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An Investigation of the Biotransformation of Organic Selenides by Fungi

HERBERT L. HOLLAND AND IAN M. CARTER

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

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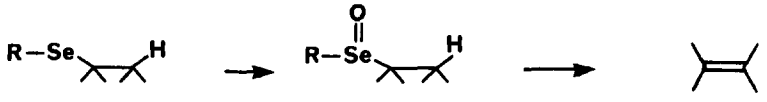
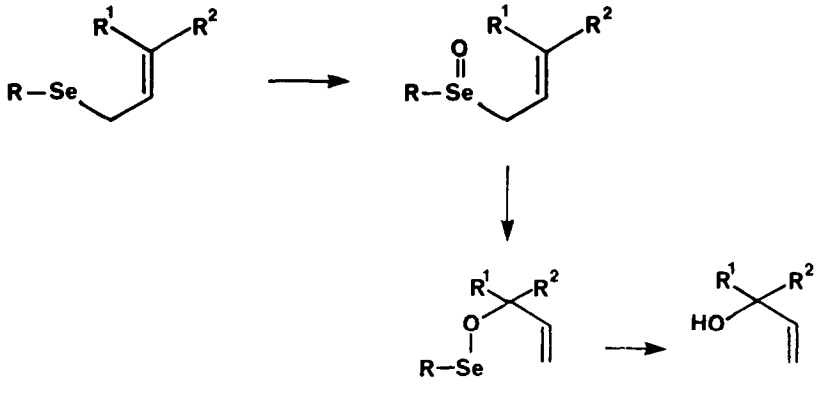
A series of alkyl phenyl selenides has been incubated with the fungi *Aspergillus niger*, *Aspergillus foetidus*, *Mortierella isabellina*, and *Helminthosporium sp*. These fungi oxidize the corresponding sulfides to sulfoxides efficiently, but in no case was any evidence obtained that the microbial oxidation of selenide to selenoxide was occurring. The fate of methyl phenyl selenide following incubation with *M. isabellina* was investigated using *methyl*-^{14}C-labeled substrate, and by quantitative selenium analysis. These techniques indicate that the selenide is taken into the fungal cell efficiently and that some metabolic cleavage of the selenium–methyl carbon bond may occur.

The *syn* elimination of selenoxides leading to olefins



[1]

provides a convenient, high yield method of synthesis of the latter (*1*); the decomposition of allyl and related selenoxides to allylic alcohols



[2]

is also a reaction of synthetic utility (*2*). In both these reactions, the unstable selenoxide is produced by oxidation of the corresponding selenide, usually with hydrogen peroxide, and is then allowed to decompose *in situ* to produce the desired product.

Although diastereomeric selenoxides of stable configuration at selenium have been synthesized in a few cases (*3–5*) chiral selenoxides are not generally accessi-

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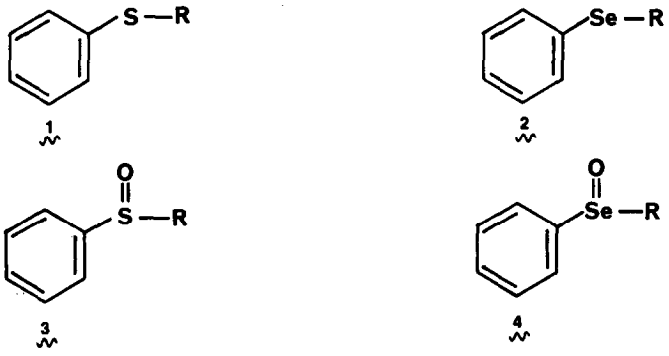
ble by normal methods of asymmetric synthesis. In view of the potential application of chiral selenoxides in the reactions of Eqs. [1] and [2], such as the possibility of asymmetric transfer from selenium to alcohol carbon in Eq. [2], and the production of olefins of defined geometry in Eq. [1] in cases where the carbon β to selenium is a methylene group, we have investigated the possibility of producing chiral selenoxides by the microbial oxidation of selenides. The racemization of selenoxides by reversible hydration (*4*) need not preclude the transfer of asymmetry from selenium to carbon: selenoxide eliminations and sigmatropic rearrangements are rapid in many cases.

