: 2 % yield, HPLC (Lux-1, *n*-hexane:2-PrOH 7:3, flow 0.5 mL/min) t_R 19.5 min; ^{1}H NMR δ 4.35 (2H, s, $–CH_2$), 7.23–7.24 (3H, m, Ar-H), 7.34–7.36 (2H, m, Ar-H), 7.51 (2H, t, $J = 7.6$, Ar-H), 7.61 (1H, t, $J = 7.2$, Ar-H), 7.92 (2H, d, $J = 7.6$, Ar-H); ^{13}C NMR δ 59.76, 128.17, 129.00, 129.10, 129.20, 131.60, 132.87, 134.04, 137.66. HR-ESI-MS: 287.0146 $[M + Na]^+$; theor. for $C_{13}H_{12}S_2O_2 + Na^+$ 287.0176.

*Phenyl Phenylsulfinylmethyl Sulfone,***7***:* 18 % yield, 50% *ee*, HPLC (Lux-1, *n*-hexane:2-PrOH 7:3, flow 1.0 mL/min) t_R 29.8 min (major) and 46.1 (minor); ^{1}H NMR δ AB-system: 4.32 (1H, d, $J = 13.6$, $–CH_{2a}$) and 4.40 (1H, d, $J = 13.6$, $–CH_{2b}$); 7.52–7.58 (3H, m, Ar-H), 7.60–7.70 (4H, m, Ar-H), 7.73 (1H, t, $J = 7.4$, Ar-H), 8.03 (2H, d, $J = 8.0$, Ar-H); ^{13}C NMR δ 80.05, 124.06, 128.60, 129.56, 129.75, 129.88, 132.15, 134.74, 138.74, 142.56. HR-ESI-MS: 303.0095 $[M + Na]^+$; theor. for $C_{13}H_{12}S_2O_3 + Na^+$ 303.0126.

(S,S)-Bis-(phenylsulfinyl)methane, **4**: 52 % yield, 68 % *ee*, 63:37 *dr*, HPLC (Lux-1, *n*-hexane:2-PrOH 7:3, flow 0.5 mL/min) t_R 17.2 min [(*S,S*)-**4**], 24.0 min [(*R,R*)-**4**] and 31.4 [(*R,S*)-*meso*-**4**]; ^{1}H NMR δ 4.00 (2H, s, $–CH_2$, *dl*), AB-system (*meso*): 4.10 (1H, d, $J = 12.8$, $–CH_{2a}$) and 4.20 (1H, d, $J = 12.8$, $–CH_{2b}$); 7.49–7.55 (6H, m, Ar-H, *dl*), 7.56–7.62 (6H, m, Ar-H, *meso*), 7.64–7.69 (4H, m, Ar-H, *dl*), 7.70–7.75 (4H, m, Ar-H, *meso*); ^{13}C NMR δ 79.76 (*meso*), 84.79 (*dl*), 123.92 (*dl*), 124.31 (*meso*), 129.63 (*meso*), 129.69 (*dl*), 131.85 (*dl*), 131.93 (*meso*), 142.09 (*meso*), 142.68 (*dl*). HR-ESI-MS: 287.0146 $[M + Na]^+$; theor. for $C_{13}H_{12}S_2O_2 + Na^+$ 287.0176. Title compound was repeatedly crystallized from EtOAc to give optically enriched (*S,S*)-**4** (95 % *ee* and 69:31 *dr*) in mother liquor, $[\alpha]_D^{23} + 215$ (*c* 0.5, acetone) (lit.[45] $[\alpha]_D^{15} + 358$ (*c* 0.19, acetone); CD: λ/nm 216 (Δε –36.98), 248 (Δε 23.28).

