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SPECIES–STRAIN DEPENDENCE OF STEREOSELECTIVITY IN MICROBIAL OXIDATION OF THIOETHERS

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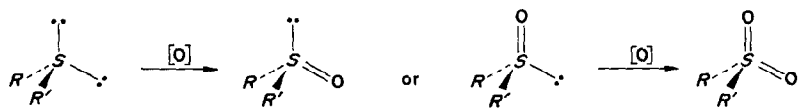
Abstract—The stereoselectivity in the aerobic, microbial oxidation of thioethers and sulphoxides is shown to be dependent on species and strain A strain of *Aspergillus niger* was used to obtain an optically active dialkyl sulphoxide

INTRODUCTION

PRODUCTS from microbial metabolic processes[1] in which oxygen atom-transfer to the substrate occurs, include phenols, alcohols, epoxides, esters and sulphoxides The last named compounds are usually obtained in a form showing optical activity when unsymmetrical thioethers, *RSR'*, are incubated in the presence of a strain of *Aspergillus niger* [2]

The optical purities of the sulphoxides obtained by this method fall in the range of 4–100%, the value depending on, (a) the structure of the thioether being oxidized, and (b) the extent of asymmetric oxidation occurring in the accompanying reaction wherein part of the initially formed sulphoxide is converted into optically inactive sulphone In the latter process preferential oxidation of one enantiomer of the sulphoxide can take place, and this has been shown to affect the optical purity of sulphoxide recovered after incubation of thioether with *A niger* to a significant degree (75% optical purity) in two examples [3] These studies have been extended in the present work, in which it has been established that the stereopreference in the oxidation of thioethers and sulphoxides depends on the strain and species of fungus used



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RESULTS AND DISCUSSION

Dependence of stereoselectivity on species

In the microbial class, Phycomycetes several strains of the genera *Rhizopus* and *Aspergillus* (**1**–**7**, Table 1) were found capable of promoting the oxidation of thioethers The combined yield of sulphoxide and sulphone isolated after incubation under standard experimental conditions was in the range 3–71% While *R stolonifer* has previously been reported to be effective for the oxidation of a methyl thiosteroid [4] no earlier report of a similar type of transformation by *R arrhizus* was found The range of optical purity of product obtained from the *Rhizopus* species was relatively low (5–25%) in comparison with that found with various strains (**3**–**7**) of *A niger* (87%) These results demonstrate that by careful choice of fungal species a higher degree of enantiomeric purity may be obtained

TABLE 1 OXIDATION OF THIOETHERS BY VARIOUS FUNGI

		Products from PhSCH Ph				Products from 'BuSCH Ph			
	Fungus	Yield	Sulphoxide Optical purity	Configuration	Sulphone Yield	Yield	Sulphoxide Optical purity	Configuration	Sulphone Yield
1	*Rhizopus arrhizus* ATCC 1145	20	25	S	S	10	10	S	
2	*R stolonifer* ATCC 6227 b	3	20	*R*	1	2	S	S	<1
3	*Aspergillus niger* NRRL 337 (subcultured in Belfast over 1 yr)	9 55 9 17	54 59	S S*	3 10	24 61	[illegible]	S	15 10
4	*A niger* NRRL 337 (subcultured in Zagreb over 9 yr)	17 S	88 66	*R**		48	6	S	10
5	*A niger* NRRL 582	0 11	86 87	*R* *R**	20	27	11	S	S
6	*A niger* NRRL 447					46	22	S	15
7	*A niger* ATCC 9142	0			15	10	S	S	6

* Results of Zagreb workers

Aromatic compounds are often metabolized to phenols in fungi and in animals A similar pattern of products is formed in either type of organism and a similar mechanism (involving mono-oxygenase enzymes) probably operates in each case [5] However, the stereoselectivity of the oxidation of thioethers to sulphoxides seems, in general, to be lower in animals (using rat liver microsomes) than in fungal processes Kexel and Schmidt[6] have shown that liver microsomal oxidation of the thioether, Me S Ph, gives only a low preference for the formation of the (*R*)-sulphoxide and we have found that the thioether, *p*-MeC_6H_4 S CH_2Ph behaves similarly under these conditions a sulphoxide only slightly enriched (1 3%) in the (*R*)-enantiomer being obtained In contrast, fungal oxidation of this thioether gives sulphoxide of 65 or 82% optical purity excess of (*R*) enantiomer (see Table 2)

Dependence on stereoselectivity on strain

A stereopreference for the oxidation of *t*-butyl benzyl thioether $^tBuSCH_2Ph$ to the

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TABLE 2 OXIDATION OF THIOETHERS AND SULPHOXIDES BY DIFFERENT STRAINS OF *Aspergillus niger*

		Products from thioether oxidation				Products from corresponding racemic sulphoxide oxidation			
		Sulphoxide		Sulphone		Sulphoxide			Sulphone
Thioether	Fungus	Yield	Optical purity	Configuration	Yield	Recovered yield	Optical purity	Configuration	Yield
$PhSCH_2Ph$	**3**	9	5	*S*	3	43	5	*R*	14
	5	30	86	*R*	20	26	75	*R*	56
$^tBuSCH_2Ph$	**3**	24	77	*S*	15	70	0	*R S*	5
	5	27	10	*S*	5	75	2	*R*	4
p Me C_6H_4 S CH_2Ph	**3**	19	82	*R*	4	32	7	*R*	32
	5	30	65	*R*	7	12	45	*R*	14
p Me C_6H_4 S Me	**3**	7	87	*R*	19	57	30	*R*	8
	5	36	67	*R*	4	66	11	*R*	20
Me S CH_2Ph	**3**	18	46	*R*	18	35	0	*R, S*	20
	5	23	4	*R*	4	12	95	*S*	57

