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Enantioconvergent Transformation of Racemic *cis*-β-Alkyl Substituted Styrene Oxides to (R,R) Threo Diols by Microsomal Epoxide Hydrolase Catalysed Hydrolysis

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Abstract: Both enantiomers of *cis*-β-ethyl, β-*n*-propyl, β-*n*-butyl, and β-*n*-hexyl substituted styrene oxides undergo microsomal epoxide hydrolase catalysed hydration at the (S) carbon to give the corresponding (R,R) *threo* diols in a > 90% e.e. A complete kinetic resolution of the racemic epoxide is also obtained with the β-ethyl substituted substrate, but not with its higher homologues.

INTRODUCTION

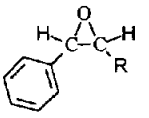
The microsomal epoxide hydrolase (mEH) is an important enzyme[1] involved in the detoxification of xenobiotic compounds, which promotes, through the formation of a diol monoester intermediate,[2] the addition of water to a broad variety of epoxides resulting from the Cytochrome P-450 catalysed biooxidation of compounds containing olefinic or aromatic functionalities.[3] Stereochemical investigations have shown that the enzyme often exhibits a remarkable substrate enantioselectivity towards racemic epoxides,[4] or product enantioselectivity with meso epoxides.[5] In the latter case the ring opening generally occurs at an (S) oxirane carbon to give (R,R) diols with high enantiomeric excesses.[5] Furthermore, the reaction is often endowed with a very high regioselectivity, the nucleophilic attack occurring at the less substituted or less hindered oxirane carbon.[6,7] The knowledge of the regio- and stereochemical course of these hydrolyses can be useful on the one hand to understand the biological effects associated to the production of xenobiotic metabolites whose biological activity is stereochemistry dependent, and, on the other, to try to obtain important chiral synthons as epoxides and 1,2-diols.

In connection with the second point, we are now reporting on the unprecedented enantioconvergent hydrolysis of racemic *cis*-β-alkyl substituted styrene oxides **1b-e** to give the (R,R) *threo*-diols **2b-e** with >90% enantiomeric excesses in a 100% yield. The results are compared with those previously reported[7] for the mEH catalysed hydrolysis of *cis*-β-methylstyrene oxide.

RESULTS AND DISCUSSION

The effect of the alkyl chain length on the ability of the rabbit liver mEH to catalyse the hydrolysis of the differently substituted *cis*-β-alkyl styrene oxides **1b-e** was preliminarily checked by measuring the saturation velocities. The epoxides were incubated with microsomal preparations at 37 °C and pH 7.4 at several substrate and protein concentrations. The reactions were stopped by extraction of the products and the extracts were analysed by HPLC. At variance with the $HClO_4$ catalysed oxirane ring opening, which led to *ca.* 8:2

mixtures of threo and erythro diols, only the threo diols **2b-e** were formed in the mEH catalysed hydrolyses of **1b-e**, showing that, as expected,[7] the oxirane ring was always opened in an anti stereospecific way.



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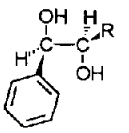

threo-**2**

a: R = CH_3, **b**: R = CH_2CH_3, **c**: R = $CH_2CH_2CH_3$ **d**: R = $CH_2(CH_2)_2CH_3$, **e**: R = $CH_2(CH_2)_4CH_3$

In the employed epoxides concentration range the diol formation was linear with the time and with the microsomal protein amount and independent of the substrate concentration, indicating enzyme saturation. The saturation rates, expressed in nmol/ (mg protein x min), were: **1b**, 2.6; **1c**, 2.2; **1d**, 3.5; **1e**, 5.3. These values were very close to those previously found for the methyl derivative (1R,2S)-**1a** (2 nmol/ (mg protein x min)) and (1S,2R)-**1a** [5 nmol/ (mg protein x min)],[7] but lower than that of styrene oxide, a typical good mEH substrate.

