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Active Site Model of Cyclohexanone Monooxygenase. 3.[1] The Case of Benzyl Sulfides Bearing Polar Groups.

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Abstract. The stereopreference of cyclohexanone monooxygenase for a series of benzyl methyl sulfides bearing in the *p*-position groups with different σ_p values has been rationalized by locating inside the enzyme active site model electron-rich and electron-poor regions.

Introduction

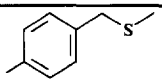
The asymmetric sulfoxidation of sulfides to sulfoxides, carried out with both chemical and enzymatic approaches, has been intensively studied. The biotic oxidation has been accomplished with different microorganisms such as *Aspergillus niger*,[2] *Mortierella isabellina*,[3] *Corynebacterium equi*[4] and *Helminthosporium* sp.[5] or with enzymes such as chloroperoxidase,[6] horseradish peroxidase[7] and cyclohexanone monooxygenase (CYMO) from *Acinetobacter* NCIMB 9871 (EC 1.14.13.22). The latter has recently been extensively investigated in our laboratories.[8-10] In a continuing programme aimed at the understanding of the properties of this enzyme we became interested in the sulfoxidation of benzyl alkyl sulfides to their corresponding sulfoxides.[9] The enantioselectivity of CYMO with the substrates was mainly influenced by the alkyl moiety which caused a switch of configuration from *(R)* to *(S)* moving from methyl to ethyl or other bulkier hydrophobic groups. In contrast, changing *p*-alkyl benzyl groups (from methyl to butyl) did not invert the enzyme's *(R)*- stereopreference. The behaviour of CYMO with these substrates was explained by us using an active site model that was able to describe and predict enzyme stereopreference.[10]

Complementary to the previous studies which dealt with hydrophobic interactions and steric hindrances, the present paper is focussed on the effects of polar groups in the sulfoxidation of *p*-substituted-benzyl methyl sulfides catalyzed by CYMO.

Results and discussion

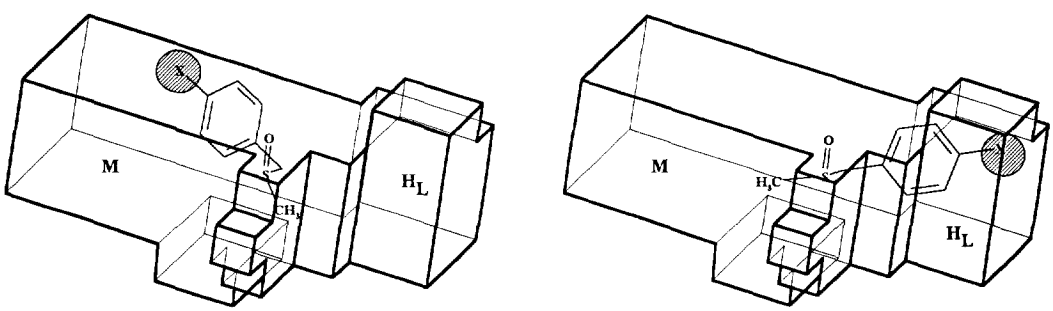
The results obtained in the CYMO catalyzed oxidation of different *p*-substituted-benzyl methyl sulfides are summarized in the **Table**. Previously we observed that the introduction of a substituent in the *para* position of the phenyl ring decreased the *(R)*-enantioselectivity in the oxidation of benzyl methyl sulfides.[9] However, unlike alkyl groups,[9] polar groups in *para* position also heavily influenced the stereochemical course of the

Table. CYMO catalyzed oxidation of sulfides to sulfoxides



	X	σ_p	Conversion (%)[a]	Ee (%)[b]	Abs.conf.
1	NH_2	-0.30	100	65	*R*
2[c]	H	0	97	54	*R*
3	OH	-0.38	50	49	*R*
4[c]	C_2H_5	-0.13	91	8	*R*
5	F	0.15	100	7	*R*
6[c]	CH_3	-0.14	97	5	*R*
7[d]	CH_3CO	0.47	95	5	*S*
8	CH_3O	-0.12	99	5	*S*
9	Cl	0.24	90	24	*S*
10	NO_2	0.81	95	50	*S*
11	CN	0.70	93	56	*S*
12	CF_3	0.53	97	56	*S*

[a]Byproducts as sulfones were negligible. [b]Uncatalyzed oxidation or racemization of the products were not observed. [c]From Ref. 9. [d]Baeyer-Villiger oxidation was not observed.



