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BIOTRANSFORMATION OF ORGANIC SULFIDES. PART 7. FORMATION OF CHIRAL ISOTHIOCYANATO SULFOXIDES AND RELATED COMPOUNDS BY MICROBIAL BIOTRANSFORMATION

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ABSTRACT: The fungi *Helminthosporium* species NRRL 4671 and *Mortierella isabellina* ATCC 42613 have been used for the biotransformation of a series of isothiocyanatoalkyl methyl sulfides and their synthetic precursors, ω-(methylthio)alkylphthalimides. *H.* species gave predominantly (*S*) sulfoxides in all cases; *M. isabellina* gave (*R*) isothiocyanatoalkyl methyl sulfoxides, but in the case of two ω-(methylthio)alkylphthalimides substantial conversion of sulfoxide to sulfone resulted in the isolation of the former with predominant (*S*) configuration. A correction is made of the previously reported configurations of two biotransformation products (Tetrahedron: Asymmetry, **1994**, *5*, 1129).

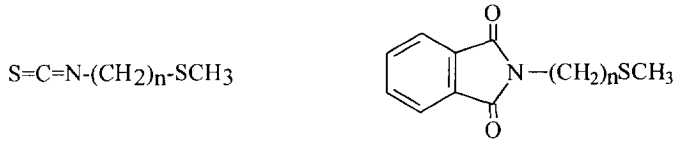
INTRODUCTION

The natural chiral sulfoxide sulforaphane ((-)-1-isothiocyanato-(4*R*)-(methylsulfinyl)butane, ((*R*)-**2a**), isolated from broccoli and present in related vegetables,[1] has been shown to act as a potent inducer of phase II detoxification enzymes in mammalian metabolism. This action has been implicated in the anti-carcinogenic properties of broccoli and also of other vegetables of the cruciform family. The related sulfoxides (-)-**1a**[2], (-)-**5a**[2] and higher analogues such as (-)-8-(methylsulfinyl)octyl isothiocyanate,[3] (-)-9-(methylsulfinyl)nonyl isothiocyanate[4] and (-)-10-(methylsulfinyl)decyl isothiocyanate[5] have also been isolated from various plant sources, where they usually occur as glucoside conjugates.

In view of the demonstrated ability of fungal species, particularly *Mortierella isabellina*[6,7] and *Helminthosporium*,[8-10] to perform enantioselective oxidation of prochiral sulfides to sulfoxides, we have examined the biotransformation of the series of prochiral isothiocyanates **1** to **4**. We have also examined the biotransformation of their synthetic precursors[11], the phthalimides **5** to **8**. We have previously reported the results of biotransformations of **2** and **6** by *Helminthosporium*:[12] the present paper serves to amplify that preliminary report and also presents, as a consequence of more detailed examination of the related series of substrates, a correction of the absolute configurations originally reported for the biotransformation products of **2** and **6** by *Helminthosporium*.

RESULTS AND DISCUSSION

The results of the biotransformations of substrates **1** to **8** (Figure 1) are presented in the accompanying table in terms of isolated yields, enantiomeric excesses and predominant configurations of sulfoxide products **1a** to **8a**, together with isolated yields of sulfones **5b** to **8b**.



1: n = 3
2: n = 4
3: n = 5
4: n = 6

5: n = 3
6: n = 4
7: n = 5
8: n = 6

1a - 8a: **1 - 8** sulfoxides. **5b - 8b**: **5 - 8** sulfones

Table. Biotransformations of sulfides 1 to 8

Substrate	Microorganism	Products	
		Sulfoxide (% yield, configuration, % e. e.)	Sulfone (% yield)
1	*Helminthosporium*	**1a** (24, *S*, 68)	-
	Mortierella	**1a** (6, *R*, 63)	-
2	*Helminthosporium*	**2a** (45, *S*, 93)	-
	Mortierella	**2a** (9, *R*, 58)	-
3	*Helminthosporium*	**3a** (34, *S*, 88)	-
	Mortierella	**3a** (7, *R*, 82)	-
4	*Helminthosporium*	**4a** (33, *S*, 90)	-
	Mortierella	**4a** (8, *R*, 37)	-
5	*Helminthosporium*	**5a** (47, *S*, ≥95)	-
	Mortierella	**5a** (77, *R*, 66)	**5b** (trace)
6	*Helminthosporium*	**6a** (61, *S*, 88)	-
	Mortierella	**6a** (72, *R*, 39)	**6b** (14)
7	*Helminthosporium*	**7a** (50, *S*, ≥95)	-
	Mortierella	**7a** (28, *S*, 48)	**7b** (60)
8	*Helminthosporium*	**8a** (62, *S*, ≥95)	-
	Mortierella	**8a** (20, *S*, 82)	**8b** (48)

