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Biotransformation of organic sulfides. Part 9. Formation of (*S*) *para*-substituted phenyl methyl sulfoxides by biotransformation using *Helminthosporium* species NRRL 4671

Herbert L. Holland *, Marc J. Bornmann, Gingipalli Lakshmaiah

Department of Chemistry, Brock University, St. Catharines, Ontario, L2S 3A1, Canada

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Abstract

Phenyl methyl sulfides substituted in the *para* position with methyl, fluoro, chloro, bromo, cyano, nitro, amino, acyl, methoxy, thiomethyl and methylsulfinyl groups have been converted to (*S*) sulfoxides by biotransformation using *Helminthosporium* species NRRL 4671. The highest yields and enantiomeric excesses were obtained with bromo, cyano, methoxy, thiomethyl and methylsulfinyl substituents and in two cases (*para*-Br and -CN) the products could be crystallized to give (*S*) sulfoxide of $\geq 96\%$ ee.

Keywords: Biotransformation; Enantiotopic selectivity; *Helminthosporium* NRRL 4671; Sulphide; Sulphoxidation

1. Introduction

The use of whole-cell fungal or bacterial biotransformation for the preparation of chiral sulfoxides by enantioselective oxidation of prochiral sulfides was first reported by Dodson et al. [1]. Since that time, the process has developed into a powerful synthetic method for the formation of chiral sulfoxides for synthetic (eg. [2,3]), mechanistic (e.g. [4]) or medicinal (eg. [5]) purposes ([6]).

The fungus *Helminthosporium* species NRRL 4671 has proved to be particularly valuable for the production of chiral sulfoxides: it typically converts sulfide to sulfoxide with little or no sulfone formation [2,3,7–11], and has been found to reliably produce sulfoxides with predominant (*S*) configuration from a wide range of benzyl alkyl and phenyl alkyl sulfides [3,8]. We have now examined this fungus for its ability to transform *para*-substituted phenyl methyl sulfides. These substrates can be converted by biotransformation using *Mortierella isabellina* to (*R*) sulfoxides [12], and we now report that the corresponding (*S*) products (Fig. 1) are accessible via biotransformation by *Helminthosporium*.

2. Results and discussion

The results of biotransformations of the substrates **1** on a gram scale are reported in Table

* Corresponding author. Tel: (+1-905)6885550 ext. 3403; fax: (+1-905)6829020; E-mail: holland@chemiris.labs.brocku.ca

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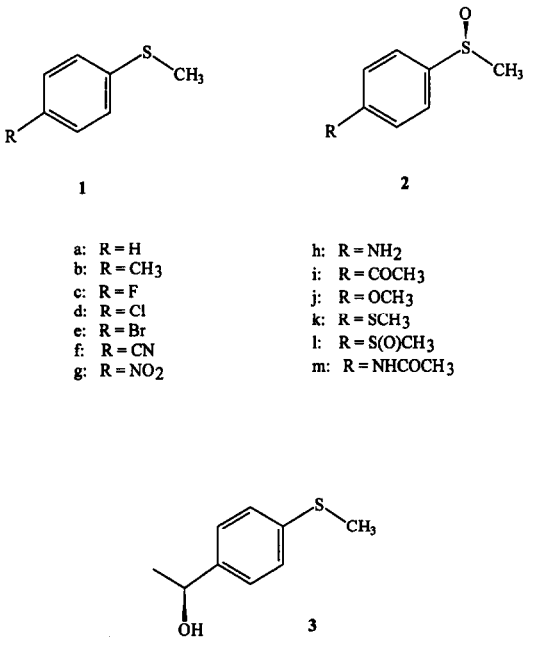

Fig. 1.