2.12. Biocatalytic oxidation of bis-(phenylthio)ethane, *3a*

Peroxygenase preparation (400 mg) was added to a suspension of **3a** (215 mg, 1.0 mmol) in phosphate buffer/TBME 75:25 v/v (18 mL). The reaction was started by adding TBHP (300 μl, 2.2 mmol) in four aliquots over 2 h and maintained under vigorous magnetic stirring at 30 °C. The reaction was monitored with TLC analysis by eluting with *n*-hexane/EtOAc 60:40 v/v mixture. After 6 h the reaction was extracted with EtOAc (3 × 10 mL) and the organic phase was dried over anhydrous Na_2SO_4. After removal of the solvent under reduced pressure, the solid was crystallized from EtOAc to give *1,2-(R,R)-bis-(phenylsulfinyl)-ethane,* **4a** in 50 % yield, >98 % *ee* and 97:3 *dr*; $[\alpha]_D^{23} + 281.1$ (*c* 0.5, EtOH) [lit. [46] $[\alpha]_D^{20} + 278$ (*c* 0.4, EtOH)]; HPLC (Amylose-1, *n*-hexane:2-PrOH 8:2, flow 1.0 mL/min) t_R 13.6 min [(*S,S*)-**4a**], 16.0 [(*R,S*)-*meso*-**4a**] and 18.9 min [(*R,R*)-**4a**]; CD: λ/nm 216 (Δε –66.59), 242 (Δε 54.69).^{1}H NMR δ 2.70–2.79 (2H, m, $–CH_{2a}$), 3.36–3.45 (2H, m, $–CH_{2b}$), 7.50 (10H, br s, Ar-H); ^{13}C NMR δ 47.70, 123.85, 129.41, 131.31, 142.30. HR-ESI-MS: 301.0301 $[M + Na]^+$; theor. for $C_{14}H_{14}S_2O_2 + Na^+$ 301.0333

3. Results and discussion

3.1. Biocatalyzed oxidation of 1,3-dithianes

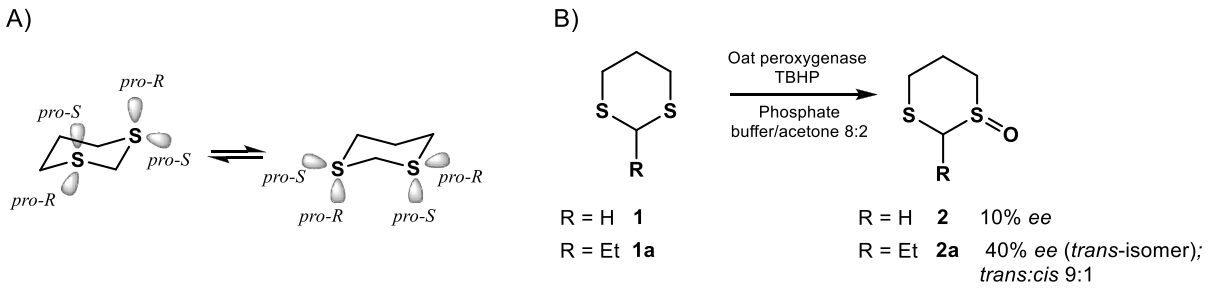
At the onset of our investigation the standard conditions previously applied for the peroxygenase-catalyzed oxidation of thioanisole (raw peroxygenase-containing preparation from oat seeds, 20 % acetone/phosphate buffer) [25] were applied to 1,3-dithiane **1** and the corresponding monosulfoxide **2** was obtained as exclusive product (Scheme 2) in 2 h, even in the presence of an excess of *t*-BuOOH (TBHP) oxidant. Sulfoxide **2** was however obtained in nearly racemic form (10 % *ee*) suggesting that the enzyme was not effective in stereodifferentiating between prochiral lone pairs (enantiotopic lone pairs) on sulfur atoms during the oxidation reaction, probably due to the conformational flexibility of 1,3-dithiane ring (Scheme 2A). Even assuming a preferential oxidation on the equatorial sulfur lone pair compared to the axial one, as it is known for chemical [47] and biocatalytic [37] oxidations of dithianes, the equatorial lone pairs of **1** are interchanged by ring-flipping and the formation of sulfoxides with opposite configuration could be expected.

It could be envisaged that a bias of this interconversion equilibrium following the introduction of a substituent on C-2 position could result in some fixation of *pro-R* and *pro-S* lone pairs on each sulfur atom and, therefore, a more stereoselective outcome of the oxidation reaction.

When the same reaction conditions were applied to dithiane derivative **1a**, a mixture of diastereoisomeric sulfoxides (*trans:cis* **2a** 90:10) was obtained, but the enantioselectivity was still unsatisfactory as 36 % *ee* was determined for the major *trans*-isomer.