The assignments of product structure relied heavily on NMR information, ^{1}H and ^{13}C spectral data being diagnostic of the oxidation state at sulfur. These, together with supporting mass spectral data, are presented at length in the experimental section. The predominant configuration and enantiomeric excesses of sulfoxide products were determined by analysis of their ^{1}H NMR spectra in the presence of the chiral shift reagent (*S*)-(+)-α-methoxyphenylacetic acid (MPAA) and confirmed by correlation with optical rotation data where available. Use of the NMR shift reagent gives consistent, configurationally dependent chemical shift patterns that have now been correlated with data obtained from both (*R*)- and (*S*)- sulfoxides of established configuration.[13] The shift reagent causes the S-methyl group signals of methyl sulfoxides to appear as a pair of singlets with $\Delta\delta$ values of the order of 0.02 - 0.03 ppm, cleanly resolved at 500 mHz. In all examples studied to date,[7-10,13,14] the signal from the methyl protons α to sulfur in the (*S*) enantiomer is at the higher field.

The configurational assignments of the table are in full agreement with the complexation model proposed for MPAA interaction with sulfoxides.[13] In those examples (**5a**, **7a**, and **8a** resulting from *Helminthosporium*-catalyzed biotransformations) where the sulfoxides were formed as essentially single enantiomers, their configurations were confirmed by incremental addition of synthetic racemic sulfoxide to the NMR chiral shift experiment. The gradual appearance of a new S(O)-CH_3 signal downfield from the original signal confirmed that the latter was due to the (*S*) enantiomer. In this way, we now conclude that the predominant configuration of (+)-**6a** produced by *Helminthosporium*, originally provisionally assigned as (*R*),[12] should be corrected to (*S*) in accord with the configurations of the other members of the series, **5a**, **7a**, and **8a**, resulting from *Helminthosporium*-catalyzed biotransformations.

The configurations of the isothiocyanato sulfoxides **1a** to **4a** were similarly determined. In this series, however, the existence of synthetic stereochemical standards for **2a**[11,15] enabled us to correlate NMR with optical rotation data. Thus the ^{1}H NMR spectrum of (-)-(*R*)-**2a**[15] produced from biotransformation of **2** by *M. isabellina* showed, in the presence of MPAA, two signals attributable to the S-methyl group at δ 2.603 and 2.620 ppm in the ratio 21(*S*):79(*R*) (ee 58%); conversely, (+)-(*S*)-**2a**[15] from *Helminthosporium* showed these same signals in the ratio 96.5:3.5, corresponding to an enantiomeric excess of 93% (*S*). We have been unable to substantiate the reported formation of (*R*)-**2a** by *Helminthosporium*:[12] with the benefit of the results from the biotransformation of the series of substrates **1** to **4** and the analysis of both enantiomers in each case, it is now clear that the biotransformation product of **2** by *Helminthosporium* is indeed (*S*)-**2a**, and that our earlier report,[12] based on a single experiment, was in error.

The natural (*R*) enantiomer of **2a**, together with the (*R*) enantiomers of the homologues **1a**, **3a**, and **4a** are, however, produced (albeit in low yield) by biotransformation of the corresponding sulfides using *Mortierella isabellina*. *Helminthosporium* species gave not only the (*S*)-sulfoxides directly from **1** to **4**, but could also be used as a source of chirality for these compounds if derived synthetically from the

phthalimide precursors **5a** to **8a**.[11] *M. isabellina*, on the other hand, gave an (*R*)-sulfoxide in the latter series only in the cases of **5**, which was converted to the *R*-**5a** without appreciable sulfone formation; and **6**, where a low yield of sulfone was accompanied by a low enantiomeric purity for the isolated sulfoxide **6a**. Biotransformation of **7** and **8** by *M. isabellina* resulted in isolation of the (*S*)-sulfoxides accompanied by substantial sulfone production in each case: this microorganism is known to perform stereoselective oxidation of sulfoxides to sulfones, with preferential oxidation of the (*R*) enantiomer in the case of methyl benzyl sulfoxides.[7] A similar phenomenon operative in the present case during the oxidations of **7** and **8** may be responsible for the isolation of (*S*) sulfoxides from these substrates.