Table 1
Biotransformation of phenyl methyl sulfides (**1**) by *Helminthosporium*

Substrate	Substituent	Products (% yield, configuration, ee)
1a	H	**2a** (30, *S*, 48) [a]
1b	CH_3	**2b** (10, *S*, 27)
1c	F	**2c** (56, *S*, 60)
1d	Cl	**2d** (35, *S*, 68)
1e	Br	**2e** (69, *S*, 90)
1f	CN	**2f** (80, *S*, 92)
1g	NO_2	**2g** (55, *S*, 48)
1h	NH_2	**1m** (10); **2h** (13, *S*, 52)
1i	$COCH_3$	**2i** (38, *S*, 32); **3** (9, *S*, 76)
1j	OCH_3	**2j** (83, *S*, 80)
1k	SCH_3	**2k** (64, *S*, 80); **21** (9, *S,S*, $>95_{S,S}$)
1l	$S(O)CH_3$	**21** (29, *R,S*/*S,S*, $>95_{S,S}$)

[a] From Ref. [8].

1. In all cases, the predominant configuration of sulfoxidation was (*S*), determined by comparison of rotation data with those available for the (*R*) sulfoxides [12] and by analysis of ^{1}H NMR spectra of the sulfoxides **2** in the presence of (*S*)-(+)-α-methoxyphenyl acetic acid (MPAA), which induces configurationally dependent shifts of protons α to sulfur in chiral sulfoxides [13]. In several cases, minor ($\leq 3\%$) amounts of sulfone were also obtained.

The highest optical purities were obtained for the *para*-bromo (**2e**) and -cyano (**2f**) sulfoxides, and in both these cases a single crystallization of the product resulted in material with ee $\geq$ 96%. High optical purities were also observed for the *para*-methoxy (**2j**) and -thiomethyl (**2k**) sulfoxides. In the latter case, *bis*-sulfoxide **2l** was also obtained, presumably by further oxidation of **2k**. Stereochemical analysis of **2l** was complicated by the fact that the product appeared by ^{1}H NMR spectral analysis to be a mixture of *meso* and enantiomeric forms, which was inseparable and not amenable to chiral

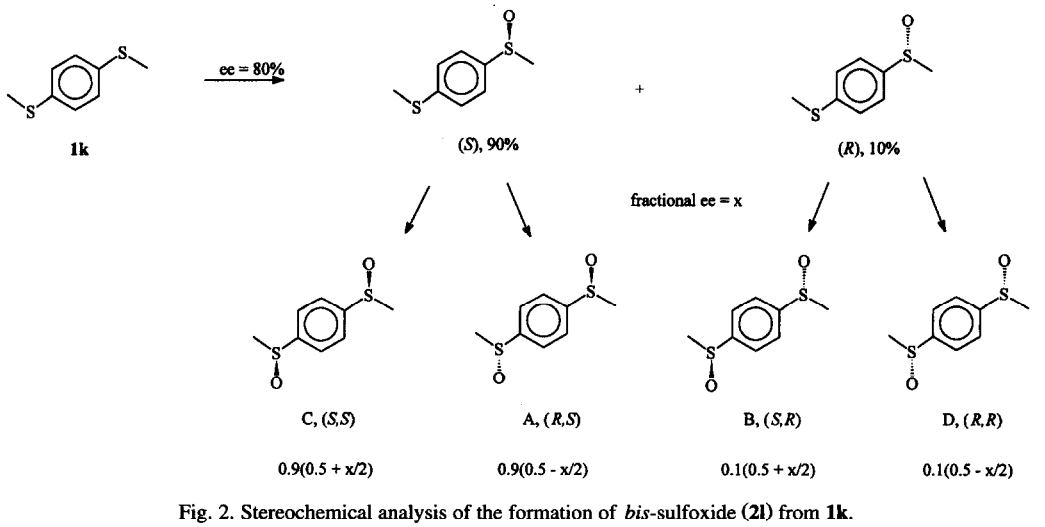

Fig. 2. Stereochemical analysis of the formation of *bis*-sulfoxide (**2l**) from **1k**.

analysis using either the MPAA or Kagan ((*R*)-(−)-*N*-(3,5-dinitrobenzoyl)-α-methylbenzylamine [14]) shift reagents.