This low degree of stereoselectivity has been also observed for the oxidation of **1** and some related 2-alkyl-1,3-dithianes in the presence of growing cultures of different fungi [37,48], while highly selective oxidation of the same substrates has been reported with cyclohexanone monooxygenase from *Acinetobacter* sp. as catalyst [38,41].

Moving to dithiane **1b**, in which the phenyl substituent is fixed in equatorial position [49], resulted in increased stereoselectivity and (1*S,2S*)-*trans*-**2b** in 72 % *ee* was obtained as major monosulfoxide together with a minor amount of diastereoisomer *cis*-**2b** in enantiopure form



Scheme 2. A) Interconversion equilibrium in 1,3-dithianes. B) Biocatalyzed oxidation of 1,3-dithianes.

(Scheme 3). The relative configuration of diastereoisomers was assigned on the basis of their NMR spectra [50-51] while the absolute (1*S, 2S*)-configuration of *trans*-isomer was by comparison of circular dichroism and optical rotation data [44] (*see infra*).

It is interesting to note that formation of *cis*-**2b** is strictly catalyzed by oat-peroxygenase since this isomer is not obtained (<2 %) from either chemical or monooxygenase-catalyzed oxidations as well as from asymmetric synthesis with different chiral catalysts.

Carrying out the biocatalyzed oxidation of **1b** in pure buffer negatively affected both diastereoselectivity and enantioselectivity in the formation of *trans*-**2b** (Table 1, compare entries 1 and 2), while using CH_3CN as co-solvent in place of acetone led to comparable results (Table 1, entry 3). The positive influence of addition of an organic co-solvent was also confirmed with *t*-BuOH (compare entries 1 and 4 in Table 1).

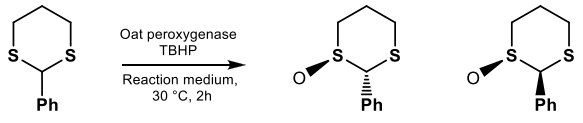
Unlike what was reported for fungal peroxygenase from *Agrocybe aegerita* [52], increasing the amount of CH_3CN as co-solvent up to 50 % (v/v) led to reduced reaction rate associated however to slightly better global stereoselectivity (compare entries 3 and 5–6 in Table 1).

Taking into the account the solid nature of **1b** and its low solubility in water, we also considered the use of a biphasic solvent system, with the organic solvent acting as reservoir of substrate. In this context, previous reports only deal with the use of aqueous-neat biphasic systems (where the second phase is the liquid substrate) for the UPO-catalyzed oxidation of ethylbenzene [53], while oat peroxygenase has been shown active in pure apolar solvents [54].

Since **1b** is not soluble in apolar solvents we considered *tert*-butyl methyl ether (TBME) or ethyl acetate as suitable solvents to set a biphasic reaction medium. In TBME/buffer system the oxidation of **1b** proceeded with the same stereoselectivity observed in homogeneous phase with 20 % co-solvent (compare entries 2, 3 and 7 in Table 1) and slightly lower reaction rate. The use of ethyl acetate/buffer led to decreased conversion of substrate compared to biphasic system with TBME (entries 7 and 8 in Table 1). The enzyme was shown active also in pure TBME but the enantioselectivity was significantly reduced (Table 1, entry 9).

Although the best conditions were obtained with 20 % (v/v) co-solvent, these data confirm that oat peroxygenase well tolerates organic solvents, in the presence of which the reduced reaction rates were in some cases counterbalanced by increased stereoselectivity.

Table 1
Effects of the reaction medium of the biocatalyzed oxidation of 1b[a].

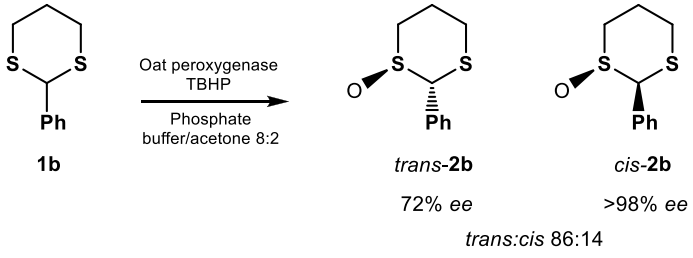


Entry	Reaction Medium	Conv. (%)[b]	*trans*-2a:*cis*-2a[b]	*ee trans*-2a (%)[b]
1	Buffer[c]	87	78:22	61
2	20 % Acetone/ Buffer	>99	86:14	71
3	20 % CH_3CN/Buffer	>99	87:13	72
4	20 % *t*-BuOH/Buffer	93	84:16	68
5	40 % CH_3CN/Buffer	74	92:8	75
6	50 % CH_3CN/Buffer	52	92:8	75
7	TMBE/Buffer[d]	80	84:16	72
8	EtOAc/Buffer[d]	56	92:8	72
9	TBME	52	86:14	68

