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Enantio and Diastereoselectivity of Cyclohexanone Monooxygenase Catalyzed Oxidation of 1,3-Dithioacetals.

Stefano Colonna[a], Nicoletta Gaggero[a]*, Giacomo Carrea[b] and Piero Pasta[b]*

[a] Centro CNR and Istituto di Chimica Organica, Facoltà di Farmacia, via Venezian 21, 20133 Milano. [b] Istituto di Chimica degli Ormoni, CNR, via Mario Bianco 9, 20131 Milano, Italy.

Abstract. The asymmetric oxidation of 2-substituted dithianes, dithiolanes and oxathiolanes catalyzed by cyclohexanone monooxygenase (CMO) has been examined. The introduction of substituents at the C-2 causes a decrease of the e.e. with the exception of 2-benzoyl-1,3-dithiane monosulfoxide (90% e.e.). With 2-monosubstituted dithioacetals CMO yields preferentially or exclusively the *trans* diastereoisomer. The binding of the 1,3-dithioacetals at the active site of the enzyme is not only controlled by the sterical hindrance of the substituents in position 2 but also by the configuration at C-2. The stereoselectivity of CMO has been compared with that reported for microsomal flavin and cytochrome P-450 monooxygenases.

INTRODUCTION

Cyclohexanone monooxygenase (CMO) from *Acinetobacter* NCIB 9871 is a versatile flavin-monooxygenase capable of transferring either an electrophilic or a nucleophilic oxygen atom to organic substrates. The enzyme was first characterized as oxidizing cyclohexanone to caprolactone in an apparent Baeyer-Villiger reaction.[1] At the same time electron rich heteroatoms are readily oxygenated by an electrophilic attack of the enzymatically generated 4-a-hydroperoxyflavin intermediate.[2]

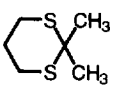
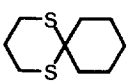
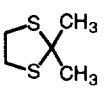
Recently we have used CMO as catalyst in the stereoselective oxidation of 1,3-dithioacetals.[3] The CMO promoted oxidation of 1,3-dithiane, 1,3-dithiolane and bis(methylthio)methane gave the enantiomerically pure (*R*) monosulfoxides with chemical yields ranging from 81 to 94%. Starting from racemic 1,3-dithiane monosulfoxide the enzyme was able to oxidize the (*S*) enantiomer to the corresponding monosulfone faster than the (*R*) enantiomer. So the enantiomerically pure (*R*)-1,3-dithiane monosulfoxide was the result of both asymmetric synthesis (v_R/v_S= 12)[4] and kinetic resolution (E = 20)[5]. The same stereochemical course was demonstrated for bis(methylthio)methane, whereas only asymmetric synthesis was operating in the case of 1,3-dithiolane since the v_R/v_S value was as high as 49.

The aim of this study was to determine the diastereotopic and enantiotopic preference for CMO mediated S-oxygenation on numerous 2,2-disubstituted and 2-monosubstituted-dithioacetals. 2-Monosubstituted substrates provide an interesting challenge for the enzyme in stereoselecting both between two prochiral lone pairs on the sulfur atom and between prochiral sulfur atoms on a carbon atom. We have also examined the effects due to the replacement of a sulfur atom with an oxygen one in the 1,3-oxathiane and 2-*p*-cyanophenyl-1,3-oxathiolane. Some of the substrates examined allow a comparison between the catalytic and stereochemical properties of cyclohexanone monooxygenase from *Acinetobacter* with those of microsomal flavin monooxygenases. When possible, the stereochemistry of the CMO catalyzed oxidation has also been compared with those of cytochrome P-450 monooxygenases, a *Helminthosporium* species, *Aspegillus niger* and *Mortierella isabellina.*

RESULTS AND DISCUSSION

As expected the increasing steric hindrance in 2,2-dialkyl dithianes and dithiolanes decreased the enantiomeric excess of the obtained monosulfoxides (**Table I**) in comparison with the unsubstituted 1,3-dithioacetals where e.e. were about 100%.[3]

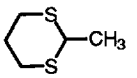
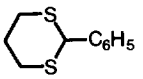
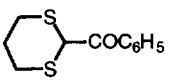
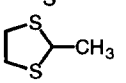
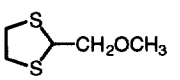
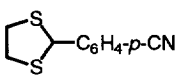
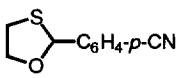
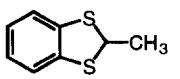
Table I. CMO catalyzed oxidation of 2,2-dialkyl dithioacetals.