It is therefore apparent that, whereas high optical purity (*S*)-sulfoxides can be readily obtained from compounds **1** to **8** by biotransformation using *Helminthosporium*, the corresponding (*R*)-sulfoxides **1a** to **4a** are produced in only low yield and moderate enantiomeric excesses by *M. isabellina*, and that the latter organism cannot be routinely used for production of (*R*) sulfoxides **5a** to **8a** without a consideration of the stereochemical consequences of the conversion of sulfoxide to sulfone during the biotransformations.

EXPERIMENTAL

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 ^{1}H) 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 ^{1}H NMR analysis in the presence of 3 equivalents of (*S*)-(+)-α-methoxyphenylacetic acid (MPAA).[4] 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.

Maintenance of microorganisms: *Helminthosporium* species NRRL 4671 was obtained from the U.S. Department of Agriculture, Northern Regional Research Laboratories, Peoria, Ill. *Mortierella isabellina* ATCC 42613 (identical with NRRL 1757) was obtained from the American Type Culture Collection, Md. Both fungi were maintained on 4% malt agar slopes, grown at 27°C and stored at 4°C.

Preparation of substrates: compounds **5** to **8** were prepared by treatment of the appropriate ω-bromoalkyl phthalimide[11] with 1.1 equivalents of sodium thiomethoxide in ethanol for 4h at reflux, followed by conventional work-up, and gave spectral data consistent with the structures. Following conversion of these intermediates to the corresponding aminoalkyl methyl thioethers using hydrazine in the usual manner,[11] the latter were converted to the isothiocyanates **1** to **4** by treatment with thiophosgene as described.[16] The following spectral data characterized these products:

1-isothiocyanato-3-(methylthio)propane, **1**; oil, ^{1}H NMR δ 2.0 (2H, q), 2.1 (3H, s), 2.6 (2H, t) and 3.7 (2H, t) ppm; ^{13}C NMR δ 15.4, 29.1, 30.8, and 43.7 ppm;[17] ms m/z(%) 147(15), 132(2), 101(100), 86(6), 72(59).

1-isothiocyanato-4-(methylthio)butane, **2**; oil, ^{1}H NMR δ 1.65-1.9 (4H, m), 2.1 (3H, s), 2.55 (2H, t) and 3.55 (2H, t) ppm; ^{13}C NMR δ 15.4, 25.9, 28.8, 33.3 and 44.7 ppm; ms m/z(%) 161(40), 146(10), 115(92), 85 (38), 72(49), 61(100).

1-isothiocyanato-5-(methylthio)pentane, **3**; oil, ^{1}H NMR δ 1.5-1.8 (6H, m), 2.1 (3H, s), 2.5 (2H, t) and 3.6 (2H, t) ppm; ^{13}C NMR δ 15.5, 25.7, 28.3, 29.6, 33.9 and 44.9 ppm; ms m/z(%) 175(50), 129(28), 101(26), 72(54), 61(100).

1-isothiocyanato-6-(methylthio)hexane, **4**; oil, ^{1}H NMR δ 1.4-1.5 (4H, m), 1.5-1.8 (4H, m), 2.1 (3H, s), 2.5 (2H, t) and 3.5 (2H, t) ppm; ^{13}C NMR δ 15.4, 26.1, 27.8, 28.8, 29.8, 33.9 and 44.9 ppm; ms m/z(%)

189(44), 174(6), 142(18), 72(45), 61(100).

Biotransformations with H. *species:* two slopes of *Helminthosporium* species NRRL 4671 were used to inoculate 15 1L 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 1M 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 (Buchner funnel), and resuspended in 15 1L Erlenmeyer flasks each containing 200 mL of distilled water, resulting in ca. 90 g (wet weight) of mycelial growth per flask. 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 treated as described below.

