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Biocatalytic oxidation of *S*-alkylcysteine derivatives by chloroperoxidase and *Beauveria* species

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Abstract

Treatment of *N*-methoxycarbonyl *C*-carboxylate ester derivatives of *S*-methyl-L-cysteine by chloroperoxidase (CPO)/hydrogen peroxide resulted in oxidation at sulfur to produce the (R_S) sulfoxide in moderate to high diastereomeric excess (DE). The (S_S) natural product sulfoxide chondrine was obtained via biotransformation of the *N*-t.boc derivative of L-4-S-morpholine-2-carboxylic acid using *Beauveria bassiana* or *Beauveria caledonica*. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Amino acid oxidation; *Beauveria bassiana*; *Beauveria caledonica*; Biocatalysis; Biotransformation; Chloroperoxidase; Chondrine; Enzyme catalysis; *S*-Methylcysteine; Sulfoxidation

1. Introduction

Natural sulfoxides of the sulfur-containing amino acids methionine and *S*-alkylcysteines (Fig. 1) have been reported to be involved in a variety of processes, ranging from aroma and flavour components (cysteine derivatives) [1–3], antibacterial activities [4,5], and age-dependant biological properties (methionine derivatives) [6,7], to a range of other oxidase enzyme-related activities [8]. The cysteine derivative chondrine, a product of the red alga *Chondria crassicaulis* [9,10] is of interest as a close relation to naturally occurring flavour precursors of onions [10,11].

The preparation of methionine sulfoxides [12] and *S*-alkylcysteine sulfoxides [13,14] with defined stereochemistry at sulfur has been achieved in low yields by chemical resolution. All the stereoisomers of methionine and ethionine sulfoxides have been prepared via biotransformation reactions involving the conversion of protected amino acid substrates to the corresponding sulfoxides by *Beauveria bassiana* [15] or *Beauveria caledonica* [8,15], while the selective formation of predominantly (*R*)-sulfoxides by sulfur oxidation of protected methionine substrates by chloroperoxidase (CPO) [16], and of the (*S*)-sulfoxide from *S*-allylcysteine by the enzyme cyclohexanone monooxygenase [3], have also been reported. The asymmetric syntheses of both diastereomers of protected (*S*)-methyl-L-cysteine and (*S*)-*n*-propyl-L-cysteine sulfoxides by using diacetone-D-glucose as a chiral transfer reagent has also been achieved [17], and the natural product chondrine (**6**) has been synthesised (in combination with its stereoisomeric sulfoxide) by oxidation of the corresponding sulfide using hydrogen peroxide [18,19].

The use of the enzyme CPO from the fungus *Caldariomyces fumago* as a catalyst for the stereoselective hydrogen peroxide-dependent oxidation of a

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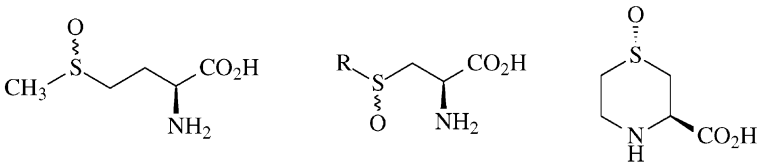


Fig. 1. Natural sulfoxides of sulfur-containing amino acids.

wide range of sulfides to chiral sulfoxides has been reported [20,21], and the application of this method for the asymmetric sulfoxidation of methionine derivatives is also known [16]. The present paper extends this reaction to the use of substrates derived from *S*-alkyl-L-cysteines, and reports its use for the preparation of protected (R_S)-*S*-alkyl-L-cysteine sulfoxides in moderate to high diastereomeric excess (DE). In addition, preparation of the (S_S) natural sulfoxide chondrine via biotransformation of the *N*-t.boc derivative of L-4-*S*-morpholine-2-carboxylic acid using *B. bassiana* or *B. caledonica* as biocatalysts is described.

2. Experimental

2.1. Materials and methods

Melting points were determined on a Kofler hot stage and are uncorrected. The ^{1}H NMR spectra were recorded on a Bruker Avance series 300 spectrometer in $CDCl_3$ using residual $CHCl_3$ as the internal standard or CD_3OD using CH_3OH as the internal standard unless otherwise stated; chemical shifts are reported (in ppm) (δ) and the signals quoted as s (singlet), d (doublet), t (triplet), q (quartet) or m (multiplet). The ^{13}C NMR spectra were recorded at 75 mHz on the same spectrometer in $CDCl_3$, CD_3OD, or D_2O solution. DE was determined by ^{13}C NMR analysis of signals α and β to sulfur. Mass spectra were obtained using a Kratos 1S spectrometer. Optical rotations were recorded at ambient temperature in the stated solvent using a Rudolph Autopol 3 polarimeter. TLC was performed on Merck silica gel F_{254} plates, 0.2 mm, and column chromatography used Merck silica gel 9385, 230–400 mesh.