The substrate and the product enantioselectivities were determined by incubating epoxides **1b-e** under saturation conditions. The reactions were stopped at different times and the residual epoxides and the formed diols were respectively analysed by GLC using a chiral column and by HPLC after transformation into the bis(MTPA) esters. The results are reported in Table 1, which also includes, for comparison porposes, the previously reported data relative to the mEH catalysed hydrolysis of **1a**.

Table 1. Enantiomeric excesses and absolute configurations of epoxides and diols obtained by mEH catalysed hydrolysis of racemic substrates **1a-e**.

Substrate	Hydrolysis	Unreacted **1**		Formed **2**	
	%	e.e.[a]	Abs. Conf.	e.e.[a]	Abs. Conf.
1a[b]	25	40	(1S,2R)	>98	(1R,2R)
	50	>98	(1S,2R)	>98	(1R,2R)
	75	>98	(1S,2R)	38	(1R,2R)
	100	-		0	
1b	25	33	(1S,2R)	>98	(1R,2R)
	50	>98	(1S,2R)	>98	(1R,2R)
	100	-		>90[c]	(1R,2R)
1c	35	20	(1S,2R)	>98	(1R,2R)
	50	35	(1S,2R)	>98	(1R,2R)
	100	-		>90[c]	(1R,2R)
1d	50	5		>98	(1R,2R)
	100	-		>90[c]	(1R,2R)
1e	50	10		>98	(1R,2R)
	100	-		>90[c]	(1R,2R)

[a] Average error of three determinations: ± 2%. At the same percent hydrolysis the e.e.s obtained with different rabbit liver microsomal preparations were reproducible to ± 2%.
[b] From ref. 7.
[c] The e.e. is lowered by some spontaneous hydrolysis due to the long incubation time.

The absolute stereochemistry of diols **2b-e** formed by complete hydrolysis of the racemic substrates was established by specific rotation and CD measurements. The relationship between specific rotation and absolute configuration was known for diol **2b**,[8] and this allowed us to establish the (R,R) configuration for laevorotatory diol arising from the enzymatic hydrolysis of **1b**. No information was instead available concerning the absolute configurations of diols (-)-**2c-e**. The same (R,R) configuration could, however, be attributed by correlation of the CD spectra of these (-)-diols, which present a positive fingerprinted Cotton effect in the 250-270 nm region, with maximum at 260 nm, with that of (+)-(S,S)-*threo*-1-phenylpropane-1,2-diol, showing a similar but negative Cotton effect in this interval. The absolute configuration of the residue epoxides **1b** and **1c** were instead deduced by the emergence order from the chiral GLC column, the (1S,2R) enantiomers being eluted before the (1R,2S) ones.[7]

The data of Table 1 show that a complete substrate enantioselection occurs in the mEH catalysed hydrolysis of epoxides **1a** and **1b**, leading to a >98% e.e. of (1S,2R)-**1a-b** and (1R,2R)-**2a-b** at 50% conversion. However, while in the case of (±)-**1a** the diol obtained at complete conversion was racemic, in agreement with a regiospecific attack at the less hindered non-benzylic methyl substituted oxirane carbon of both enantiomers,[7] a nearly enantiomerically pure (1R,2R) diol was formed from (±)-**1b**. This shows that for **1b** the oxirane ring opening occurs essentially at the (S) carbon of both enantiomers. Longer alkyl chains determined a drastic decrease in the substrate enantioselectivity, which was rather low for the propyl derivative (±)-**1c** and practically absent for the *n*-butyl and *n*-hexyl derivatives (±)-**1d** and (±)-**1e**. From all these substrates, however, the corresponding (R,R) diols with >90% e.e. were always stereoconvergently formed, showing that also for these long chain epoxides opening occurs at the (S) configurated carbons of both enantiomers.