In order to assess the substrate and product enantioselectivity of the formation of **2l**, we examined substrate **1l** (equivalent to **2k**); **1l** recovered from biotransformation by *Helminthosporium* showed no detectable enantiomeric enrichment, implying that *bis*-sulfoxide **2l** resulting from oxidation of **1l** contained ca. 50% *meso* material. In this event, the fractional enantiomeric excess inherent in the oxidation of **1l** to **2l** can be estimated as follows: if the measured rotation (−72.3) of **2l** produced from **1l** is due to a sample containing 50% *meso* material, then the rotation due to the enantiomeric component of this sample should be −144.6. If this material is formed with a fractional ee of x, then the rotation for an optically pure sample of **2l**, y, is given by $-144.6/x$.

The stereochemical analysis of the biotransformation of **1k** is given in Fig. 2. From this, it can be seen that: total *meso* component, A + B is

$$0.9(0.5 - x/2) + 0.1(0.5 + x/2) = 0.5 - 0.4x$$

total enantiomeric component, C + D, is

$$0.9(0.5 + x/2) + 0.1(0.5 - x/2) = 0.5 + 0.4x$$

The observed rotation of **2l** produced from **1k** is −124.5, so that the rotation of the enantiomeric component of this mixture, y, is given by $-124.5/(0.5 + 0.4x)$. For **2l** produced from **1l**, however, $y/x = -144.6$: combining these equations gives a value for x of 1.08 (compared to a maximum of 1.0), corresponding to an ee approaching 100% for conversion of **1l** into **2l**, resulting in the formation of *bis*-sulfoxide **2l** with ee > 95%.

Table 1 also lists the formation of two products other than sulfoxides from the sulfides **1**. The *para*-amino substrate **1h** was converted to the acetamide **1m** in low yield, and the carbonyl group of **1i** was reduced to give the (*S*) alcohol **3** in 76% enantiomeric excess: this latter result is consistent with the known ability of *Helminthosporium* to perform redox reactions on (*S*)-benzylic alcohols/acetophenones [15].

A complete analysis of all features of the *Helminthosporium*-catalyzed sulfoxidation of alkyl aryl sulfides has not yet been undertaken. For the present series, however, the ee values listed in Table 1 are difficult to rationalize other than to state that they are maximal in the case of large, polar but non-ionic substituents (eg Br, CN, OCH_3). The presence of a direct electronic effect of the *para* substituent on the ee of sulfoxidation, such as that observed in *M. isabellina*-catalyzed oxidations of analogous substrates [12] cannot be eliminated. Such an effect does not complicate the analysis of sulfoxidation of *para*-substituted benzyl sulfides however, where high ee's are additionally observed in the case of *para*-nitro and -amino substrates [3]. A predictive model for the use of *Helminthosporium* in the production of (*S*) alkyl aryl sulfoxides by biotransformation of the corresponding prochiral sulfides is under development and will shortly be presented.

3. Experimental

3.1. Apparatus, materials and methods

Melting points were determined on a Kofler heating stage. Infrared spectra were recorded with an Analect 6260FX spectrometer. NMR spectra were recorded at 200 MHz (routine ^1H) or 50 MHz (^{13}C) with a Bruker AC200 spectrometer using $CDCl_3$ as solvent and $CHCl_3$ as internal standard. Enantiomeric ratios were determined at 500 MHz (Bruker AC500) by ^1H NMR analysis in the presence of 3 equivalents of (*S*)-(+)-α-methoxyphenylacetic acid (MPAA reagent), two equivalents of (*R*)-(−)-*N*-(3,5-dinitrobenzoyl)-α-methylbenzylamine (Kagan reagent), or sufficient tris-[3-(heptafluoropropylhydroxymethylene)-*d*-camphorato] europium (III) ($Eu(thfc)_3$) to render the peaks under examination as baseline-resolved signals at δ 15–20

ppm. Optical rotations were obtained in the stated solvent at ambient temperature with a Rudolph Autopol III polarimeter. Mass spectra (EI mode) were obtained with a Kratos 1S instrument. Thin layer chromatography was performed on Merck silica gel 60F-254 and flash column chromatography used silica gel, 230–400 mesh.

3.2. Maintenance of microorganisms

Helminthosporium species NRRL 4671 was obtained from the US Department of Agriculture, Northern Regional Research Laboratories, Peoria, IL, and was maintained on 4% malt agar slopes, grown at 27°C and stored at 4°C.