CPO from *C. fumago* was obtained from the Sigma Chemical Co. (catalogue no. C-0278). *B. bassiana* ATCC 7159 and *B. caledonica* ATCC 64970 were obtained from the American Type Culture Collection, and maintained on 4% malt agar slopes.

2.2. Preparation of substrates: L-cysteine derivatives

2.2.1. N-protection

N-Acetyl-*S*-methyl-L-cysteine, *N*-chloroacetyl-*S*-methyl-L-cysteine and *N*-phthaloyl-*S*-methyl-L-cysteine [8,16], and *S*-ethyl-L-cysteine [22], *S*-*n*-propyl-L-cysteine [23] and *S*-allyl-L-cysteine [3] were prepared as described.

N-methoxycarbonyl (*N*-MOC)-*S*-methyl, *S*-ethyl, *S*-*n*-propyl and *S*-allyl-L-cysteines were prepared by the following standard procedure. A stirred solution of *S*-methyl-L-cysteine (5.02 g) in aqueous sodium hydroxide (80 ml, 2 M) at 0 °C was treated with methyl chloroformate (20.5 ml) in 1 ml increments over 1 h. The pH was maintained above 10.5 by addition of 2 M sodium hydroxide as required. After the final aliquot of methyl chloroformate (0.5 ml) was added, the reaction mixture was allowed to reach room temperature then washed with an equal volume of ether. The aqueous layer was then cooled to 0 °C, acidified to pH 2.3 with 10% HCl, and extracted with ethyl acetate (3 × 200 ml). The extract was dried ($MgSO_4$) and evaporated to yield *N*-MOC-*S*-methyl-L-cysteine (4.15 g, 58%), oil. NMR data are listed below. Unless stated otherwise all products gave *m*/*z* MH^+ ions using FAB-NBA spectra.

2.2.1.1. N-MOC-S-methyl-L-cysteine. ^{1}H NMR ($CDCl_3$): δ 2.16 (3H, s), 3.0 (2H, m), 3.74 (3H, s), 4.62 (1H, q), 5.65 (1H, br.d), and 6.45 (1H, br.s) ppm; ^{13}C NMR ($CDCl_3$): δ 16.6, 36.7, 53.1, 53.7, 157.2 and 175.6 ppm; MS (EI); *m*/*z* (%) 193 (3), 161 (6), 118 (19), 61 (100).

*2.2.1.2. N-MOC-S-ethyl-*L*-cysteine.* ^{1}H NMR (CD_3 OD): δ 1.24 (3H, t), 2.58 (2H, q), 2.8–3.0 (2H, m), 3.65 (3H, s), 4.43 (1H, t) and 4.85 (1H, br.s) ppm; ^{13}C NMR (CD_3OD): δ 14.0, 26.0, 33.3, 51.8, 54.4, 157.6 and 171.0 ppm.

*2.2.1.3. N-MOC-S-n-propyl-*L*-cysteine.* ^{1}H NMR (CD_3OD): δ 0.99 (3H, t), 1.59 (2H, m), 2.53 (2H, t), 2.75–3.0 (2H, m), 3.72 (3H, s), 4.36 (1H, m), and 4.82 (1H, br.s) ppm; ^{13}C NMR (CD_3OD): δ 13.6, 23.8, 34.6, 35.3, 52.8, 55.5, 156.8 and 173.3 ppm.

*2.2.1.4. N-MOC-S-allyl-*L*-cysteine.* ^{1}H NMR δ (CD_3OD): 2.7–3.0 (2H, m), 3.18 (2H, d), 3.70 (3H, s), 4.38 (1H, m), 5.10 (2H, m), 5.74 (1H, m), and 7.45 (1H, br.s) ppm; ^{13}C NMR (CD_3OD): δ 32.0, 34.6, 51.8, 54.2, 116.9, 134.4, 157.3 and 172.1 ppm.

2.2.2. Esterification

The esters of N-protected amino acids were all obtained by the following standard procedure using the appropriate alcohol. *N*-MOC-*S*-methyl-L-cysteine (1.13 g) was dissolved in methanol (20 ml) and a catalytic amount of hydrochloric acid added to adjust the solution to pH 3.0. The solution was heated under reflux for 12 h, then evaporated. Water (50 ml) was added and the product extracted with ethyl acetate. The solution was washed with saturated $NaHCO_3$, dried and evaporated to give *N*-MOC-*S*-methyl-L-cysteine methyl ester as an oil (0.92 g, 75%). NMR data are listed later. Unless listed otherwise, all products gave *m*/*z* MH^+ ions using FAB-NBA spectra.