Microbial oxidation of sulfides using actively growing or resting cultures of fungi can provide a convenient, high yield method for producing sulfoxides of high enantiomeric purity (*6–8*):

[3]

In the present study, we have therefore selected four fungi capable of performing efficient asymmetric oxidation of alkyl aryl sulfides, and examined the use of alkyl aryl selenides as substrates for this oxidation.

The fungi *Aspergillus niger, Aspergillus foetidus,* and *Helminthosporium sp.* all convert methyl phenyl sulfide (**1a**) to the corresponding sulfoxide (**3a**) in high chemical yield and enantiomeric purity (*6–8*). The corresponding selenide, **2a**, whose oxide **4a** is stable under the conditions of the incubation, was used as substrate with these fungi. Incubations were performed under conditions identical to those used for biotransformation of **1a**, but in no case was any methyl phenyl selenoxide (**4a**) detected following extraction of the incubation medium and fungus. The only selenium-containing component of the extract was **2a**, recovered in 50–70% isolated yield.



1–4 a; R = CH_3

b; R = CH_2—CH=CH—CH_3

c; R = CH_2— 

More extensive investigation was carried out using the fungus *Mortierella isabellina*, also capable of efficient asymmetric oxidation of **1a** (*8, 9*). Incubation of **2a** with *M. isabellina* (Table 1) resulted in the recovery of starting material as the only identifiable material. The selenoxide **4a** was not detected among the products. Control experiments involving the incubation of **4a** with *M. isabellina* followed by its reextraction confirmed that selenoxide produced by oxidation of **2a** should be stable and extractable under the conditions employed.

The inability of *M. isabellina* to oxidize **2a** is not due to deactivation of the oxidative enzymes by the selenide. This conclusion follows from experiments in which the sulfide **1a** was added to the fungus after the latter had been in contact with selenide **2a** for 2 hr. Subsequent incubation for a further 75 hr resulted in the conversion of sulfide **1a** to sulfoxide **3a** as usual. Analogous experiments with sulfides and selenides **1b/2b** and **1c/2c** gave similar results, summarized in Table 1.

The low recoveries of **2a** from the fungal incubations prompted an investigation of the distribution of substrate at the end of the incubation period, using [*methyl*-^{14}C]methyl phenyl selenide as substrate (Table 2). A considerable fraction (36%) of the radioactivity remained unextracted from the fungal mycelia; the extractable portion corresponded to recovered starting material. Only 61% of the total activity of the substrate could be accounted for at the end of the incubation period. This value differs from that obtained by estimation of selenium remaining after incubation, as determined by DC plasma emission analysis of the medium and mycelial extracts (Table 3), and suggests that metabolism by cleavage of the selenium–methyl bond by the fungus may be occurring. The fraction of radioactivity ac-

TABLE 1

INCUBATIONS OF **1–4** WITH *M. isabellina*

Substrate	Weight per flask (mg)	Time (hr)	Compound isolated (%)
1a	100	24–36	**3a** (see Ref. *9*)
2a	60	48	**2a** (62)
2a	80	48	**2a** (54)
2a + **1a**	20 + 75	2 + 48	**3a** (50)
2a	70	36	see Table 3
[*methyl*-^{14}C]**2a**	40	39	see Table 2
4a	70	24	**4a** (50)
1b	50	48	**3b** (52)
2b	50	48	**2b** (40); **6** not formed
2b + **1a**	60 + 50	48 + 24	**3a** (42)
1c	120	72	**3c** (62)
2c	60	48	**2c** (50); **5** not formed[a]
2c + **1a**	60 + 60	72 + 48	**3a** (40)
5	100	48	**5**[a]
6	100	48	**6** (74)

[a] Isolated by continuous ether extraction.