3.3. Preparation of substrates

Sulfides **1a** and **1b** were commercial samples. Compounds **1c–1g**, **1i** and **1j** were prepared by reaction of the corresponding thiol with iodomethane as described [16]. Amine **1h** was obtained by reduction of **1g** as reported [17]. All compounds gave satisfactory spectral and analytical data.

*1,4-Di(methylthio)benzene (**1k**):* sodium borohydride (1.02 g) was added slowly to a stirred solution of (±)-4-(methylsulfinyl)thioanisole (**1l**) (0.5 g) and cobalt chloride hexahydrate (1.2 g) in 95% ethanol at 10–15°C. The mixture was then stirred for a further 2 h at room temperature, diluted with water (8 ml), and then heated on a steam bath for 5–10 min. The mixture was then poured into water, the resulting solution extracted with ether and the extract dried and evaporated to give **1k** as a white solid, mp 79°C; ^{1}H NMR δ 2.47 (6H, s, S-CH_3) and 7.17 (4H, s, Ar-H) ppm; ^{13}C NMR δ 16.4, 127.7, 135.5 ppm; ms m/z(%) 170(100), 155(77).

*(±)-4-(Methylsulfinylphenyl) methyl sulfide (**1l**):* the Grignard reagent prepared from 4-bromothioanisole (1.1 g) and magnesium (0.125 g) in dry ether (10 ml) was added at −8°C over a period of 15 min to a stirred solution of cyclohexyl methyl sulfinate (0.875 g) [18] in dry ether (12 ml). The mixture was then refluxed for 6 h, cooled and worked up by the addition of saturated ammonium chloride and extraction with ether. The extract was dried and evaporated to yield 0.52 g (52%) of (**1l**); mp 88–91°C; ^{1}H NMR δ 2.47 (3H, s, SCH_3), 2.66 (3H, s, $S(O)CH_3$), 7.3 and 7.51 (4H, ABq, Ar-H) ppm; ^{13}C NMR δ 16.1, 44.5, 123.9, 126.4, 141.3, 142.8 ppm; MS *m/z* (%) 186(32), 171(100), 155(6), 139(11).

Biotransformations with Helminthosporium species: two slopes of *Helminthosporium* species NRRL 4671 were used to inoculate 15 1 dm^3 Erlenmeyer flasks each containing 200 ml of an autoclaved medium composed of V-8 vegetable juice (200 ml) and calcium carbonate (3 g) per l of distilled water, adjusted to pH 7.2 prior to sterilization by the addition of 1 M sodium hydroxide. The flasks were allowed to stand overnight at 27°C, then placed on a rotary shaker at 180 rpm, and growth continued for a further 72 h at 27°C. The fungus was then harvested by vacuum filtration (Büchner funnel), and resuspended in 15 1 dm^3 Erlenmeyer flasks each containing 200 ml of distilled water. Substrate (1 g in 30 ml of 95% ethanol) was then distributed among the flasks, which were replaced on the rotary shaker at 180 rpm, 27°C for a further 48 h. The fungus and aqueous medium were then separated by filtration as before, the aqueous medium extracted with dichloromethane (continuous extraction, 72 h), and the fungus discarded. Concentration of the medium extract gave the crude product, which was examined by TLC, using ether or 10% methanol/ether as solvent, and then submitted to flash chromatography using a benzene–ether 10% stepwise gradient, followed by an ether–methanol 5% stepwise gradient. The yields and ee values quoted in the tables refer to purified, homogeneous material and, unless otherwise stated, arise from the combination of (only) homogeneous column fractions without further purification (e.g. crystallization) that could lead to changes in stereo-

chemical enrichment values. Products were identified by a combination of NMR and mass spectral analysis. Significant or hitherto unreported spectral and optical rotation data for products obtained in this study are listed below under the appropriate substrate heading.

*p-Tolyl methyl sulfide (**1b**):* *p*-tolyl methyl sulfoxide (**2b**); $[\alpha]_D$ −47.8 (c = 0.46, $CHCl_3$) (*S*), ee 27% based on $[\alpha]_D$ +179 for the (*R*) enantiomer [12].

