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MONOOXYGENASE-CATALYSED METABOLISM OF THIOETHERS AND SELENOETHERS BY FUNGI

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Abstract—Evidence for mono-oxygenase activity during the metabolism of thioethers by the fungus *Aspergillus niger* is presented. Attempts to obtain selenoxides as microbial metabolic products are described.

THE ISOLATION from plants of sulphoxides as secondary metabolites,[1] allied to reports of oxidations at sulphur (in thioethers) by animals[2-5] and micro organisms,[6] prompted further study of the oxygenating system of a fungus. Many of the oxygen atom transfer reactions that occur in animal livers are catalysed by mono-oxygenase enzymes.[7] Sulphoxidations ($RSR' \rightarrow RSOR'$) in these circumstances require the presence of dioxygen (molecular oxygen) and a reducing agent (NADPH).[2] Previous work here has shown that the fungus *Aspergillus niger* promotes various oxygen transfer reactions including aliphatic and aromatic hydroxylations, epoxidations and dealkylations.[8] In addition to the chemical behavioural similarity between animal liver and *A. niger* systems, a common oxygenating mechanism often appears to be operative.[8]

Initial experiments were concerned with finding out if dioxygen was necessary for sulphoxidation to occur in the presence of *A. niger*. The thioether, $Ph \cdot CH_2 \cdot S \cdot Bu^t$, was shaken with an aqueous suspension of the acetone powder from the fungus under various $N_2 : O_2$ atmospheres as in Table 1. The results show that the yield of sulphoxide depends on the concentration of dioxygen, indicating that this will be the source of oxygen in the product.

Subsequent experiments were carried out using the easily prepared freeze dried mycelium of *A. niger*, the yields of sulphoxide being comparable with those from the acetone powder technique but the reproducibility being considerably better. The above thioether, $Ph \cdot CH_2 \cdot S \cdot Bu^t$, was shaken at 30° with a suspension of freeze dried mycelium in water under dioxygen containing 98% of $^{18}O_2$. The resultant sulphoxide contained $^{18}O_2$ (34%). Oxygen exchange between the product and the solvent ($H_2\ ^{16}O$) and residual ^{16}O present in the mycelium could account for the proportion of ^{18}O in the sulphoxide being lower than the theoretical

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value of 98%. Nevertheless the degree of incorporation of ^{18}O is sufficiently high to show that a mono-oxygenase enzyme system is present.

Selenoethers, RSeR′, resemble thioethers in that, under appropriate chemical oxidation conditions, an atom of oxygen can be incorporated into each type of substance to give selenoxides and sulphoxides respectively. As with sulphoxides, stable enantiomeric forms of selenoxides are known.[9]

TABLE 1

Initial gaseous composition in culture flask (N_2:O_2)	Thioether recovered (%)	Sulphoxide obtained (%)
100:0	56	0
80:20	41	23
0:100	23	49

Attempts to use the dioxygen-*A. niger* system to convert two selenoethers into (optically active) selenoxides were not successful. The selenide, $Ph \cdot CH_2 \cdot Se \cdot Ph$, was recovered largely unchanged from attempted oxidation using the acetone powder procedure. However, under the same conditions, the related selenide, $Ph \cdot CH_2 \cdot Se \cdot C_6H_4Me$-*p*, was oxidized to toluene-*p*-seleninic acid, $HO_2Se \cdot C_6H_4Me$-*p*, in 45% yield. This conversion does not apparently proceed via the selenoxide because this substance (as its racemate) was not metabolized to seleninic acid under the same conditions although a significant proportion of selenoxide was recovered. In previous work,[6] the presence or absence of a *p*-methyl group was also found to have a considerable effect on the course of enzymatic reactions—in that case the oxidation of a pair of thioethers.

The oxidation of the selenide, $Ph \cdot CH_2 \cdot Se \cdot C_6H_4Me$-*p*, to toluene-*p*-seleninic acid is a dealkylation (debenzylation) process, and is in contrast with the behaviour of inorganic selenites and selenates in the presence of *A. niger* where alkylation occurs to give dimethyl selenide (in unstated yields).[10]

EXPERIMENTAL

Aspergillus niger. NRRL 337 was obtained from the ARS culture collection, Northern Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture, Peoria, Illinois. MS analysis was performed with an AEI MS 902 spectrometer.

Effect of dioxygen concentration. Acetone powder was prepared from *A. niger* mycelium as described before.[6] *t*-Butyl benzyl sulphide (110 mg) and acetone powder (1 g) were shaken in water (100 ml) in a 1 l. flask under gas of known composition (see Table 1) for 5 days No sulphone was detected in the products.

Incorporation of ^{18}O. *t*-Butyl benzyl sulphide (80 mg) was added to a 1 l flask (fitted with two inlet taps) containing H_2O (100 ml) and mycelium (4 g) (freeze-dried at 2 mm for 60 hr). Air was flushed out with N_2 and then the flask was connected to a 1 l. flask containing an equivolume mixture of ^{18}O gas (>90% ^{18}O) and N_2. The reaction mixture was shaken for 4 days at 30°. The sulphoxide formed was isolated with CH_2Cl_2 and purified by preparative TLC using silica gel plates and EtoAc–toluene (1·1). Mass spectral analysis (from peaks at 196 and 198) showed that the sulphoxide contained 34% ^{18}O.

Microbial transformation of selenides. Each selenide (110 mg) was shaken with *A. niger* acetone powder (1 g) in H_2O (100 ml) at pH 4·5–6 and 30° for 5 days The following results were obtained.

Stability of benzyl p-*tolyl selenoxide* The selenoxide (starting with a 50 mg sample) was recovered, (a) in 80% yield after being shaken in H_2O (100 ml) at 30° for 5 days, and (b) in 15% yield after being shaken in H_2O (100 ml) containing acetone powder (1 g) under the same conditions

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Substrates		Products: Seleninic acid	Products: Recovered selenide
$PhCH_2 \cdot Se\ C_6H_4Me$-*p*	(i)	6	61
	(ii)	45	15
$PhCH_2 \cdot Se \cdot Ph$	(i)	0	65
	(ii)	0	52

Selenoxides were not detected.

The compounds referred to in this part of the work were obtained by lit. methods: phenyl benzyl selenide, b.p. 203°/15 mm [lit.[11] b.p. 200–202°/15 mm], phenyl benzyl selenoxide, m.p. 135° (from acetone) [lit.[12] m.p. 135°], *p*-tolyl benzyl selenide, b.p. 140°/0 4 mm, m.p. 33° [lit.[13] m.p. 33°], *p*-tolyl benzyl selenoxide, m.p. 146–147° (Found: C, 60·6; H, 5·2. $C_{14}H_{14}OSe$ requires: C, 60·7; H, 5·1%) and *p*-tolyl seleninic acid, m.p. 160–164° (from water) [lit.[14] m.p. 171°]. The selenoxides, formed by treatment of the selenides with hydrogen peroxide (30% w/w) at 0°, were purified by chromatography over silica gel and crystallization.

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