Scheme. Binding modes in the enzyme active site model of *p*-X-benzyl methyl sulfoxides with a *R* (left) and *S* (right) configuration. The dashed area in the left side represents an electron-rich hydrogen bonding region. The dashed area in the right side represents an electron-poor region.

sulfoxidation catalyzed by CYMO. Indeed, the enantiomeric purity of the products ranged from 65% ee and *(R)*-configuration (**1**) to 56% ee and *(S)*-configuration (**12**). The inversion of stereopreference cannot be ascribed only to the different hindrance of the substituents, because bulkier *p*-alkyl derivatives (e.g. *t*-butyl and

n-butyl) showed a clear *(R)*-stereopreference (23 and 80% ee).[9] Moreover, if we look at the active site model of the enzyme, we can see that for each compound both *(R)* and *(S)*-sulfoxides can fit confortably inside the pockets (**Scheme**).

These initially puzzling results can be reasonably explained by considering the parameter σ_p for the substituents, described in the Hammett relationship,[11] which represents a contribution of factors such as resonance, field and inductive effects. The **Table** shows that there was an *(R)*-stereopreference with negative values of σ_p, while the opposite occurred with positive values of σ_p. Instead, when σ_p. was close to zero the correspondent sulfoxide was nearly racemic. A marked exception to this trend was represented by the CH_3CO substituent, where a higher *(S)*-stereopreference would have been expected. The **Figure** gives a more direct illustration of the correlation existing between ee values of products and σ_p values of substituents. It can be seen that the compounds according to their substituents can be placed in two groups, strong H bond donors (OH and NH_2, *R*-preference) and strong H bond acceptors (CN, CF_3 and NO_2, *S*-preference), with weaker H bond acceptors (Cl, CH_3O, F and acyl) showing a slight (*S*) preference. For the first group of compounds there was a negative ρ value while for the second group the ρ value was positive.

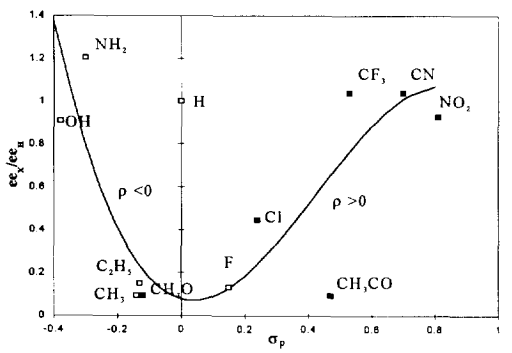


Figure. Correlation of substituent constant σ_p with the ratio between the enantiomeric excess of *p*-substituted-benzyl methyl sulfides (ee_X) and that of benzyl methyl sulfide (ee_H). The empty symbols represent compounds with *(R)*-stereopreference (❑) and the solid symbols the compounds with *(S)*-stereopreference (■).

The active site model proposed for CYMO predicts two dispositions for *(R)*- and *(S)*- enantiomers, with their benzyl moiety pointing towards two distinct pockets (M and H_L respectively). The results suggest that electron-releasing groups (σ_p <0) stabilize the *(R)*-intermediate in the M pocket, while electron-withdrawing groups (σ_p >0) stabilize the *(S)*-intermediate in the H_L pocket. As the different behaviour of the two pockets should reflect also their environment we propose that in the M pocket near the *p*-position of the benzyl derivative there is an electron-rich hydrogen bonding region while in the H_L pocket there is an electron-poor region.