The different regio- and enantioselectivity observed within this class of compounds can be rationalised on the basis of the previously proposed topology of the enzyme active site, for which two hydrophobic pockets of different shape and size, situated at the right and left back side of the epoxide binding site, have been postulated.[7] In the case of **1a** and **1b** the phenyl group, but not the small alkyl group, can give rise to a strong binding into the lipophilic pocket on the right side, determining the formation of a more stable enzyme-substrate complex for the epoxide having an (R) configuration at the benzylic carbon. This causes a competitive inhibition of the (1R,2S) enantiomer on the hydrolysis of its antipode, which is responsible for the observed kinetics resolution. On the other hand, in **1d** and in **1e** both the phenyl group of one enantiomer and the long alkyl chain of the other can form a binding to the right side pocket, the other substituent being accommodated into the left side one, giving equally stable complexes. No competitive inhibition is therefore possible for these substrates, and no relevant kinetic resolution is observed. Furthermore, while the enzyme can discriminate between the steric effects due to a phenyl and a small methyl group, this ability is lost with longer alkyl groups, so that only the preference for the nucleophilic attack at the (S) oxirane carbon, exhibited in the hydrolysis of meso epoxides, determines the regio- and stereochemistry of the ring opening of both enantiomers of epoxides **1b-e**.

CONCLUSIONS

The results of this investigation show that cis-β-alkyl substituted styrene oxides are hydrolysed by microsomal epoxide hydrolase to diols in an anti stereospecific way and, with the exception of the methyl derivative, in a stereoconvergent way to give >90% enantiomerically pure *threo*-(1R,2R) diols at complete

hydrolysis. Furthermore, depending on the size of the alkyl chain the reaction can occur with an high substrate enantioselection to give at half hydrolysis an enantiomerically pure unreacted epoxide and an enantiomerically pure diol. This once again indicates that the microsomal epoxide hydrolase can be useful for small scale kinetic resolution of epoxides and for the preparation of chiral vicinal diols, and that it may became of much greater utility when the enzyme produced by biotechnologies will become available in large amounts.

EXPERIMENTAL SECTION

Optical rotations were measured with a Perkin-Elmer 241 polarimeter. The ^1H and ^{13}C NMR spectra were registered in $CDCl_3$ with a Bruker AC 200 instrument using TMS as the internal reference. CD spectra were registered with a JASCO J-40AS spectropolarimeter. HPLC analyses for the quantification of diols were carried out with a Waters 600E apparatus equipped with a diode array detector, using a Nitrile S5 column, with hexane/2-propanol (95:5) as the eluent at a flow rate of 1 ml/min, monitoring at 220 nm. The e.e.s of the diols were determined, after conversion to the diastereoisomeric bis(MTPA) esters, by HPLC analysis using a Nitrile S5 column with hexane/2-propanol (99.5:0.5) as the eluent at a flow rate of 1 ml/min. The yields of recovered epoxides and their e.e.s were obtained by GlC analysis using a Carlo Erba HRGC 5300 instrument equipped with a 10 m Chiraldex G-TA (ASTEC) column, evaporator and detector 245 °C, helium flow 1 ml/min, at the following temperatures: **1b** 100 °C; **1c** 100 °C; **1d** 109 °C; **1e** 120 °C.

