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Synthesis of Chiral Benzyl Alkyl Sulfoxides by Cyclohexanone Monooxygenase from *Acinetobacter* NCIB 9871

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Abstract. Benzyl alkyl sulfides with alkyl groups from methyl to hexyl, *para*-alkylbenzyl groups from methyl to butyl, 2-phenylethyl methyl sulfide and 3-phenylpropyl methyl sulfide have been oxidized by cyclohexanone monooxygenase from *Acinetobacter* NCIB 9871. Sulfoxides with enantiomeric excesses ranging from 80% for the (*R*)-configuration to 96% ee for the (*S*)-configuration were obtained.

INTRODUCTION

Both chemical[1] and biological[2] approaches have been actively tested to stereoselectively oxidize organic sulfides to the corresponding sulfoxides. Enantiomerically pure sulfoxides, in fact, are important chiral synthons[3] and powerful stereodirecting groups.[4]

Previously, we have shown that cyclohexanone monooxygenase (CMO) from *Acinetobacter* NCIB 9871 can catalyze the asymmetric sulfoxidation of numerous phenyl alkyl sulfides, dialkyl sulfides and dialkyl disulfides.[5] The structure of the sulfide dramatically influences not only the enantioselectivity, but also the stereochemical course of the reaction, giving sulfoxides ranging from 99% ee with (*R*)-configuration to 93% ee with (*S*)-configuration.[5] Similar results were obtained in the asymmetric oxidation of phenyl alkyl sulfides with the alkyl chain functionalized with Cl, CN, vinyl or hydroxyl groups.[6]

In the present paper, we have extended our investigation of CMO-catalyzed sulfoxidations to numerous benzyl alkyl sulfides.

RESULTS AND DISCUSSION

The oxidation of benzyl alkyl sulfides catalyzed by CMO (reaction 1) was coupled to a second enzymatic reaction (reaction 2) catalyzed by glucose-6-phosphate dehydrogenase (G6PDH) in order to regenerate NADPH.[5]

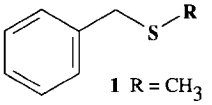
$$\text{Ar-S-R} + \text{NADPH} + \text{O}_2 + \text{H}^+ \xrightarrow{\text{CMO}} \text{Ar-SO-R} + \text{NADP}^+ + \text{H}_2\text{O} \quad \text{(reaction 1)}$$

$$\text{glucose-6P} + \text{NADP}^+ + \text{H}_2\text{O} \xrightarrow{\text{G6PDH}} \text{gluconate-6P} + \text{NADPH} + \text{H}^+ \quad \text{(reaction 2)}$$

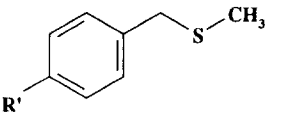
The Table shows the results obtained in the enzymatic oxidation of sulfides **1-14** in terms of degrees of conversion into sulfoxides, and enantiomeric excesses and predominant absolute configurations of the products. The conversions were in all cases ≥ 75%, which indicates that the catalytic efficiency of CMO is not very sensitive to the structural variations of the substrates. The formation of sulfones was in all cases very low (≤4%), which means that the overwhelming contribution to the chirality of produced sulfoxides comes from an asymmetric first oxidation step and that the kinetic resolution in the further oxidation, if present, is negligible.

Enzyme enantioselectivity was markedly influenced by the structure of sulfides. Considering compounds **1-7**, it can be seen that when the alkyl chain is bulkier than the methyl group, the predominant configuration of the product changes from (*R*) to (*S*), with the highest ee of (*S*)-product (96%) obtained in the case of the *n*-propyl group. The low enantioselectivity obtained with the *n*-butyl group (15% ee) appears in disagreement with the trend. The same inversion of configuration on increasing the length of the alkyl chain occurs also with phenyl alkyl sulfides. However, in the latter case the phenomenom is observed with the *n*-propyl group which gives (*S*)-configuration and 68% ee (determined in this work but not included in the Table) whereas the ethyl group still affords (*R*)-configuration (47% ee).[5]

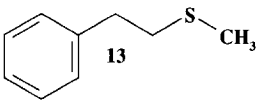
The introduction of a *para*-alkyl chain in the phenyl ring decreased the (*R*)-enantioselectivity for benzyl sulfide oxidation, with the exception of *n*-butyl (**11**), which yielded an (*R*)-sulfoxide with 80% ee (Table). The increase of the length of the chain linking the phenyl group to sulfur had an (*S*)-orienting effect. In fact, whereas with benzyl methyl sulfide (**1**) the configuration of the product was (*R*) and ee 54%, with 3-phenylpropyl methyl sulfide (**14**) the configuration was (*S*) and ee 48%.