[a] Reaction conditions: Peroxygenase-containing preparation (30 mg), substrate (19.6 mg, 0.1 mmol), TBHP (16 μL, 0.12 mmol), solvent (8 mL), 30 °C, 2h
[b] Determined by chiral HPLC (see Experimental section)
[c] 50 mM phosphate buffer at pH 7.5.
[d] Two phase system, TBME (2 mL)/phosphate buffer (6 mL).

Pre-incubation experiments in which the enzyme was exposed to the reaction medium for some time intervals before starting the reaction by addition of thioanisole, chosen as reference substrate, revealed an initial loss of the enzyme activity in 40 % CH_3CN/buffer or in TBME/buffer, which however maintained almost constant up over 24 h, compared to pure buffer or 20 % CH_3CN/buffer solvent (Figure S1).

Having determined 20 % CH_3CN/buffer as the best reaction medium for the biocatalyzed oxidation of **1b**, we extended our study to some other dithiane derivatives **1c-1f**, obtained by condensation of 1,3-propandithiol with the suitable aldehydes according to a reported procedure [43]. Introducing a *para*-substituent on the phenyl ring of **1b** resulted in decreased reaction rate for all the tested compounds, but increased stereoselectivity was found for substrates **1c** and **1d**, bearing an electron donating (for mesomeric effects) *para*-substituent. On the other hand,



Scheme 3. Biocatalyzed oxidation of 2-phenyl-1,3-dithiane.

the introduction of the electron withdrawing nitro group in dithiane **1f** negatively affected both stereoselectivity and reactivity (Table 2).

The selectivity towards the formation of *1S,2S*-enantiomer of *trans*-sulfoxides **2c**-**2f** was maintained in all the cases and higher diastereoisomeric ratios were determined in comparison with **2b**. The absolute configurations were assigned by correlation with the known (+)-(1*S*,2*S*)-2-phenyl-1,3-dithiane-1-oxide **2b** [44] (see *infra*).

3.2. Assignment of the absolute configuration of 2-phenyl-1,3-dithiane-1-oxides

The absolute configuration of monosulfoxides **2c**-**2f** has been previously assigned by comparison of chiral HPLC retention times with literature data and only for sulfoxide *trans*-(1*S*,2*S*)-(+)-**2b** the configuration was ascertained by X-Ray analysis and associated to its chiroptical properties (optical rotation and circular dichroism) [44]. However, HPLC data for *trans*-**2b** on the same Chiralcel OD column were conflicting since Lattanzi et al. [55] reported a (1*S,2S*)-configuration for the first eluting enantiomer without any additional support, while in a subsequent work by Wu et al. [31] the first eluting compound of *trans*-**2b** was associated to the (1*R,2R*)-enantiomer as confirmed by its negative optical rotation. In the same work of Wu et al. [31], even for *para*-substituted sulfoxides **2c**-**2f** the first eluted enantiomers show negative optical rotation, but in the case of *trans*-**2c** and *trans*-**2d** the authors assigned (1*S*,2*S*)-configuration to these enantiomers following the HPLC assignment reported by Lattanzi et al. [55].

In order to shed light on the assignment of absolute configuration of these compounds, we planned to isolate the target compounds from biocatalyzed oxidation reactions, optically enrich them by crystallization and measure their optical rotation and circular dichroism (CD) spectra. In our hands, the major enantiomer of *trans*-**2b** eluted as the second peak in chiral HPLC analyses on a Lux-1 column, which is packed with the same cellulose-based chiral stationary phase as Chiralcel OD and is therefore equivalent to it. A positive optical rotation was measured for this enantiomer, which agrees with its (1*S,2S*)-configuration [44].