*2.2.2.1. N-MOC-S-methyl-*L*-cysteine methyl ester (**1a**).* ^{1}H NMR ($CDCl_3$): δ 2.02 (3H, s), 2.85 (2H, m), 3.60 (3H, s), 3.70 (3H, s), 4.49 (1H, q) and 5.70 (1H, br.s) ppm; ^{13}C NMR ($CDCl_3$): δ 16.5, 37.0, 52.8, 53.0, 53.7, 156.8 and 171.8 ppm.

*2.2.2.2. N-MOC-S-methyl-*L*-cysteine ethyl ester (**1b**).* ^{1}H NMR ($CDCl_3$): δ 1.24 (3H, t), 2.08 (3H, s), 2.90 (2H, m), 3.64 (3H, s), 4.18 (2H, q), 4.50 (1H, q), and 5.58 (1H, br.s) ppm; ^{13}C NMR ($CDCl_3$): δ 12.1, 16.6, 22.5, 37.3, 52.8, 53.8, 66.3, 157.0 and 171.6 ppm.

*2.2.2.3. N-MOC-S-methyl-*L*-cysteine n-propyl ester (**1c**).* ^{1}H NMR ($CDCl_3$): δ 0.95 (3H, t), 1.66 (2H, s), 2.10 (2H, m), 2.96 (2H, m), 3.70 (3H, s), 4.11 (2H, t), 4.56 (1H, q) and 5.62 (1H, br.s) ppm; ^{13}C NMR ($CDCl_3$): δ 10.7, 16.6, 22.2, 37.1, 52.8, 53.9, 67.7, 156.8 and 171.4 ppm.

*2.2.2.4. N-MOC-S-methyl-*L*-cysteine n-butyl ester (**1d**).* ^{1}H NMR ($CDCl_3$): δ 0.88 (3H, t), 1.35 (2H, m), 1.62 (2H, m), 2.08 (3H, s), 2.90 (2H, m), 3.67 (3H, s), 4.14 (2H, t), 4.53 (1H, q) and 5.52 (1H, br.s) ppm; ^{13}C NMR ($CDCl_3$): δ 14.0, 16.6, 19.4, 30.9, 37.1, 52.8, 53.9, 66.0, 156.8 and 171.4 ppm.

*2.2.2.5. N-MOC-S-methyl-*L*-cysteine n-pentyl ester (**1e**).* ^{1}H NMR ($CDCl_3$): δ 0.92 (3H, t), 1.31 (2H, m), 1.34 (2H, m), 1.68 (2H, m), 2.10 (3H, s), 2.91 (2H, m), 3.70 (3H, s), 4.15 (2H, t), 4.56 (1H, q) and 5.60 (1H, br.s) ppm; ^{13}C NMR ($CDCl_3$): δ 14.3, 16.6, 22.6, 28.3, 28.5, 37.1, 52.8, 53.8, 66.3, 156.8 and 171.4 ppm.

*2.2.2.6. N-Acetyl-S-methyl-*L*-cysteine methyl ester.* ^{1}H NMR ($CDCl_3$): δ 2.04 (3H, s), 2.1 (3H, s), 2.94 (2H, m), 3.76 (3H, s), 4.81 (1H, m) and 6.42 (1H, br.s) ppm; ^{13}C NMR ($CDCl_3$): δ 16.5, 23.4, 36.8, 52.0, 53.0, 170.3 and 171.9 ppm.

*2.2.2.7. N-Chloroacetyl-S-methyl-*L*-cysteine methyl ester.* ^{1}H NMR ($CDCl_3$): δ 2.1 (3H, s), 2.85–3.0 (2H, m), 3.76 (3H, s), 4.08 (2H, s), 4.71 (1H, m) and 7.2 (1H, br.s) ppm; ^{13}C NMR ($CDCl_3$): δ 16.5, 36.8, 42.8, 52.0, 53.0, 166.3 and 171.9 ppm.

*2.2.2.8. N-MOC-S-ethyl-*L*-cysteine methyl ester (**2a**).* ^{1}H NMR (CD_3OD): δ 1.25 (3H, t), 2.58 (2H, q), 2.75–3.0 (2H, m), 3.65 (3H, s), 3.73 (3H, s), 4.38 (1H, m) and 4.83 (1H, br.s) ppm; ^{13}C NMR (CD_3OD): δ 14.0, 26.0, 33.1, 51.8, 51.9, 54.4, 158.1 and 172.1 ppm.