Biotransformations with Mortierella isabellina: these were performed as described above, with the following modifications. The fungal growth medium was composed of glucose (40 g), soybean flour (5 g), yeast extract (Sigma, 5 g), sodium chloride (5 g) and dibasic potassium phosphate (5 g) per L. The fungus was separated from the medium by centrifugation (IEC chemical centrifuge), resuspended as above resulting in 85 g (wet weight) of biomass per flask, and biotransformation carried out for a period of 24 h (substrates **5** to **8**) or 40 h (substrates **1** to **4**).

Isolation and characterization of products: the crude biotransformation extracts obtained as described above were 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 stereochemical enrichment values. Products were identified by a combination of NMR and mass spectral analysis. Spectral and optical rotation data for products obtained in this study are listed below under the appropriate substrate heading.

1-isothiocyanato-3-(methylthio)propane, **1**; 1-isothiocyanato-3-(methylsulfinyl)propane, **1a**; oil; ^{1}H NMR δ 2.0 (2H, g), 2.4 (3H, s), 2.6 (2H, t) and 3.5 (2H, t) ppm; ^{13}C NMR δ 23.4, 38.7, 44.0 and 50.7 ppm; ms m/z(%) 163(5), 116(20), 100(22), 72(100); $[\alpha]_D$, *H.* species, +28 (c = 0.62, chloroform); $[\alpha]_D$, *M. isabellina*, −26.1 (c = 0.92, chloroform).

1-isothiocyanato-4-(methylthio)butane, **2**; 1-isothiocyanato-4-(methylsulfinyl)butane, **2a**; oil; ^{1}H NMR δ 1.8-2.0 (2H, m), 2.6 (3H, s), 2.73 (2H, m) and 3.60 (2H, t) ppm; ^{13}C NMR δ 20.0, 29.0, 38.7, 44.6 and 53.4 ppm; ms m/z(%) 177(1), 160(68), 114(12), 72(100); $[\alpha]_D$, *H.* species, +69.4 (c = 0.7, chloroform); $[\alpha]_D$, *M. isabellina*, −45.5 (c = 0.45, chloroform).

1-isothiocyanato-5-(methylthio)pentane, **3**; 1-isothiocyanato-5-(methylsulfinyl)pentane, **3a**; oil; ^{1}H NMR δ 1.6-2.0 (6H, m), 2.6 (3H, s), 2.7 (2H, t) and 3.6 (2H, t) ppm; ^{13}C NMR δ 21.8, 25.7, 29.4, 38.5, 44.7 and 54.0 ppm; ms m/z(%) 191(2), 174(85), 128(19), 72(100); $[\alpha]_D$, *H.* species, +56 (c = 1.27, chloroform); $[\alpha]_D$, *M. isabellina*, −51.8 (c = 0.4, chloroform).

1-isothiocyanato-6-(methylthio)hexane, **4**; 1-isothiocyanato-6-(methylsulfinyl)hexane, **4a**; oil; ^{1}H NMR δ 1.4-1.6 (4H, m), 1.6-1.9 (4H, m), 2.6 (3H, s), 2.7 (2H, q), 3.5 (2H, t) ppm; ^{13}C NMR δ 22.3, 26.1, 27.7, 29.6, 38.6, 44.9 and 54.3 ppm; $[\alpha]_D$, *H.* species, +63 (c = 1.06, chloroform); $[\alpha]_D$, *M. isabellina*, −24 (c = 0.25, chloroform).

N-(3-(methylthio)propyl)phthalimide, **5**; N-(3-(methylsulfinyl)propyl)phthalimide, **5a**; mp 120-122°C; ^{1}H NMR δ 2.20 (2H, m), 2.58 (3H, s), 2.76 (2H, d of t), 3.87 (2H, s) and 7.7-7.9 (4H, m) ppm; ^{13}C NMR δ 22.3, 36.8, 38.7, 52.0, 123.4, 131.9, 134.1 and 168.3 ppm; ms m/z(%) 251(4), 235(25), 188(100), 160(77); $[\alpha]_D$, *H.* species, +75.6 (c = 0.8, ethanol); $[\alpha]_D$, *M. isabellina*, −48.5 (c = 0.52, ethanol).