* Results obtained using **3** (*A niger* NRRL 337, Belfast) are taken from previously published data[2,3] and are compared with those presently obtained using **5** (*A niger* NRRL 382)

(*S*)-enantiomer of *t*-butyl benzyl sulphoxide was observed for all strains of *A niger* (**3–7**, Table 1), however, a wide range of optical purity was found among the products (6–77%) The greatest difference (6% compared with 77%) was unexpectedly found between two specimens (**3** and **4**) of the same strain, NRRL 337, both specimens having originated from the same culture collection (ARS Culture Collection, Northern Utilization Research and Development Division, Agricultural Research Service, U S Dept of Agriculture) The behaviour of phenyl benzyl thioether, $PhSCH_2Ph$, towards these two specimens was also different, the Zagreb subculture of the strain giving a preponderance of the (*R*)-sulphoxide whereas a slight preference for the formation of (*S*)-sulphoxide was observed with the Belfast variety The only difference between these two subcultures of *A niger* appears to be in the time interval during which the organisms were subcultured Form (**4**) was subcultured in Zagreb over a period of 9 yr, whereas (**3**) was used in Belfast within 1 yr of receipt Oxidation of the thioether, $PhSCH_2Ph$, occurred in the same (*S*)-direction when a sample of strain (**3**) was sent to Zagreb for oxidation experiments The differences in the optical purity of products from strains (**3**) and (**4**) may be due to mutation and change in the monooxygenase activity especially over the 9-year period Thus, in trying to reproduce microbial transformation results reported in the literature, it may sometimes be necessary to obtain slants and detailed culture conditions from the authors of the report However, the reproducibility of procedure and results that is possible in different laboratories (Zagreb and Belfast) was demonstrated with another strain of *A niger*, NRRL 382 oxidation of phenyl benzyl thioether gave (*R*)-sulphoxide of 86–87% optical purity in each location

Asymmetric metabolism of sulphoxides

As mentioned earlier the optical purity of a sulphoxide obtained by microbial oxidation of a thioether can also depend on the selectivity in its partial, concomitant metabolism to sulphone However, from previous work with fungus (**3**) (NRRL 337), this factor was a relatively minor one in the oxidations of the two thioethers chosen for study of species and strain dependence (Table 1) The results for five thioethers and sulphoxides, using NRRL 337, are summarized in Table 2, they show that the optical purities of sulphoxides isolated after partial oxidation of racemic sulphoxide range from 0 to 7% with the exception of the sulphoxide, *p*-Me C_6H_4 S Me, where a 30% optically pure product was isolated However, some greater enrichments were obtained using fungus (**5**) (NRRL 382),

here the optical purities of the five recovered sulphoxides were 2, 11, 45 75 and 95% (Table 2), the last entry being the best example of an enzymatic metabolic resolution of a sulphoxide to date It is of interest that the reverse reaction, preferential reduction of one sulphoxide enantiomer, can also occur in the *A niger* system,[6] and this is yet another factor that requires to be taken into account in further quantitative studies

An optically active dialkyl sulphoxide from microbial oxidation

Dialkyl sulphoxides are difficult to obtain in a high state of optical purity by chemical techniques due to the instability and low m ps of intermediate sulphinates One dialkyl sulphoxide (*n*-butyl methyl sulphoxide) has, however, been resolved [7] In an attempt to apply the microbial technique to this problem a range of strains of *A niger* was grown in the presence of *n*-butyl methyl thioether One strain successfully produced (*R*)-sulphoxide in low yield ($\sim 1\%$) with an optical purity of 26% The relatively low yield was probably a result of growth inhibition by the high concentration of substrate required to isolate a sufficient quantity of the water-soluble product Nevertheless this experiment demonstrates that this technique may also be successfully applied to simple thioethers

The present and previous results[2,3] show that microbial procedures can be used to obtain sulphoxides of high (82–99%) optical purity (*p*-Me C_6H_4 SO R where $R = CH_2Ph$ and Me, $PhCH_2$ SO R where $R = {}^tBu$, Ph and Me) and 100% optical purity (*p*-Me C_6H_4 SO R where $R = {}^tBu$ and *p*-Me $C_6H_4CH_2$) Most of the sulphoxides in the 82–99% optical purity range can be recrystallized to 100% purity

EXPERIMENTAL

Optical rotations were determined at 659 nm using both a visual and a Perkin-Elmer 141 automatic polarimeter Fungi were originally obtained from the American Type Culture Collection (ATCC) 12301 Parklawn Drive Rockville, Maryland 20852 or the Northern Utilization Research and Development Division (NRRL) Agricultural Research Service U S Department of Agriculture Peoria Illinois Thioethers sulphoxides and sulphones were synthesized and characterized as reported previously [2,3]

Liver microsomal sulphoxidation of benzyl *p*-tolyl sulphide was carried out at pH8 using a crude microsomal preparation [8] The product sulphoxide was isolated and purified by preparative TLC (silica-gel)

Microbial oxidations were carried out using Czapek Dox liquid medium and a platform shaker at a temp of 28–30 , in the manner described previously [2,3] The procedure was modified in the oxidation of *n*-butyl methyl sulphide where a high concentration of substrate was used, the sulphide sulphoxide product mixture was separated by cation exchange chromatography

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