*2.2.2.9. N-MOC-S-n-propyl-*L*-cysteine methyl ester (**2b**).* ^{1}H NMR (CD_3OD): δ 1.0 (3H, t), 1.56 (2H, m), 2.54 (2H, t), 2.75–3.0 (2H, m), 3.64 (3H, s), 3.72 (3H, s), 4.38 (1H, m), and 4.82 (1H, br.s) ppm; ^{13}C NMR (CD_3OD): δ 13.6, 23.8, 34.5, 35.2, 52.8, 52.9, 55.5, 157.6 and 173.4 ppm.

*2.2.2.10. N-MOC-S-allyl-*L*-cysteine methyl ester (**2c**).* ^{1}H NMR (CD_3OD): δ 2.65–2.95 (2H, m), 3.12 (2H, m), 3.62 (3H, s), 3.71 (3H, s), 4.38 (1H, m), 5.11 (2H,

m), 5.73 (1H, m), and 7.35 (1H, br.s) ppm; ^{13}C NMR (CD_3OD): δ 32.0, 34.6, 51.7, 51.8, 54.2, 116.8, 134.4, 158.1 and 172.0 ppm.

2.3. Preparation of standard protected sulfoxides

Standard diastereomeric sulfoxide mixtures of esters of N-protected cysteines were all prepared by the following representative procedure.

N-MOC-*S*-methyl-L-cysteine methyl ester (1.90 g) was dissolved in methanol (40 ml) in an Erlenmeyer flask (100 ml). In a separate flask, hydrogen peroxide (1.13 g of 30% aqueous solution, 1.1 molar equivalents) was dissolved in methanol (15 ml). Both solutions were cooled to −20 °C, then mixed and allowed to stand at −20 °C overnight. The solvent was then removed by vacuum to give a quantitative yield of a 1:1 diastereomeric mixture of *N*-MOC-L-cysteine methyl ester sulfoxides (oil).

2.3.1. N-MOC-S-methyl-L-cysteine methyl ester sulfoxides

^{1}H NMR (CD_3OD): δ 2.70 (3H, s), 3.28 (2H, m), 3.68 (3H, s), 3.77 (3H, s), and 4.58 (1H, m) ppm; ^{13}C NMR (CD_3OD): δ 37.8, 49.6, 51.81/51.95, 52.4, 55.35/55.71, 157.9 and 171.4 ppm.

2.3.2. N-MOC-S-methyl-L-cysteine ethyl ester sulfoxides

^{1}H NMR (CD_3OD): δ 1.30 (3H, t), 2.71 (3H, s), 3.22 (2H, m), 3.68 (3H, s), 4.20 (2H, q) and 4.58 (1H, m) ppm; ^{13}C NMR (CD_3OD): δ 13.4, 37.8, 49.7, 51.80/51.94, 55.40/55.79, 62.1, 158.0 and 170.9 ppm.

2.3.3. N-MOC-S-methyl-L-cysteine n-propyl ester sulfoxides

^{1}H NMR (CD_3OD): δ 0.97 (3H, t), 1.66 (2H, m), 2.70 (3H, s), 3.20 (2H, m), 3.65 (3H, s), 4.10 (2H, m) and 4.58 (1H, m) ppm; ^{13}C NMR (CD_3OD): δ 9.6, 21.9, 37.8, 49.7, 51.77/51.89, 55.31/55.73, 67.6, 157.9 and 170.9 ppm.

2.3.4. N-MOC-S-methyl-L-cysteine n-butyl ester sulfoxides

^{1}H NMR (CD_3OD): δ 0.95 (3H, t), 1.41 (2H, m), 1.65 (2H, m), 2.70 (3H, s), 3.22 (2H, m), 3.65 (3H, s), 4.20 (2H, m) and 4.58 (1H, m) ppm; ^{13}C NMR (CD_3OD): δ 13.0, 19.1, 35.7, 37.8, 49.7, 51.79/51.91, 55.32/55.75, 65.8, 157.9 and 170.9 ppm.

2.3.5. N-MOC-S-methyl-L-cysteine n-pentyl ester sulfoxides

^{1}H NMR (CD_3OD): δ 0.93 (3H, t), 1.35 (4H, m), 1.68 (2H, m), 2.70 (3H, s), 3.20 (2H, m), 3.66 (3H, s), 4.14 (2H, m) and 4.59 (1H, m) ppm; ^{13}C NMR (CD_3OD): δ 13.3, 22.3, 28.1, 28.3, 37.7, 49.7, 51.82/51.95, 55.24/55.64, 66.1, 158.0 and 170.9 ppm.

