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PREPARATION OF (*R*)-SULFORAPHANE BY BIOTRANSFORMATION USING *HELMINTHOSPORIUM* SPECIES NRRL 4671

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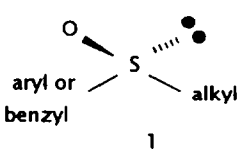
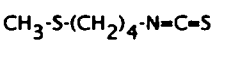
ABSTRACT: The fungus *Helminthosporium* species NRRL 4671 oxidizes 1-isothiocyanato-4-(methylthio)butane to (-)-1-isothiocyanato-(4*R*)-(methylsulfinyl)butane (sulforaphane). *Helminthosporium* also converts 1-methylthio-4-(N-phthalimidyl)butane to the (*R*) sulfoxide.

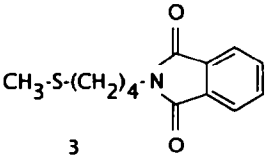
In recent years, chiral sulfoxides have become increasingly available as a result of developments in asymmetric synthesis[1] and biotransformation.[2] We have carried out a systematic investigation of the ability of the fungus *Helminthosporium* species NRRL 4671 to perform asymmetric oxidation of a range of prochiral sulfides,[3-5] and have found that this fungus efficiently oxidizes many phenyl and benzyl alkyl sulfides to give chiral sulfoxides with the stereochemistry shown in **1**, below.



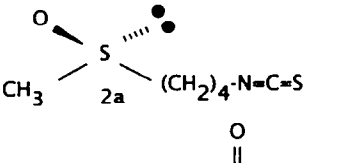
1

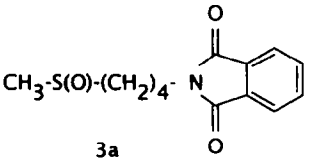


2



3





3a

The chiral sulfoxide sulforaphane ((-)-1-isothiocyanato-(4*R*)-(methylsulfinyl)butane, (**2a**)), isolated from broccoli where it occurs at the level of ca. 1ppm,[6] has been shown to act as a very potent inducer of phase II detoxication enzymes in mammalian metabolism, and this induction has been cited as a significant component in the anticarcinogenic action of broccoli and related cruciform vegetables. In view of the correspondence in absolute configuration between **2a** and the chiral sulfoxides which we have obtained by biotransformation using *Helminthosporium*, we have used this fungus for biotransformation of the prochiral isothiocyanato sulfide **2**.[7] We have also investigated biotransformation of the phthalide **3**, a synthetic precursor of **2**,[7] and the results of both experiments are presented on the following page.

Biotransformations of **2** and **3** by *Helminthosporium* species NRRL 4671

Substrate	Product	yield (%)	$[\alpha]_D$	configuration	EE (%)
2	**2a**	45	-69.4 (c 0.7, $CHCl_3$)	(*R*)	86
3	**3a**	61	+64.2 (c 0.9, EtOH)	(*R*)	88

The absolute configuration and enantiomeric purity of the resulting sulforaphane, **2a**, was determined by comparison of rotation data with literature values,[7] and confirmed by analysis of its ^{1}Hnmr spectrum in the presence of (*S*)-α-methoxyphenylacetic acid. The latter method was also used to determine the EE and provisionally assign (*R*) configuration to the phthalimide sulfoxide **3a** using the complexation model of Buist and Marecak.[8] As **3a** can be converted to sulforaphane,[7] its production in chiral form presents an alternative route to the latter. The enantiomeric excess of sulforaphane produced by biotransformation of **2** is identical to that reported for the natural material[6] and this conversion, while not a high yield process, nevertheless represents a more efficient means for the preparation of sulforaphane than the existing method of resolution of a synthetic intermediate, convertible into the final product in less than 20% yield.[7]

Biotransformations with H. species: two slopes of *Helminthosporium* species NRRL 4671, obtained from the U.S. Department of Agriculture, Northern Regional Research Laboratories, Peoria, Ill., USA 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 by 1M sodium hydroxide. The flasks were allowed to stand overnight at 27°C, then placed on a rotary shaker at 180 rpm, 27°C for a further 72h. The fungus was harvested by filtration, and resuspended in 15 1L Erlenmeyer flasks each containing 200 mL of distilled water. Substrate (1 g in 30 mL 95% ethanol) was distributed among the flasks, which were replaced on the rotary shaker at 180 rpm, 27°C for a further 48h. The fungus was removed by filtration, the aqueous medium continuously extracted for three days with dichloromethane and the concentrated extract submitted to flash chromatography (silica gel; benzene-ether 10% stepwise gradient, followed by an ether-methanol 5% stepwise gradient) to give the product.

ACKNOWLEDGEMENTS

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