The CD spectrum of (1*S,2S*)-*trans*-**2b** was then recorded in methanol and a negative band at about 214 nm followed by two more intense and positive Cotton effects centered at 227 and 247 nm were observed (Fig. 1) [44]. All the other *trans*-sulfoxides **2c**-**2f** obtained by biocatalyzed oxidation showed the same characteristics as (1*S,2S*)-*trans*-**2b**, i.e. the major enantiomer was eluted as the second peak in chiral HPLC analysis, had positive optical rotation and gave the same sign of bands in CD spectra, so that the (1*S*,2*S*)-configuration was assigned to all these compounds (Table S1).

The first band in CD spectra (around 210 nm), which have been attributed to an allowed σ-σ* transition associated with the sulfoxide

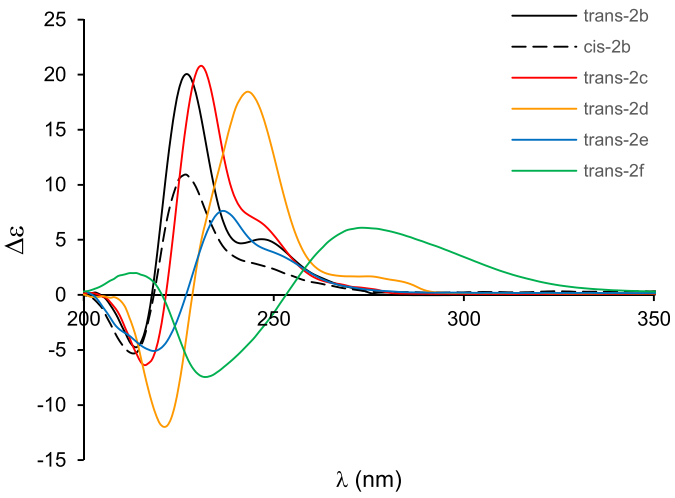


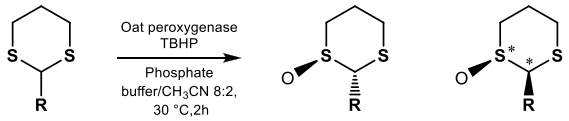
Fig. 1. CD spectra of 2-substituted-1,3-dithiane 1-oxides.

chromophore [37,56-57], moved slightly to longer wavelengths going from **2b** to **2e** while the other Cotton effects in the range 225–245 nm, attributed to sulphide chromophore [37] and aromatic π-π* transitions [56], were found more sensitive to the *para*-substituent on phenyl ring. In the case of compound **2f**, the electron-withdrawing and conjugative effects between the nitrogroup and the benzene ring resulted in a strong bathochromic shift of the absorption bands, as observed in different classes of compounds following the presence of nitro-chromophore [56, 58]. Taking into the account the chromatographic behavior and the sign of optical rotation of *trans*-**2f**, we were confident in assigning the 1*S*, 2*S*-configuration also to this sulfoxide obtained by peroxygenase-catalyzed oxidation.

In the CD spectrum of *cis*-**2b** the bands associated with sulfoxide/sulfide chromophores showed the same sign and λ_{max} as observed for (1*S,2S*)-*trans*-**2b**, but with lower intensity, suggesting that these Cotton effects are little affected by the C-2 configuration, as reported for other *cis-trans* diastereoisomeric sulfoxides [57]. On this basis the 1*S* configuration was assigned to sulfoxide in *cis*-**2b,** resulting in a 1*S*,2*R* configuration for this compound.

During this study, we also noticed that the elution of *trans*-sulfoxides **2b**-**2f** on amylose-based chiral stationary phase (CSP) was reversed with respect to that observed on Chiralcel OD column (or its equivalent Lux-1 column), which is a cellulose-based CPS, thus highlighting the need for careful comparison of peak elution order in the absolute configuration assignment.

Table 2
Peroxygenase-catalyzed oxidation of 2-phenylsubstituted 1,3-dithianes[a].