2.3.6. N-Acetyl-S-methyl-L-cysteine methyl ester sulfoxides

^{1}H NMR (CD_3OD): δ 2.0 (3H, s), 2.73 (3H, s), 3.13–3.45 (2H, m), 3.78 (3H, s) and 4.75 (1H, m) ppm; ^{13}C NMR (CD_3OD): δ 21.4, 37.8, 38.1, 55.3, 55.4, 170.69/170.99 and 172.27/172.55 ppm.

2.3.7. N-Chloroacetyl-S-methyl-L-cysteine methyl ester sulfoxides

^{1}H NMR (CD_3OD): δ 2.65 (3H, s), 2.95–3.2 (2H, m), 3.78 (3H, s), 4.10 (2H, s), and 4.61 (1H, m) ppm; ^{13}C NMR (CD_3OD): δ 37.5, 43.0, 51.83/52.08, 53.43/53.58, 55.3, 166.5 and 172.5 ppm.

2.3.8. N-MOC-S-ethyl-L-cysteine methyl ester sulfoxides

^{1}H NMR (CD_3OD): δ 1.33 (3H, t), 2.75–3.0 (2H, m), 3.1–3.3 (2H, m), 3.66 (3H, s), 3.77 (3H, s) and 4.64 (1H, m) ppm; ^{13}C NMR (CD_3OD): δ 6.0, 45.61/45.81, 49.50/49.61, 51.9, 52.85/52.93, 54.5, 157.8 and 171.4 ppm.

2.3.9. N-MOC-S-n-propyl-L-cysteine methyl ester sulfoxides

^{1}H NMR (CD_3OD): δ 1.0 (3H, t), 1.62 (2H, m), 2.81 (2H, t), 3.1–3.35 (2H, m), 3.66 (3H, s), 3.77 (3H, s) and 4.58 (1H, m) ppm; ^{13}C NMR (CD_3OD): δ 13.4, 17.30/17.36, 52.78/52.87, 53.5, 54.45/54.51, 55.20/55.50, 55.9, 156.8 and 173.0 ppm.

2.3.10. N-MOC-S-allyl-L-cysteine methyl ester sulfoxides

^{1}H NMR (CD_3OD): δ 2.95–3.20 (2H, m), 3.32 (2H, m), 3.65 (3H, s), 3.73 (3H, s), 4.48 (1H, m), 5.21 (2H, m) and 5.73 (1H, m) ppm; ^{13}C NMR (CD_3OD): δ 49.42/49.55, 51.8, 51.9, 54.18/54.27, 55.6, 123.6, 125.98/126.04, 158.1 and 171.4 ppm.

2.4. Preparation of N-t.boc-L-4-S-morpholine-2-carboxylic acid

L-4-*S*-Morpholine-2-carboxylic acid. To a solution of *S*-(2-chlororethyl)-L-cysteine hydrochloride (1.0 g, 0.0045 mol) in dry DMF (75 ml) was added anhydrous triethylamine (8.3 ml). A white solid precipitated and the mixture was heated under a constant stream of argon (anhydrous conditions) for 2.5 h at 92–95 °C and then concentrated in vacuo to give a brown solid. This was dissolved in 13 ml water and passed through a column of DOWEX 50 (H^+) (16 mm × 50 mm), washed with 300 ml water and 250 ml 1.5 M NH_4OH. The ammoniacal eluent was collected and evaporated in vacuo to give a brown solid [18,24]. ^{1}H NMR (D_2O): δ 2.67 (1H, m), 2.7–3.1 (3H, m), 3.19 (1H, m, $J = 3.2$ and 11.98 Hz), 3.58 (1H, dt) and 3.73 (1H, dd, $J = 3.2$ and 10.2 Hz) ppm; ^{13}C NMR (D_2O): δ 23.8, 27.5, 45.3, 59.2 and 172.4 ppm; MS: *m*/*z* (%) 148 (100), 102 (30).