Entry	Substrate	Conversion (%)	e.e. (%)	Absolute configuration
1		100	68	(*S*)
2		27	25	–
3		100	65	–

2,2-Dimethyl-1,3-dithiane yielded preferentially the (*S*) monosulfoxide, whereas the opposite enantiomer was obtained from 1,3-dithiane. This result indicates that the stereochemistry of the enzymatic sulfoxidation is highly dependent on substrate structure also with the conformationally more rigid cyclic 1,3-dithioacetals and this is consistent with our previous results obtained with acyclic sulfides.[6] The microbial transformation of 2,2-dimethyl-1,3-dithiane by a *Helminthosporium* species, *Mortierella isabellina* and *Aspergillus foetidus* gave the sulfoxide with (*S*) absolute configuration and e.e. ranging from 21 to 36%. The lack of literature data prevented us from determining the stereochemical course of the reaction for 1,5-dithiaspiro[5,5]undecane **2** and 2,2-dimethyl-1,3-dithiolane **3**. The low chemical yield of **2** is to ascribe in part to a poor solubility of the solid substrate in the reaction buffer.

The results of the sulfoxidation of monosubstituted dithianes, dithiolanes and oxathiolanes **4-11**, collected in **Table II**, show the preferential or exclusive formation of the *trans* monosulfoxides.

Table II. CMO catalyzed oxidation of monosubstituted substrates.

Entry	Substrate	Conversion (%)	*Trans* : *Cis*[1]	e.e.(%) *Trans*	e.e.(%) *Cis*
4		31 100	7 : 1 10 : 1	75 (1*R*, 2*R*) 95 (1*R*, 2*R*)	not determined
5		100	≥ 50 : 1	28 (1*R*, 2*R*)[2]	
6		90	≥ 50 : 1	90	
7		100	≥ 50 : 1	50 (1*R*, 2*R*)	
8		90	≥ 50 : 1	56	
9			15 : 1	-[3]	100 (1*S*, 2*R*)
10			34 : 1	10 (1*S*, 2*S*)	100 (1*R*, 2*S*)
11			4 : 1	50 (1*S*, 2*S*)	8 (1*R*, 2*S*)

[1] The diastereoisomeric ratio was determined after 3 and 16 hours for substrate 4. For all other substrates the reaction time was 16 h.

[2] D. R. Boyd, private communication.

[3] It was impossible to determine a reliable value of the e.e. because of the poor HPLC separation.

With 2-methyl-1,3-dithiane **4** the time progress of the reaction was monitored. For long reaction times the *trans* : *cis* ratio increased and the preferential kinetic resolution of the *trans* diastereoisomer had the same stereochemical course as the asymmetric synthesis, thus causing an increase of the e.e. of the recovered sulfoxide as already found for 1,3-dithiane.[3] The ketoderivative **6** was oxidized to the *trans* monosulfoxide with high enantiomeric excess. This result is particularly significant, since Page and coworkers[8] have used the monosulfoxide of this and related compounds as a highly selective element of stereocontrol for reactions involving the carbonyl function such as enolate alkylation, nucleophilic addition, reductions, conjugate addition and cycloadditions.

CMO exhibited a high diastereopreference for the *trans* isomer also with 2-*p*-cyanophenyl-1,3-oxathiolane **10** but the e.e. was high only for the less abundant *cis* monosulfoxide. The capability of cyclohexanone monooxygenase to transform 1,3-oxathioacetals was ascertained also with 1,3-oxathiane (datum not shown in

the Tables), that gave the corresponding monosulfoxide in 90% chemical yield (90% e.e.). Prior to this work, 1,3-oxathiane-3-oxide was reported in the literature only as a racemic form.[9] The sulfinyl group has a different conformational preference from 1,3-dithiane-1-oxide.[10] In fact, in 1,3-oxathiane-3-oxide the SO function is preferentially axial, whereas 1,3-dithiane-1-oxide presents only 15% of the axial form. 1,3-Oxathiane is a good substrate for CMO in terms of chemical and optical yield. This leads to the conclusion that the replacement of a sulfur atom with an oxygen in the starting material is without appreciable consequences for its interaction with the enzyme active site and that the amount of asymmetric synthesis is independent from the conformational preference of the reaction products. Because of the instability of 2-alkyl-1,3-oxathiane S-oxides[11] the study has not been extended to other substrates.