Materials

cis-1-Phenyl-1-butene, *cis*-1-phenyl-1-pentene, and *cis*-1-phenyl-1-hexene were obtained from the corresponding commercial alkynes by reduction with catechol borane according to the reported procedure.[9] *cis*-1-Phenyl-1-octene was similarly prepared from 1-phenyl-1-octyne, obtained from lithium phenylacetylide (1 M in THF) and hexyl bromide following the reported procedure.[10] *cis*-1-Phenyl-1-butene: ^1H NMR δ ppm: 1.0 (t, J = 7.2 Hz, 3H, CH_3), 2.5 (m, 2H, CH_2), 5.6 (d of t, J = 12 and 7.2 Hz, 1H, =CH), 6.35 (d, 12 Hz, 1H, =CH), 7.1-7.3 (m, 5H, aromatic protons). *cis*-1-Phenyl-1-pentene: ^1H NMR δ ppm: 0.95 (t, J = 7.5 Hz, 3H, CH_3), 1.45 (m, 2H, CH_2), 2.30 (m, 2H, CH_2), 5.7 (d of t, J = 11.5 and 7.2 Hz, 1H, =CH), 6.35 (d, 11.5 Hz, 1H, =CH), 7.1-7.3 (m, 5H, aromatic protons). *cis*-1-Phenyl-1-hexene: ^1H NMR δ ppm: 0.95 (t, J = 7.5 Hz, 3H, CH_3), 1.5 (m, 4H, $(CH_2)_2$), 2.4 (m, 2H, CH_2), 5.6 (d of t, J = 11.5 and 7.2 Hz, 1H, =CH), 6.35 (d, 11.5 Hz, 1H, =CH), 7.1-7.3 (m, 5H, aromatic protons). *cis*-1-Phenyl-1-octene: ^1H NMR δ ppm: 0.87 (t, J = 7.5 Hz, 3H, CH_3), 1.5 (m, 8H, $(CH_2)_4$), 2.4 (m, 2H, CH_2), 5.6 (d of t, J = 11.5 and 7.2 Hz, 1H, =CH), 6.35 (d, 11.5 Hz, 1H, =CH), 7.1-7.3 (m, 5H, aromatic protons).

cis-1-Phenyl-1-butene oxide, **1b**, *cis*-1-phenyl-1-pentene oxide, **1c**, *cis*-1-phenyl-1-hexene oxide, **1d**, and *cis*-1-phenyl-1-octene oxide, **1e**, were synthesized by epoxidation of the corresponding olefins with *m*-chloroperoxybenzoic acid and KF[11] in dichloromethane for 24h, followed by Kugelrohr short-path distillation. **1b**: oil, ^1H NMR δ ppm: 0.9 (t, J = 7.2 Hz, 3H, CH_3), 1.25 (m, 1H, CH_2), 1.43 (m, 1H, CH_2), 3.15 (d of t, J = 4.2 and 7.2 Hz, 1H, CH), 4.1 (d, J = 4.2 Hz, 1H, CH), 7.2-7.3 (m, 5H, aromatic protons). **1c**, oil: ^1H NMR δ ppm: 0.77 (t, J = 7.0 Hz, 3H, CH_3), 1.35 (m, 4H, $(CH_2)_2$), 3.15 (m, 1H, CH), 4.00 (d, J = 4.2 Hz, 1H, CH), 7.1-7.3 (m, 5H, aromatic protons). **1d**: oil, ^1H NMR δ ppm: 0.8 (t, J = 7.3 Hz, 3H, CH_3), 1.30 (m, 6H, $(CH_2)_3$), 3.20 (m, 1H, CH), 4.05 (d, J = 4.2 Hz, 1H, CH), 7.2-7.3 (m, 5H, aromatic protons). **1e**: ^1H NMR δ ppm: 0.82 (t, J = 7.0 Hz, 3H, CH_3), 1.35 (m, 10H, $(CH_2)_5$), 3.15 (m, 1H, CH), 4.05 (d, J = 4.2 Hz, 1H, CH), 7.1-7.3 (m, 5H, aromatic protons).