2.4.1. N-t.boc-L-4-S-morpholine-2-carboxylic acid (4)

To a stirred solution of L-4-*S*-morpholine-2-carboxylic acid (0.25 g, 1.70 mmol) in 5 ml water was added NaOH (0.075 g, 1.87 mmol) and *t*-BuOH, (2 ml), followed by 1.05 equivalents of di-*t*-butyl dicarbonate (0.39 g, 1.79 mol). The reaction mixture was allowed to stand at room temperature overnight and then extracted with pentane (10 ml). The pentane layer was re-extracted using saturated $NaHCO_3$ solution (10 ml) and the two aqueous layers combined. The pH of the combined aqueous layers was adjusted to 1–2 and the solution then extracted with ethyl acetate (3 × 25 ml). The combined organic layers were dried over $MgSO_4$ and the solvent removed in vacuo to give a clear oil (0.22 g, 52%) [24]. ^{1}H NMR (CD_3OD): δ 1.47 (9H, s), 2.45 (1H, m), 2.71 (1H, m), 2.92 (1H, m), 3.07 (1H, m), 3.27 (1H, m), 4.30 (1H, dd), 5.07 (0.5H, br.s) and 5.32 (0.5H, br.s) ppm; ^{13}C NMR (CD_3OD): δ 27.5, 28.7, 42.2, 43.5, 54.0, 55.4, 81.6, 155.4, 156.0 and 175.7 ppm; MS: *m*/*z* (%) 248 (15), 192 (50), 148 (58), 132 (7), 102 (40), 57 (100).

N-t.boc-L-4-*S*-Morpholine-2-carboxylic acid, sulfoxides (**5** + **7**). A solution of *N*-t.boc-L-4-*S*-morpholine-2-carboxylic acid (**4**) (0.29 g) in methanol (25 ml) was cooled to −20 °C, then treated with a solution of hydrogen peroxide (0.13 g of 30%) also at −20 °C. The resulting mixture was maintained at −20 °C overnight, then evaporated to give a quantitative mixtures of the sulfoxides (**5** + **7**). ^{1}H NMR (CD_3OD): δ 1.47 (9H, s), 2.70 (1H, m), 2.95 (1H, m), 3.30 (2H, m), 3.75 (1H, m), 4.30 (1H, dd) and 5.17 (0.1H, br.s) ppm; ^{13}C NMR (CD_3OD): δ 27.4, 29.8, 31.0, 37.5, 38.4, 43.6, 43.9, 53.3, 53.8, 81.4, 81.8, 155.1 and 170.7 ppm.

2.5. Biotransformation procedures

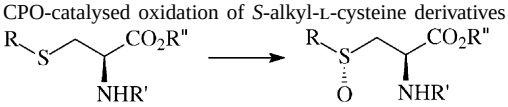
2.5.1. Chloroperoxidase-catalysed oxidations

The reactions were all carried out by the following standard procedure. *N*-MOC-L-*S*-methylcysteine methyl ester (31 mg, 0.15 mM) was dissolved in 16.6 ml of disodium citrate buffer (0.1 M, pH 5.0) in a 50 ml flask. The solution was stirred slowly at room temperature, and CPO (2000 U) added. A solution of hydrogen peroxide (56.6 ml of 30% aqueous solution) in disodium citrate buffer (1.6 ml, 0.1 M, pH 5.0) was then added via a syringe pump over 70 min, with addition occurring directly below the surface level of the reaction mixture. The reaction was stirred for a further 5 min, quenched by addition of aqueous saturated sodium sulfite (0.5 ml) and then continuously extracted with dichloromethane for 3 days. Evaporation of the extract gave a quantitative recovery of amino acid material which was analysed directly by ^{1}H and ^{13}C NMR. Yield of product and configuration at sulfur of *N*-MOC-L-*S*-alkylcysteine ester sulfoxides were identified by NMR analysis and is presented in Table 1.

2.5.2. Beauveria-catalysed oxidations

2.5.2.1. Beauveria bassiana. A growth medium (3 l) composed of glucose (10 g) and corn steep liquor (20 g) per litre of distilled water, adjusted to pH 4.85 with 1 M NaOH was distributed in 15 1 l Erlenmeyer flasks which were stoppered with foam plugs and sterilised by autoclaving at 121 °C for 20 min. The flasks were allowed to cool, then inoculated under sterile conditions with *B. bassiana* taken from a 3-day-old agar slope. The flasks were allowed to stand overnight at 27 °C, then placed on a rotary shaker (1 in. orbit) at 180 rpm, 27 °C. After 3 days, a solution of the appropriate substrate (1.0 g) in 95% ethanol (30 ml) was added and growth allowed to continue for a further

Table 1
CPO-catalysed oxidation of *S*-alkyl-L-cysteine derivatives



Substrate	R	R′	R″	Conversion (%)	DE (%)	Sulfoxide stereochemistry
1a	CH_3	MOC	CH_3	85	84	*R*
1b	CH_3	MOC	C_2H_5	90	90	*R*
1c	CH_3	MOC	*n*-C_3H_7	81	86	*R*
1d	CH_3	MOC	*n*-C_4H_9	80	66	*R*
1e	CH_3	MOC	*n*-C_5H_{11}	50	60	*R*
	CH_3	ClAc	CH_3	85	12	
	CH_3	Ac	CH_3	<5	–	–
2a	C_2H_5	MOC	CH_3	21	86	*R*
2b	*n*-C_3H_7	MOC	CH_3	<5	–	–
2c	Allyl	MOC	CH_3	<5	–	–

3 days. The fungal mass was removed by filtration, and the filtrate adjusted to pH 3, and continuously extracted with dichloromethane for 4 days. The extract was treated with decolourising carbon in chloroform solution, and then evaporated to give a residue that was treated as described later.