The comparison of our stereochemical data for 2-*p*-cyanophenyl-1,3-dithiolane **9** and 2-*p*-cyanophenyl-1,3-oxathiolane **10** with those obtained with crude or isolated microsomal mammalian FMO and mammalian P-450 monooxygenases[11,13] shows a preference towards the *trans* diastereoisomer for all the three enzymatic systems. In the case of CMO a high enantioselectivity was observed only with the minor *cis* monosulfoxide deriving from dithioacetal **9** (100% e.e.), whereas the other monooxygenases yield high enantioselectivity for both diastereoisomers. Both CMO and FMO show a stereopreference for the *pro-S* sulfur atom with oxathiolane **10**, whereas they behave differently with dithiolane **9** for reasons difficult to rationalize.

2-Methyl-1,3-benzodithiole-S-oxide **11**, that provides a suitable probe for stereochemical studies, has been examined using a range of chemical, prokaryotic and enzyme-catalyzed oxidations.[14] *Aspergillus foetidus*, *Pseudomonas putida* , rabbit lung FMO and hog liver FMO (in the presence of octylamine) afforded preferentially *cis*-1,3-benzodithiole-S-oxide with e.e. ranging from 80 to 100% and a preference for the *pro-S* sulfur atom. CMO oxidizes **11** with low enantioselectivity and *trans* diastereoselection. Our results obtained with 2-methyl-1,3-benzodithiole are not to be ascribed to the steric hindrance of the methyl group on C-2 and, in fact, modest e.e. (41%) was achieved also with 1,3-benzodithiole (datum not reported in the Tables).

2-Methyl-1,3-dithiane **4** and 2-methyl-1,3-dithiolane **7** oxidized by grown cultures of a *Helminthosporium* species, *Mortierella isabellina* and *Aspergillus niger* gave preferentially the *trans* diastereoisomer with low e.e.[7,15] Compared to CMO the three fungal species are less efficient with unsubstituted, mono and disubstituted 1,3-dithioacetals.

The asymmetric sulfoxidation method by the Sharpless reagent is only poorly succesful for 1,3-dithianes and dithiolanes with simple alkyl group substitutions at C-2.[16,17] To achieve high enantiomeric excesses by chemical means it is necessary to operate on the 2-acyl-2-alkyl derivative a two step reaction consisting of the modified Sharpless oxidation followed by an alkaline hydrolisis of the acyl moiety. This method gives as major reaction product the *cis* 2-alkyl-1,3-dithiane monosulfoxide.[8] In terms of enantiomeric excess and diastereoselectivity the CMO catalyzed oxidation of substituted 1,3-dithioacetals gave similar results to the direct Sharpless oxidation, but it was much more satisfactory in the case of unsubstituted substrates.

CONCLUSIONS

Cyclohexanone monooxygenase from *Acinetobacter* shows a wide substrate selectivity towards organic sulfur compounds. Indeed it is able to oxidize alkyl aryl sulfides, disulfides, dialkyl sulfides, cyclic and acyclic 1,3-dithioacetals and 1,3-oxathioacetals to the corresponding monosulfoxides. It yields high enantioselectivity with alkyl aryl sulfides and in this regard it is comparable to mammalian FMO.[18] Total enantioselectivity is obtained in CMO mediated oxidation of simple cyclic and acyclic thioacetals. The introduction of substituents at

the C-2 causes a decrease of the e.e. with the exception of 2-benzoyl-1,3-dithiane monosulfoxide **6** (90% e.e.). With 2-monosubstituted dithioacetals CMO gives preferentially or exclusively the *trans* diastereoisomer and at least in the case of substrates **10** and **11** the enzyme oxidizes preferentially the *pro-S* sulfur atom on the prochiral carbon atom. The binding of the 1,3-dithioacetals to the active site of the enzyme is not only controlled by the steric hindrance of the substituents in position 2 but also by the configuration at C-2.