Experimental

Materials. Sulfides were synthesized as previously reported.[12] NADPH, glucose-6-phosphate and glucose-6-phosphate-dehydrogenase were bought from Sigma. CYMO was prepared as previously reported.[8a] The enzyme had an activity of 1 unit/ mg with phenyl methyl sulfide as substrate.

Enzymatic oxidations. The sulfide (**1, 3, 5, 7-12**) (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, 6 units of CYMO and 50 units of Gluc-6-P-DH. 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 their enantiomeric excesses were determined by chiral HPLC on a Chiracel OB column, using the proper mixture of *n*-hexane and propan-2-ol as mobile phase (90/10 **3**, **9**, **12**; 80/20 **1**, **5**, **7**, **8**, **10**, **11**). The elution order was *(S)* before *(R)* and the absolute configuration was determined by comparison with authentic samples.[12]

References

1. Part 2. Ottolina, G.; Carrea, G.; Colonna, S.; Rückemann, A.; *Tetrahedron: Asymmetry*, **1996**, *7*, 1123.
2. Auret, B.J.; Boyd, D.R.; Henbest, H.B.; Ross, S.; *J. Chem. Soc.*, **1968**, 2371.
3. Holland, H.L.; Popperl, H.; Ninniss, R.W.; Chenchaiah, P.C.; *Can. J. Chem.*, **1985**, *63*, 1118.
4. Otha, H.; Okamoto, Y.; Tsuchihashi, G.I.; *Agric. Biol. Chem.*, **1985**, *49*, 671.
5. a) Rossi, C; Fauve, A.; Madesclarire, M.; Roche, D.; Davies, F.A.; Reddy, R.T.; *Tetrahedron: Asymmetry*, **1992**, *3*, 629. b) Holland, H.L.; Brown, F.; Larsen, B.G.; *Tetrahedron: Asymmetry*, **1994**, *5*, 1241.
6. Colonna, S.; Gaggero, N.; Casella, L.; Carrea, G.; Pasta, P.; *Tetrahedron: Asymmetry*, **1992**, *3*, 95.
7. Colonna, S.; Gaggero, N.; Casella, L.; Carrea, G.; Pasta, P.; *J. Chem. Soc. Chem Commun.*, **1992**, 357.
8. a) Carrea, G; Redigolo, B.; Riva, S.; Colonna, S.; Gaggero, N.; Battistel, E.; Bianchi, D.; *Tetrahedron: Asymmetry*, **1992**, *3*, 1063. b) Secundo, F.; Carrea, G.; Dallavalle, S.; Franzosi, G.; *Tetrahedron: Asymmetry*, **1993**, *4*, 1981. c) Secundo, F.; Carrea, G; Riva, S.; Battistel, E.; Bianchi, D.; *Biotechnol Lett.*, **1993**, *15*, 865. d) Colonna, S.; Gaggero, N.; Bertinotti, A.; Carrea, G.; Pasta, P.; Bernardi, A.; *J. Chem. Soc. Chem Commun.*, **1995**, 1123. e) Colonna, S.; Gaggero, N.; Carrea, G.; Pasta, P.; *Tetrahedron: Asymmetry*, **1996**, *7*, 565.
9. Pasta, P.; Carrea, G.; Holland, H.L.; Dallavalle, S.; *Tetrahedron: Asymmetry*, **1995**, *6*, 933.
10. Ottolina, G.; Pasta, P.; Carrea, G.; Colonna, S.; Dallavalle, S.; Holland, H.L.; *Tetrahedron: Asymmetry*, **1995**, *6*, 1375.
11. Exner, O.; in *Correlation Analysis in Chemistry*, N.B. Chapman and J. Shorter eds., Plenum Press, New York, 1978, Chap. 10.
12. Holland, H.L.; Brown, F.M.; Larsen, B.G.; *Tetrahedron: Asymmetry*, **1995**, *6*, 1561.

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