TABLE 2

DISTRIBUTION OF RADIOACTIVITY FOLLOWING INCUBATION OF [*methyl*-^{14}C]METHYL PHENYL SELENIDE WITH *M. isabellina*

Sample	Percentage total activity of substrate
Medium after extraction	0.35
Extract of medium	0.01
Mycelia after extraction	35.9
Extract of mycelia	24.5
Total recovered activity	60.8

counted for at the end of the incubation was presumably lost by formation of volatile one-carbon metabolic products.

In order to investigate the possibility that selenoxide was being formed by fungal oxidation, but was all remaining associated with the fungal membrane, and was therefore not extractable, the incubations of crotyl phenyl selenide (**2b**) and cyclohexylmethyl phenyl selenide (**2c**) with *M. isabellina* were studied. The selenoxide **4c**, derived by chemical oxidation of **2c**, is reported to decompose spontaneously to produce methylene cyclohexane (**5**) (*10*), and we have confirmed (see Experimental) that oxidation of **2b** produces, via decomposition of **4b**, the allylic alcohol **6** in good yield.

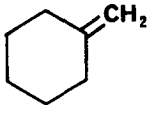
In neither case could **5** or **6** be detected by GC/MS analysis of the extracts resulting from the incubations of **2c** and **2b**, respectively, with *M. isabellina*. Incubation of **2c** was performed in a closed system; GC/MS analysis of the head space gave no indication that **5** was present. In both cases, recovered starting material was the only identifiable product. Control experiments (see Table 1) have confirmed that both **5** and **6** are stable and extractable under the conditions employed for the selenide incubations, and that the *S* oxidizing capability of *M. isabellina* is not impaired by the prescence of the selenides **2b** or **2c** (*vide supra*).

TABLE 3

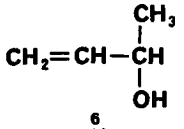
DISTRIBUTION OF SELENIUM FOLLOWING INCUBATION OF **2a** WITH *M. isabellina*

Sample	Percentage total selenium of substrate
Total medium	11
Mycelia after extraction	46
Extract of mycelia	41
Total recovered selenium	98

In control experiments, the alkene **5** could be detected in both the head space and extracts of the incubation medium.



5



6

We therefore conclude that the fungi employed in this study, in spite of being efficient oxidizers of alkyl aryl sulfides, are not capable of performing the analogous oxidation of alkyl aryl selenides to selenoxides, and that the prognosis for the asymmetric microbial oxidation of selenides at selenium is poor.

EXPERIMENTAL

Apparatus, Materials, and Methods

Melting points were determined with a Gallenkamp apparatus and are uncorrected. Infrared spectra were recorded with a Perkin–Elmer 237B spectrometer. ^{1}H nmr spectra were obtained at 60 MHz with a Varian A-60 or Bruker WP-60, or at 80 MHz with a Bruker WP-80CW, and ^{13}C nmr spectra at 15.18 MHz with a Bruker WP-60, using $CDCl_3$ as solvent and TMS as internal standard. Mass spectra were obtained with an AE1 MS30 interfaced to a Kratos DS55 data system. Column chromatography was performed on a silica gel (60–200 mesh), and thin-layer chromatography on Merck silica gel 60F-254 (0.2 mm). Radioactivity was assayed by liquid scintillation counting (Searle Delta 300 counter). Samples were dissolved in methanol and dispersed in Aquasol. Duplicate samples were counted under comparable conditions of quenching. Selenium analyses were performed on appropriately diluted samples using a Beckman Spectraspan V DC plasma emission spectrometer.

M. isabellina NRRL 1757, *A. niger* ATCC 9142, and *A. foetidus* NRRL 337 were maintained on malt agar slopes. *Helminthosporium sp.* NRRL 4671 was maintained on V-8 juice agar slopes.

Preparation of Substrates

The following were prepared by published procedures and gave satisfactory spectral and analytical data: crotyl phenyl sulfide (**1b**) (*11*), cyclohexylmethyl phenyl sulfide (**1c**) (*12*), methyl phenyl selenide (**2a**) (*13*), crotyl phenyl selenide (**2b**) (*14*), and cyclohexylmethyl phenyl selenide (**2c**) (*10*). Methyl phenyl sulfide (**1a**) was a commercial sample.