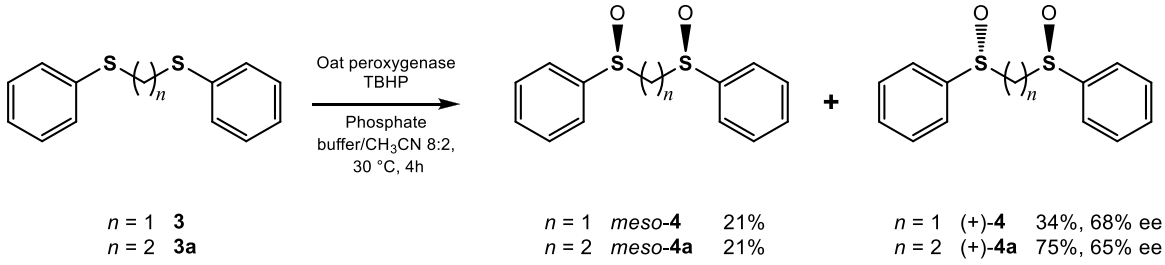
R	Substrate	Conv. (%)[b]	Product	*trans:cis*[b]	*ee trans* (%)[b]
Ph	**1b**	>99	**2b**	87:13	72
4-Me-Ph	**1c**	71	**2c**	91:9	88
4-OMe-Ph	**1d**	41	**2d**	94:6	90
4-Br-Ph	**1e**	53	**2e**	94:6	66
4-NO_2-Ph	**1f**	17	**2f**	90:10	57

[a] Reaction conditions: Peroxygenase-containing preparation (30 mg), substrate (0.1 mmol), *t*-BuOOH (16 μl, 0.12 mmol), phosphate buffer pH 7.5/CH_3CN 8:2 (8 mL), 30 °C, 2 h.
[b] Determined by chiral HPLC.

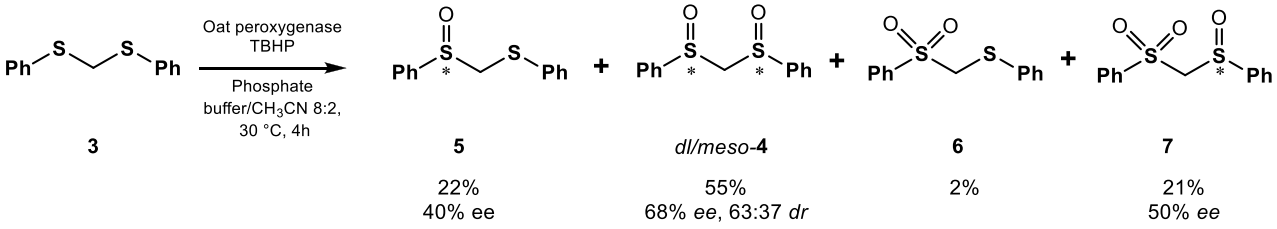
3.3. Biocatalyzed oxidation of bis-(phenylthio)alkanes

Preliminary reactions of peroxygenase-catalyzed oxidation of *bis*-(phenylthio)methane, **3** and 1,2-*bis*-(phenylthio)ethane, **3a** in 20 % CH_3CN/phosphate buffer in the presence of oxidant excess showed that, unlike as observed in dithianes, both sulfur atoms in the substrates underwent oxidation, resulting in the formation of the corresponding *bis*-sulfoxides **4** and **4a,** both obtained as a mixture of (*R,S*)-*meso*- and C_2-symmetric isomers (Scheme 4).

The oxidation of **3**, however, gave rise to a complex reaction mixture (Scheme 5) due to the presence of over-oxidation products, which are formed from the beginning of the reaction and not in a sequential manner so that it was not possible to control their formation by decreasing the quantity of oxidant. While the substrate was rapidly consumed, the intermediate monosulfoxide **5** was present throughout the reaction together with *bis*-sulfoxide **4**, monosulfone **6** and sulfone-sulfoxide **7**, all identified by combination of their NMR and ESI spectra with chiral HPLC elution profiles (Figure S2). The presence of different reaction products each with its own enantiomeric excess



Scheme 4. Biocatalyzed oxidation of *bis*-(phenylthio)alkanes.



Scheme 5. Composition of the reaction mixture in the biocatalyzed oxidation of *bis*-(phenylthio)methane.

suggests that each oxidation step is catalyzed by peroxygenase and the occurrence of kinetic resolution processes (*e. g.* from **5** to **4** or from **4** to **7**) cannot be excluded. The composition of the reaction mixture, therefore, is the result of different activities and stereoselectivities shown by the enzyme during each oxidation step and depends on the time and the amount of available oxidant.