threo-1-Phenyl-1,2-butanediol, **2b**, *threo*-1-phenyl-1,2-pentanediol, **2c**, *threo*-1-phenyl-1,2-hexanediol, **2d**, and *threo*-1-phenyl-1,2-octanediol, **2e**, were obtained in mixtures with the corresponding erythro diols in ratios of *ca.* 8:2 by $HClO_4$ (0.05 M) promoted hydrolysis of (±)-**1b-e** in 60:40 THF/H_2O for 12 h. The relative stereochemistry of the formed diols was established on the basis of the lower value of vicinal coupling constant of the erythro isomers with respect the threo ones in $CDCl_3$.[12] **2b**: oil, ^1H NMR δ ppm: 0.95 (t, J = 7.5 Hz, 3H, CH_3), 1.35 (m, 2H, CH_2), 3.6 (m, 1H, C<u>H</u>OH), 4.45 (d, J = 7.0 Hz, 1H, C<u>H</u>OH), 7.2-7.3 (m, 5H, aromatic protons). **2c**, oil: ^1H NMR δ ppm: 0.83 (t, J = 7.0 Hz, 3H, CH_3), 1.5 (m, 4H, $(CH_2)_2$), 3.65 (m, 1H, C<u>H</u>OH), 4.40 (d, J = 7.0 Hz, 1H, C<u>H</u>OH), 7.1-7.3 (m, 5H, aromatic protons). **2d**: oil, ^1H NMR δ ppm: 0.83 (t, J = 7.3 Hz, 3H, CH_3), 1.35 (m, 6H, $(CH_2)_3$), 3.60 (m, 1H, C<u>H</u>OH), 4.35 (d, 1H, J = 6.9 Hz, C<u>H</u>OH), 7.2-7.3 (m, 5H, aromatic protons). **2e**, oil: ^1H NMR δ ppm: 0.84 (t, J = 7.0 Hz, 3H, CH_3), 1.25 (m, 10H, $(CH_2)_5$), 3.65 (m, 1H, C<u>H</u>OH), 4.40 (d, J = 6.9 Hz, 1H, C<u>H</u>OH), 7.1-7.3 (m, 5H, aromatic protons).

Rates of mEH and cEH catalysed hydrolysis of epoxides 1b-e. Aliquots (20 μl) of ethanolic stock solutions of (±)-**1b-e** were added to 2 ml of diluted microsomal preparation[7] containing 2 or 4 mg of protein/ml, in a such way as to obtain a 7.5 or 30 mM final substrate concentration, and the mixtures were incubated with shaking at 37 °C. After 15 and 30 min the reactions were stopped by extraction with ethyl acetate (2ml) and centrifuged. The organic phases were diluted to an exactly known volume and the formed diols were quantified by HPLC analysis using calibration curves obtained for each diol. All experiments were carried out in duplicate. Only *threo* diols **2a-e** were always detected. The diol formation was linear with the time and the protein concentration, and was independent of the substrate concentration, indicating enzyme saturation. The average saturation rates [in nmol/(mg protein x min)] obtained for the various substrates were: **1b**, 2.6; **1c**; 2.2; **1d**, 3.5; **1e**, 5.3.

Enantiomeric excesses and absolute configurations..

Epoxides **1b-e** were added as neat liquids (*ca.* 30 mg) to 20 ml of the microsomal preparation containing 10 mg of protein/ml. At prefixed times the reactions were stopped by extraction with ethyl acetate and the extracts were diluted to an exactly known volume. *o*-Methylbenzaldehyde was added as an internal standard to aliquots of the extracts, which were subjected to GLC analysis using the chiral column for the quantification of the unreacted epoxides and the determination of their e.e.s. At least three determinations were made at each time. In all cases the conversions were > 95% within 20 h. The absolute configurations of the two enantiomers of the unreacted epoxides **1b** and **1c** were attributed by comparison of their emergence order with that of (-)-(1R,2S)-**1a** and (+)-(1S,2R)-**1a**.

The remaining ethyl acetate extracts were evaporated. The residues were treated with (+)-(S)-α-methoxy-α–trifluoromethylphenylacetylchloride in pyridine for two days,[5b] and subjected to HPLC analysis in order to determine the e.e.s of the formed diols. The pure diols obtained at complete conversion were subjected to optical rotation in chloroform and to CD measurements in acetonitrile. All diols **2b-e** were laevorotatory [**1b** $[\alpha]_D$= - 28.5, (c = 0.01); **1c** $[\alpha]_D$= - 23.5, (c = 0.01); **1d** $[\alpha]_D$= - 21.5, (c = 0.01); **1e** $[\alpha]_D$= - 14.7, (c = 0.01)][13] and exhibited a fingerprinted positive Cotton effect between 250 and 270 nm, with a maximum at 260 nm. Diol (+)-(1S,2S)-**2a** showed a similar CD curve but with a negative Cotton effect in the same wavelength interval ($\Delta\varepsilon_{260}$ = -1.89 $M^{-1}cm^{-1}$). The results are reported in Table 1.

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