EXPERIMENTAL SECTION

Materials. $NADP^+$, NADPH, glucose-6-phosphate dehydrogenase (G6PDH) (Type XXIV) were obtained from Sigma. All other chemicals were reagent grade. 2,2-Dimethyl-1,3-dithiane **1**, 1,5-dithiaspiro[5,5]undecane **2**, 2,2-dimethyl-1,3-dithiolane **3**, 2-methyl-1,3-dithiane **4**, 2-phenyl-1,3-dithiane **5**, and 2-methyl-1,3-dithiolane **7** were synthetized by the method of Hoppmann et al.[19] 1,3-Oxathiane was synthetized according to Carlson and Helquist[9] and 1,3-benzodithiole and 2-methyl-1,3-benzodithiole **11** were prepared according to Boyd et al.[21] 2-*p*-Cyanophenyl-1,3-dithiolane **9** and 2-*p*-cyanophenyl-1,3-oxathiolane **10** were prepared by the method used by Holland and Munoz[11] and had the physical properties reported in literature.[12,13] 2-Benzoyl-1,3-dithiane was synthetized according to Page et al[22] and 2-methoxymethyl-1,3-dithiane **8** was a generous gift of Prof. V. Aggarwal (University of Sheffield).

Cyclohexanone monooxygenase. *Acinetobacter* NCIB 9871 (from NCIMB) was grown (20 l colture) as described by Donogue et al.[1] The cells were disrupted by ultrasonication and cell debris were removed by centrifugation. The supernatant was subjected to fractionation with $(NH_4)_2SO_4$ and the fraction precipitated between 50 and 80% saturation retained. It was redissolved in 0.02 M potassium phosphate buffer, pH 7, dialyzed against the same buffer and lyophilized. The enzymatic activity was analyzed by monitoring NADPH consumption at 340 nm using as the assay buffer 0.05 Tris-HCl, pH 8.6, containing 0.6 mM methyl phenyl sulfide and 0.12 mM NADPH. The total activity obtained from 20 l colture was of 860 units.

Characterization of the sulfoxides. 2,2-Dimethyl-1,3-dithiane-1-oxide,[7] 2,2-dimethyl-1,3-dithiolane-1-oxide,[16] *trans*-2-methyl-1,3-dithiane-1-oxide,[7] *trans*-2-phenyl-1,3-dithiane-1-oxide,[16] *trans*-2-benzoyl-1,3-dithiane-1-oxide,[22] *trans*-2-methyl-1,3-dithiolane-1-oxide,[15] *trans* and *cis*-2-*p*-cyanophenyl-1,3-dithiolane-1-oxide,[12] *trans* and *cis*-2-*p*-cyanophenyl-1,3-oxathiolane-1-oxide,[13] *trans* and *cis*-2-methyl-1,3-benzodithiole-1-oxide[14] and 1,3-benzodithiole-1-oxide[21] were already known in the optically pure form and the physical properties of our specimens were in agreement with those reported in literature. *Trans*-2-methoxymethyl-1,3-dithiolane-1-oxide is unknown in literature in the optical active form. Our optically active sample had the same physical properties as the racemic sample provided by Prof. V. Aggarwal. Optically active 1,3-oxathiane-3-oxide is not reported in literature but the physical properties of our optically active specimen were in agreement with those of the racemic form.[9] Optically active and racemic 1,5-dithiaspiro [5,5] undecane-1-oxide are unknown in literature. Our racemic sample had p.f. 72-73°C; Mass Spec. (FAB+): $M+H^+$: Calc. 205, Found 205.

Enzymatic oxidation: typical procedure. 1.5 Mmoles of glucose-6-phosphate (G6P), 0.02 mmoles of NADP, 54 U of glucose-6-phosphate dehydrogenase (G6PDH) (G6P and G6PDH dehydrogenase served to regenerate NADPH) and 15-30 U of CMO were dissolved in 14 ml of Tris-HCl buffer solution at pH 8.6. The dithioacetal (0.25 mmoles) was added and the reaction mixture was stirred for 16 hours. The product was extracted using 15 ml of ethylacetate or dichloromethane. The organic phase was separated by centrifugation and analyzed by chiral GLC or HPLC. The degree of conversion of 1,3-dithioacetals into the corresponding

sulfoxides and sulfones, the diastereoselection and the e.e. of the sulfoxides were determined on ethylacetate extracts by GLC with a CP-cyclodextrin-β-2,3,6-M19 capillary column (50 m, 0.25 mm, Chromopack) with H_2 as carrier gas for substrates **2, 3, 4, 7,** and 1,3-dithiolane. The reaction products of the substrates **1, 5, 6, 8, 9, 10, 11**, 1,3-dithiane, bis-(methylthio)-methane, 1,3-benzodithiole and 1,3-oxathiane were analyzed by HPLC on a chiralcel OD column (Daicel) using the proper mixture of *n*-hexane/propan-2-ol as the mobile phase. All sulfoxide enantiomers were base-line separated except *trans*-2-*p*-cyano phenyl-1,3-dithiolane-1-oxide.

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