Comparable reaction profile was observed changing the reaction medium (40 % CH_3CN/buffer; TBME/buffer) and, furthermore, *meso*-**4** was found chromatographically inseparable from the C_2-symmetric isomer. However, it was possible to obtain enantiomerically enriched *bis*-sulfoxide (+)-**4** (95 % *ee*) together with 31 % of *meso*-isomer in mother liquors of repeated crystallizations from EtOAc and its absolute configuration was assigned as (*S,S*) on the basis of its reported optical rotation [45].

On the other hand, *bis*-sulfide **3a** underwent a complete and fairly stereoselective oxidation to the corresponding *bis*-sulfoxide, obtained as a mixture of *meso*-**4a** (21 %) and (*R,R*)-**4a** with 65 % enantiomeric excess, without any further side-product. Interestingly, when the same biocatalyzed reaction was carried out in TBME/buffer biphasic system the amount of formed *meso*-compound decreased at 13 % while the enantiomeric excess of (*R,R*)-**4a** increased at 83 %. These conditions were then applied for the preparation of (*R,R*)-**4a** that, although chromatographically inseparable from the *meso*-isomer, was obtained in enantiopure form and *dr* 97:3 by direct crystallization from the whole reaction mixture. The absolute configuration of the obtained (*R,R*)-**4a** was assigned by comparison of its CD spectrum and optical rotation with literature data [46,59].

When the CD spectrum of (*R,R*)-**4a** was recorded in CH_2Cl_2 for comparison with literature data [59] a single positive Cotton effect with λ_{max} 242 nm was observed, while running the spectrum in methanol revealed that this band is part of a bisignate −/+ couplet centered at 228 nm (Figure S3).

Beyond the opposite descriptors for the absolute configuration, due to the priority rules, the optically active sulfoxides **4** and **4a** have the same spatial arrangement around the chiral sulfur atoms, that is also in agreement with the stereoselectivity observed in the oxidation of thioanisole [20], of which **3** and **3a** could be considered the dimers.

4. Conclusions

In this study the catalytic activity of peroxygenase from oat was investigated in the oxidation of 1,3-dithianes and *bis*-(phenylthio)alkanes with the aim to gain information about the recognition features of the enzyme in discriminating two prochiral reactive centers in the substrate. In the case of dithianes, the stereoselective outcome of oxidation, which affects only one of the two sulfur atoms, improved by introducing a substituent in position 2 of the substrates. When the reaction was applied to a series of 2-phenylsubstituted-1,3-dithianes some influence of the aromatic substituent on the yield and stereoselectivity was observed, so that the corresponding (1*S*,2*S*)-*trans*-1-oxides were obtained in moderate to good optical purity. On the other hand, in the reaction with *bis*-(phenylthio)methane both sulfur atoms in the substrate underwent oxidation and over-oxidation products were also formed, resulting in a decreased yield of the target chiral C_2-sulfoxide, obtained in mixture with its *meso*-isomer. Peroxygenase-driven oxidation of 1,2-*bis*-(phenylthiol)ethane afforded the expected *bis*-sulfoxide as exclusive product and enhanced diastereo- and enantioselectivity were observed changing the reaction medium from 20 % CH_3CN/buffer to *tert*-butyl methyl ether/buffer biphasic system.

The use of a crude peroxygenase-containing preparation of plant origin has the advantage of easy availability of the enzyme source and represents a valid and economical alternative to the use of whole-cell biocatalysts or purified enzymes, which require specialized microbiology laboratories or time-consuming steps for enzyme purification. On the other hand, modulation of enzymatic activity (stereopreference and substrate scope) by protein-engineering techniques is more easily achievable for microbial peroxygenases, as demonstrated by the broad spectrum of efficiency reached with nonspecific fungal peroxygenases (UPOs).

The results here described could contribute to better understand the potential of peroxygenase in organic synthesis and offer an alternative to metal-based catalysts in the preparation of optically active sulfoxides.

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CRediT authorship contribution statement

Claudia Sanfilippo: Writing – review & editing, Investigation,

Conceptualization. **Federica Cernuto:** Investigation. **Angela Patti:** Writing – review & editing, Writing – original draft, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Angela Patti reports financial support was provided by Italian Ministry for University and Research. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.mcat.2024.114440.

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