1 R = CH_3
2 R = C_2H_5
3 R = *n*-C_3H_7
4 R = *n*-C_4H_9
5 R = *i*-C_4H_9
6 R = *i*-C_5H_{11}
7 R = *n*-C_6H_{13}



8 R'= CH_3
9 R'= C_2H_5
10 R'= *i*-C_3H_7
11 R'= *n*-C_4H_9
12 R'= *t*-C_4H_9



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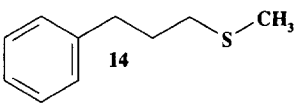

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Comparing the results obtained with CMO with those obtained using the fungus *Helminthosporium*[7,8] and the same substrates used in this study, it can be observed that the fungus gave almost exclusively sulfoxides with the (*S*)-configuration (ee 16-85%) whereas CMO gave both (*R*)- and (*S*)-sulfoxides with ee ranging from 96% ee and (*S*)-configuration (**3**) to 80% ee and (*R*)-configuration (**11**).

It should be noted that the same results, in terms of enantioselectivity, were obtained using either crude or purified CMO. This demonstrates that: a) the high sensitivity of CMO to structural variations of the substrate is an intrinsic property of a single enzyme and b) in the crude preparation other enzymes capable of oxidizing sulfoxides are not present. Work is in progress to develop an active site model in order to rationalize the results obtained in the oxidation by CMO of these and many other sulfides.[5,6]

Table. CMO catalyzed oxidation of benzyl alkyl sulfides to sulfoxides

	sulfoxide		
sulfide	conversion %	ee %	configuration
1	97	54	*R*
2	80	67	*S*
3	90	96	*S*
4	98	15	*S*
5	90	90	*S*
6	95	80	*S*
7	75	56	*S*
8	97	5	*R*
9	91	8	*R*
10	88	4	*R*
11	76	80	*R*
12	87	23	*R*
13	95	40	*R*
14	79	48	*S*

EXPERIMENTAL SECTION

Materials. Sulfides were synthesized as previously reported.[7,8] NADPH, glucose-6-phosphate and glucose-6-phosphate dehydrogenase were bought from Sigma. Crude cyclohexanone monooxygenase from *Acinetobacter* NCIB 9871 was prepared as previously described[5] and purified by DEAE chromatography and affinity chromatography on Matrix Gel Red 4 (Amicon), as reported by Latham and Walsh.[9] The enzyme had an activity of 3 units/mg of protein with phenyl methyl sulfide as the substrate.

Enzymatic oxidations. The sulfide (**1-14** and phenyl *n*-propyl sulfide) (0.1 mmol) was magnetically stirred in 4 ml of 0.05 M Tris-HCl buffer, pH 8.6, containing 2 μmol NADPH, 0.4 mmol glucose-6-phosphate, 5 units of CMO and 10 units of glucose-6-phosphate dehydrogenase. After overnight reaction, the solution was extracted with 4 portions (4 ml each) of ethyl acetate and the organic extract was dried and evaporated.

Determination of degree of conversion and enantiomeric excess. The degrees of conversion of sulfides into sulfoxides and sulfones were determined on the ethyl acetate extracts by GLC with a 25 m HP-1 capillary column coated with methylsilicone gum (Hewlett Packard) with H_2 as carrier gas. The enantiomeric excesses of sulfoxides were determined by chiral HPLC on a Chiralcel OB (sulfoxides obtained from **1,4,6,7,8,9,13,14** and phenyl *n*-propyl sulfide) or OD (sulfoxides obtained from **2,3,5,10,11,12**) column, using the proper mixture of *n*-hexane and propan-2-ol as the mobile phase. It should be noted that, whereas for the sulfoxides previously investigated[5,6] and the majority of those studied in the present work the elution order was (*S*) before (*R*) with the OB column, and (*R*) before (*S*) with the OD column, the sulfoxides obtained from **2-6** showed the opposite elution order. The absolute configuration of sulfoxides was determined by comparison with authentic samples[7,8] using chiral HPLC.

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