[*methyl-*^{14}*C-*]*Methyl Phenyl Selenide*

[*methyl-*^{14}C]Methyl iodide (0.248 mg, 100 μCi) was diluted with cold material (2.48 g), and the total added to a solution of phenyl selenol (2.72 g) in ethanol (70

ml) containing sodium hydroxide (0.692 g) and water (5 ml). The resulting mixture was heated under reflux for 1 hr using a cold trap to prevent loss of any volatile radioactive material to the atmosphere. The solution was then cooled, diluted with water (200 ml), and extracted with ether (3 × 50 ml). The extract was washed (10% sodium hydroxide, 75 ml), dried (sodium sulfate), and evaporated to give [*methyl*-^{14}C]methyl phenyl selenide, sp act 7.8 μCi/mmol (2.15 g, 73%), identical with an authentic sample of unlabeled material. The integrity of label was confirmed by radioscanning of a TLC plate of the product, which confirmed that all the label was located in methyl phenyl selenide.

Preparation of Sulfoxides

The following sulfoxides were prepared from the corresponding sulfides using sodium metaperiodate as oxidant (*15*) and gave satisfactory spectral and analytical data: methyl phenyl sulfoxide (**3a**) (*16*), crotyl phenyl sulfoxide (**3b**) (*17*), and cyclohexylmethyl phenyl sulfoxide (**3c**) (*12*).

Oxidation of Selenides

Methyl phenyl selenide (**2a**). A solution of sodium metaperiodate (2.06 g) in water (20 ml) was added slowly to a stirred solution of **2a** (1.5 g) in methanol (200 ml) at 0°C, and the resulting mixture stirred at 0°C for 4 hr. The solution was then filtered, and the volume of the filtrate reduced *in vacuo* to 30 ml. Distilled water (100 ml) was added, and the mixture then extracted with chloroform (3 × 75 ml). The organic extract was dried and evaporated, and the residue purified by column chromatography to yield 0.85 g (52%) of **4a**, mp 46–49°C (lit. (*19*) mp 53–54°C). ^{1}H nmr included signals at δ 2.68 (3H,s) and 7.42 (5H,m) ppm.

Crotyl phenyl selenide (**2b**). A solution of sodium metaperiodate (0.96 g) in water (20 ml) was added dropwise over 1 hr to an ice-cooled, stirred solution of **2b** (0.8 g) in methanol (100 ml). The resulting mixture was stirred for 16 hr at 0°C, and then the precipitate removed by filtration. The filtrate was reduced in volume *in vacuo*, diluted with water, and then extracted with chloroform. The extract was dried and evaporated to yield a residue (0.6 g) which was analyzed by GC/MS. The major volatile component was identified as 3-buten-2-ol (**6**) by comparison of GC/MS data with those for authentic material.

Incubations with A. niger and A. foetidus

Both fungi were grown in Czapek Dox medium in 1-liter Erlenmeyer flasks, using the techniques previously described (*9*). A solution of methyl phenyl selenide (**2a**) (60 mg) in methanol (1 ml) was added to a 48-hr-old growth of fungus suspended in distilled water (150 ml). Incubation was carried out for a further 48 hr, after which time extraction of the cultures resulted in the recovery of starting material (typically 70%) as the only identifiable material.

Incubation with Helminthosporium sp.

This fungus was grown in a medium composed of V-8 tomato juice (200 ml) and

calcium carbonate (3 g) in water (1 liter), adjusted to pH 7.2. Incubation of **2b** (50 mg) with a 3-day-old growth of fungus in replacement culture (150 ml) for 48 hr resulted in the recovery of starting material (50%) as the only identifiable product. Incubation of the sulfide **1a** under identical conditions gave the sulfoxide **3a.**

Incubations with M. isabellina

This fungus was grown and incubations performed as previously described (*9*). The results of incubations with *M. isabellina* are summarized in Table 1. In all cases, products and recovered starting materials were identified by comparison of spectral and analytical data with those of authentic samples.

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