*4-Fluorophenyl methyl sulfide (**1c**):* 4-fluorophenyl methyl sulfoxide (**2c**); oil; ^{1}H NMR δ 2.53 (3H, s, SCH_3), 7.25 and 7.55 (each 2H, m, Ar-H) ppm; ^{13}C NMR δ 44.2, 116.5/116.9, 125.8/126.0, 141.4 and 161.9/166.9 ppm; MS m/z (%) 158(66), 143(100); $[\alpha]_D$ −81.9 (c = 1.545, EtOH), (*S*), ee 60% based on MPAA and $[\alpha]_D$ +138 for the (*R*) enantiomer [12].

*4-Chlorophenyl methyl sulfide (**1d**):* 4-chlorophenyl methyl sulfoxide (**2d**); oil; ^{1}H NMR δ 2.70 (3H, s, SCH_3), 7.50 and 7.60 (4H, ABq, Ar-H) ppm; ^{13}C NMR δ 44.1, 125.0, 129.7, 137.0, and 144.2 ppm; MS m/z (%) 176/174(65/24), 161/159(37/100); $[\alpha]_D$ −79.8 (c = 1.275, EtOH), (*S*), ee 68% (MPAA).

*4-Bromophenyl methyl sulfide (**1e**):* 4-bromophenyl methyl sulfoxide (**1e**); ^{1}H NMR δ 2.71 (3H, s, SCH_3), 7.52 and 7.68 (4H, ABq, Ar-H) ppm; ^{13}C NMR δ 44.0, 125.2, 125.5, 132.6 and 145.1 ppm; MS m/z (%) 220/218(58/57), 205/203(100/98); $[\alpha]_D$ −97.1 (c = 1.06, EtOH), (*S*), ee 90% based on MPAA and $[\alpha]_D$ +110 for the (*R*) enantiomer [12]. One crystallization from ethyl acetate/hexane gave a sample with mp 68–70°C $[\alpha]_D$ −106 (c = 1.375, EtOH), (*S*), ee 96%.

*4-Cyanophenyl methyl sulfide (**1f**):* 4-cyanophenyl methyl sulfoxide (**2f**); ^{1}H NMR δ 3.08 (3H, s, SCH_3), 7.88 and 8.10 (4H, ABq, Ar-H) ppm; ^{13}C NMR δ 43.8, 124.3, 133.0, 138.0 ppm; MS m/z (%) 165(91), 150(100), 134(9), 122(32); $[\alpha]_D$ −120 (c = 1.07, EtOH), (*S*), ee 92% based on Kagan and $[\alpha]_D$ +131 for the (*R*) enantiomer [12]. One crystallization from ethyl acetate/hexane gave a sample with mp 81–83°C $[\alpha]_D$ −127.9 (c = 1.375, EtOH), (*S*), ee 97%.

*4-Nitrophenyl methyl sulfide (**1g**):* 4-nitrophenyl methyl sulfoxide (**2g**); mp 110–111°C; ^{1}H NMR δ 2.78 (3H, s, SCH_3), 7.83 and 8.40 (4H, ABq, Ar-H) ppm; ^{13}C NMR δ 43.9, 124.5, 124.7, 149.6 and 153.4 ppm; MS m/z (%) 185(100), 170(42), 140(11); $[\alpha]_D$ −100.5 (c = 1.4, EtOH), (*S*), ee 48% based on MPAA and $[\alpha]_D$ +205 for the (*R*) enantiomer [12].

*4-Aminophenyl methyl sulfide (**1h**):* 4-acetamidophenyl methyl sulfide (**1m**); mp 126–128°C (lit. mp 128°C [19]); ^{1}H NMR δ 2.15 (3H, s, $COCH_3$), 2.48 (3H, s, SCH_3), 7.15 and 7.40 (4H, ABq, Ar-H) ppm; MS m/z (%) 181(85), 167(25), 139(69), 124(100). 4-Aminophenyl methyl sulfoxide (**2h**); oil; ^{1}H NMR δ 2.70 (3H, s, SCH_3), 4.05 (2H, br.s, exchanges D_2O, NH_2), 6.75 and 7.42 (4H, ABq, Ar-H) ppm; ^{13}C NMR δ 43.8, 115.1, 125.7, 140.0 and 149.6 ppm; MS m/z (%) 155(22), 140(100), 124(24), 108(16); $[\alpha]_D$ −69.8 (c = 1.45, EtOH), (*S*), ee 52% (Kagan).