2.5.2.2. Beauveria caledonica. This was grown in 1 l Erlenmeyer flasks as described above in a medium of potato dextrose broth at 24–26 °C. The substrate was added 6 days after inoculation of the culture, and biotransformation allowed to proceed for a further 3 days. Subsequent procedures were as described earlier. *B. caledonica* could also be grown and used for biotransformation in the glucose/corn steep liquor medium described for *B. bassiana*. No qualitative or quantitative differences in product produced using the two media were observed. The biotransformation extracts were analysed by the spectral data listed later. The conversion and DE of products are listed in Table 2.

Table 2
Biotransformations with *Beauveria* species

Substrate	Biocatalyst	Conversion (%)	DE (%)	Sulfoxide stereochemistry
3a	*B. bassiana*	35	<5	–
3a	*B. caledonica*	40	<5	–
3b	*B. bassiana*	<5	–	–
3b	*B. caledonica*	<5	–	–
4	*B. bassiana*	>90	>95	*S*
4	*B. caledonica*	>90	>95	*S*

2.6. Synthesis of chondrine

N-t.boc-L-4-*S*-Morpholine-2-carboxylic acid sulfoxide (**5**). Biotransformation of *N*-t.boc-L-4-*S*-morpholine-2-carboxylic acid (**4**) (0.1 g) by *B. caledonica* as described earlier gave the sulfoxide (**5**) (0.82 g), mp: 148–151 °C, $[\alpha]_D$: −43.1 (c 1.01, EtOH). ^{1}H NMR (CD_3OD): δ 1.47 (9H, s), 2.70 (1H, m), 2.95 (1H, m), 3.30 (2H, m), 3.75 (1H, m), 4.30 (1H, dd) and 5.17 (0.1H, br.s) ppm; ^{13}C NMR (CD_3OD): δ 27.4, 29.8, 31.0, 37.5, 38.4, 43.6, 43.9, 53.3, 53.8, 81.8, 155.1 and 170.7 ppm.

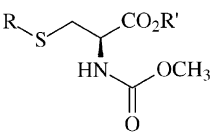
L-4-*S*-Morpholine-2-carboxylic acid sulfoxide (chondrine) (**6**). A solution of *N*-t.boc-L-4-*S*-morphline-2-carboxylic acid sulfoxide obtained by biotransformation using *B. caledonica* (58 mg) in water (3 ml) was treated with 1.3 ml (6 eq.) of 1 M H_2SO_4. The resulting solution was stirred overnight at room temperature, then adjusted to pH 5.75 with 1 M $Ba(OH)_2$, clarified by filtration (celite and Millipore^{TM} filtration) and evaporated to dryness to give a sample of L-4-*S*-morpholine-2-carboxylic acid sulfoxide (chondrine) (**6**) (31 mg, 85%), mp: 245–250 °C, $[\alpha]_D$: +19.5 (c = 0.8, H_2O) ([10,18] mp: 252 °C (sealed), $[\alpha]_D$; +19 and +20.91 (H_2O)). ^{1}H NMR (D_2O): δ 2.87 (1H, m), 2.7–3.1 (2H, m), 3.15–3.3 (2H, m), 3.58 (1H, dt) and 3.73 (1H, dd, J = 3.2 and 10.2) ppm; ^{13}C NMR (D_2O): δ 31.8, 39.1, 41.9, 52.1 and 170.8 ppm.

3. Results and discussion

Table 1 summarises the optimum use of substrate protecting groups for the CPO-catalysed oxidation of *S*-alkyl-L-cysteine derivatives, with the result that maximum yield and diastereomeric excesses were obtained with the *N*-MOC *C*-carboxylate ester derivatives **1a**–**1d**. The *S*-ethyl substrate **2a** was also acceptable for CPO-catalysed oxidation, but the larger *S*-alkyl or *S*-alkenyl substrates (**2b** and **2c**) were not.