N-(4-(methylthio)butyl)phthalimide, **6**; N-(4-(methylsulfinyl)butyl)phthalimide, **6a**; mp 129-131°C; ^{1}H NMR

δ 1.7-1.95 (4H, m), 2.58 (3H, s), 2.85 (2H, t), 3.74 (2H, t) and 7.7-7.9 (4H, m) ppm; ^{13}C NMR δ 19.9, 27.7, 37.1, 38.7, 53.9, 123.3, 131.8, 134.0 and 168.4 ppm; ms m/z(%) 265(0.1), 248(18), 202(30), 160(100); $[\alpha]_D$, *H.* species, +65.3 (c = 0.9, ethanol); $[\alpha]_D$, *M. isabellina*, −28.9 (c = 0.35, ethanol). N-(4-(methylsulfonyl)butyl)phthalimide, **6b**; mp 143-145°C; ^{1}H NMR δ 1.85-2.0 (4H, m), 2.90 (3H, s), 3.13 (2H, t), 3.74 (2H, t) and 7.7-7.9 (4H, m) ppm; ^{13}C NMR δ 19.7, 27.2, 36.7, 40.2, 53.9, 123.1, 131.8, 134.0 and 168.2 ppm; ms m/z(%) 281(18), 188(16), 174(34), 160(100).

N-(5-(methylthio)pentyl)phthalimide, **7**; N-(5-(methylsulfinyl)pentyl)phthalimide, **7a**; mp 106-108°C; ^{1}H NMR δ 1.3-1.8 (6H, m), 2.44 (3H, s), 2.58 (2H, d of t), 3.57 (2H, t) and 7.6-7.8 (4H, m) ppm; ^{13}C NMR δ 21.7, 25.6, 27.7, 37.0, 38.2, 54.0, 122.7, 131.7, 133.2 and 167.8 ppm; ms m/z(%) 279(1), 262(16), 216(39), 160(100); $[\alpha]_D$, *H.* species, +58.4 (c = 0.7, ethanol); $[\alpha]_D$, *M. isabellina*, +28.0 (c = 0.59, ethanol). N-(5-(methylsulfonyl)pentyl)phthalimide, **7b**; mp 101-103°C; ^{1}H NMR δ 1.4-2.0 (6H, m), 2.89 (3H, s), 3.0 (2H, t), 3.7 (2H, t) and 7.7-7.9 (4H, m) ppm; ^{13}C NMR δ 22.0, 25.6, 28.0, 37.3, 40.5, 54.6, 123.3, 132.1, 134.0 and 168.2 ppm; ms m/z(%) 295(5), 216(34), 160(100).

N-(6-(methylthio)hexyl)phthalimide, **8**; N-(6-(methylsulfinyl)hexyl)phthalimide, **8a**; mp 104-106°C; ^{1}H NMR δ 1.2-1.8 (8H, m), 2.47 (3H, s), 2.60 (2H, d of t), 3.56 (2H, t) and 7.6-7.8 (4H, m) ppm; ^{13}C NMR δ 22.1, 26.1, 28.0 (2C), 37.4, 38.3, 54.3, 122.8, 131.8, 133.6 and 168.0 ppm; ms m/z(%) 293(0.4), 277(18), 262(9), 230(50), 160(100); $[\alpha]_D$, *H.* species, +57.2 (c = 0.8, ethanol); $[\alpha]_D$, *M. isabellina*, +44.5 (c = 0.425, ethanol). N-(6-(methylsulfonyl)hexyl)phthalimide, **8b**; mp 100-102°C; ^{1}H NMR δ 1.3-1.9 (m, 8H), 2.9 (3H, s), 3.0 (2H, t), 3.7 (2H, t) and 7.65-7.85 (4H, m) ppm; ^{13}C NMR δ 22.4, 26.3, 27.9, 28.2, 37.6, 40.5, 54.7, 123.1, 132.2, 134.0 and 168.4 ppm; ms m/z(%) 309(4.5), 230(30), 216(4), 174(11), 160(100).

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17. In this and other ^{13}C NMR spectra of isothiocyanates, the isothiocyanato carbon resonance was typically not observed or was extremely weak when using standard acquisition conditions (pulse delay of 1.1 sec.). Upon extension of the pulse delay time to >5 seconds, this carbon resonance appeared in the 125-132 ppm region of the spectrum.

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