*4-Thiomethylacetophenone (**1i**):* 1-(4′-thiomethylphenyl)ethanol (**3**); mp 42–44°C ^{1}H NMR δ 1.50 (3H, d, CH_3), 2.50 (3H, s, SCH_3), 4.86 (1H, q, CHOH), 7.23 and 7.30 (4H, ABq, Ar-H) ppm; ^{13}C NMR δ 16.0, 25.0, 69.9, 125.9, 127.0, 137.3 and 142.9 ppm; MS m/z (%) 168(69), 153(100), 135(9), 125(20), 109(25); $[\alpha]_D$ −11.7 (c = 0.48, EtOH), (*S*), ee 76% ($Eu(thfc)_3$). 4-Methylsulfinylacetophenone (**2i**); mp 96–98°C (lit. mp (racemate) 104.5–107.5°C [20]); ^{1}H NMR δ 2.66 (3H, s, CH_3), 2.80 (3H, s, SCH_3), 7.74 and 8.10 (4H, ABq, Ar-H) ppm; ^{13}C NMR δ 26.6, 43.7, 123.7, 129.0, 139.1, 150.9 and 196.7 ppm; MS m/z (%) 182(81), 167(100), 152(80), 139(18); $[\alpha]_D$ −55.6 (c = 0.25, EtOH), (*S*), ee 32% (MPAA).

*4-Methoxyphenyl methyl sulfide (**1j**):* 4-methoxyphenyl methyl sulfoxide (**2j**); oil; ^{1}H NMR δ 2.71 (3H, s, SCH_3), 3.83 (3H, s, OCH_3), 7.04 and 7.60 (4H, ABq, Ar-H) ppm; ^{13}C NMR δ 44.0, 55.5, 114.9, 125.5, 139.2 and 161.5 ppm; MS m/z (%) 170(21), 155(100),

139(11), 123(10); $[\alpha]_D$ −134 (c = 0.65, EtOH), (*S*), ee 80% based on MPAA and $[\alpha]_D$ +168 for the (*R*) enantiomer [12].

*1,4-Di(methylthio)benzene (**1k**):* 4-(methylsulfinylphenyl) methyl sulfide (**1l**); mp 50–54°C; spectral data identical with those reported above for synthetic **1l** except for $[\alpha]_D$ −126 (c = 0.65, EtOH), (*S*), ee 80% (Kagan). 1,4-Di(methylsulfinyl)benzene (**2k**); mp 106–108°C; ^{1}H NMR δ 2.751 and 2.753 (total 3H, each s, SCH_3) and 7.80 (4H, s, Ar-H) ppm; ^{13}C NMR δ 43.7, 124.2, 149.0 ppm; MS m/z (%) 202(56), 187(100), 171(26), 141(81); $[\alpha]_D$ −124.5 (c = 0.4, EtOH), (meso + *S,S*), $ee_{S,S}$ > 95% based on a value of −144 for the pure (*S,S*) enantiomer (see text).

*(±)-4-(methylsulfinylphenyl) methyl sulfide (**1l**):* recovered (±)-4-(methylsulfinylphenyl) methyl sulfide (**1l**) (56%); spectral data identical with those reported above. 1,4-Di(methylsulfinyl) benzene (**2l**); mp 105–108°C; ^{1}H NMR δ 2.749 and 2.751 (total 3H, each s, SCH_3) and 7.80 (4H, s, Ar-H) ppm; ^{13}C NMR δ 43.6, 124.2, 148.9 ppm; MS m/z (%) 202(61), 187(100), 171(88), 141(57); $[\alpha]_D$ −72.3 (c = 0.6, EtOH), (meso + *S,S*), de 0, $ee_{S,S}$ > 95% based on a value of −144 for the pure (*S,S*) enantiomer (see text).

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