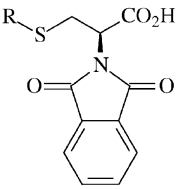
The configuration at sulfur of the resulting sulfoxides was determined by comparison of ^{1}H and ^{13}C NMR data with those of an authentic sample of the (S_S)-sulfoxide of *N*-MOC-*S*-methyl-L-cysteine methyl ester (**1a** sulfoxide), prepared by the standard application of protecting groups to (S_S) sulfoxide of L-cysteine [13]. Although the ^{1}H and ^{13}C NMR chemical shifts for the *S*-methyl carbons were not always distinguishable for the two diastereomers (S_S, $R_{C\text{-}2}$) and (R_S, $R_{C\text{-}2}$) of sample **1**, the ^{13}C NMR chemical shifts for C-2 and C-3 for **1** and **2** consistently appeared as a pair of signals for the diastereomeric samples. When compared with the ^{13}C NMR of the standard (S_S) sulfoxide **1a**, the pairs of signals assigned to C-2 and C-3 of the sulfoxides of **1** and **2** produced by biotransformation were consistently separated, so that those from the (S_S)-sulfoxide always appeared at a higher field position than those for the (R_S)-sulfoxide.

Biotransformation of the *N*-phthaloyl substrates **3a** and **3b** by *Beauveria* species (Table 2), in comparison with the successful S-oxidation of analogous methionine derivatives [8], was not observed for the cysteine derivatives. The sulfoxidation of substrate **4** was performed by *Beauveria* species (Fig. 2) in order to produce **5**, an intermediate in the synthesis of the natural product chondrine, (**6**), and the results are summarised in Table 2.



1a, R = R' = CH_3
1b, R = CH_3, R' = C_2H_5
1c, R = CH_3, R' = n.C_3H_7
1d, R = CH_3, R' = n.C_4H_9
1e, R = CH_3, R' = n.C_5H_{11}

2a, R = C_2H_5, R' = CH_3
2b, R = n.C_3H_7, R' = CH_3
2c, R = $CH_2CH{=}CH_2$, R' = CH_3



3a, R = CH_3
3b, R = $CH_2CH{=}CH_2$

Although both species of *Beauveria* gave a complete conversion of substrate to the same single product, the use of *B. caledonica* ATCC 64970 as a practical biocatalyst was slightly preferable as its simpler growth medium resulted in a cleaner extract and slightly higher isolated yield of sulfoxide **5**. The configuration of the resulting sulfoxide was assigned as the axial (S_S) sample **5** in preference to the equatorial

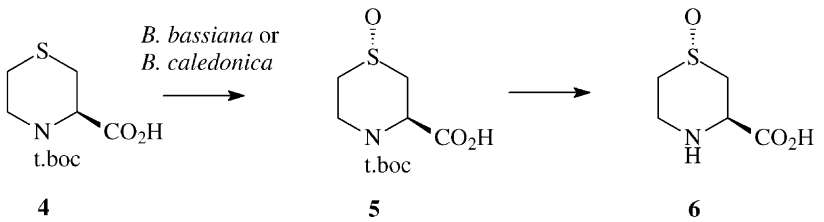


Fig. 2. Synthesis of chondrine.

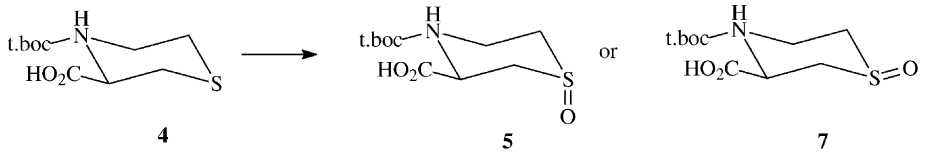


Fig. 3. Stereochemistry of sulfoxidation of the *N*-t.boc derivative of L-4-*S*-morpholine-2-carboxylic acid.

sulfoxide **7** (Fig. 3) by comparison of its ^{13}C NMR spectrum with that of the substrate **4**. The average chemical shift differences for the carbons β to sulfur of **4** and **5** (−14.5 ppm) more closely match those regularly attributed to axial (−12.3 ppm) rather than equatorial (−4.5 ppm) addition of oxygen to thia-cyclohexanes [25]. A mixture of both **5** and **7** was obtained by oxidation of **4** by hydrogen peroxide, a similar result to that obtained by peroxide oxidation of the *N*-deprotected analogue of **4** [18].

The absolute configuration of the biocatalysis product **5** was reconfirmed by removal of the t.boc protecting group to give the natural product chondrine (**6**) possessing the (S_S) sulfoxide (Fig. 2) [18].

In contrast to the straightforward removal of the protecting group from the biotransformation product **5**, attempted removal of the protecting groups from the sulfoxides resulting from the cysteine substrates **1a** to **1e** and **2a** by a variety of standard chemical methods has not been successful. A similar difficulty in the removal of ester and N-protecting groups from *S*-alkylcysteine sulfoxides was recently reported by others [17], and warrants further examination.

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