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Enantioconvergent Transformation of Racemic *cis*-Dialkyl Substituted Epoxides to (R,R) Threo Diols by Microsomal Epoxide Hydrolase Catalysed Hydrolysis‡

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Abstract: Both enantiomers of (±)-9,10-epoxystearic acid (**1b**), *cis*-(±)-5,6-epoxyhexadecane (**1c**) and *cis*-(±)-11,12-epoxyhexadecan-1-ol (**1d**) as well as the meso *cis*-9,10-epoxyoctadecane (**1a**) undergo microsomal epoxide hydrolase catalyzed hydration at the (S) carbon to give the corresponding (R,R) *threo* diols in a >90 e.e. Copyright © 1996 Elsevier Science Ltd

Enantiopure epoxides and vicinal diols are important intermediates in the synthesis of chiral compounds owing to their ability to react with a broad variety of nucleophiles and to behave as chiral synthons. Different synthetic methods have been reported in the last ten years to prepare these classes of compounds.[1] Although high enantiomeric purities have often been obtained, more recently biocatalytic methods have been investigated and developed[2] for their preparation.

Epoxide hydrolases (EH) are important enzymes, present in microbial,[3] fungine,[4] plant, [5] insect,[6] and mammalian systems,[7] both in the microsomal and soluble forms. They are primarily involved in the detoxification of xenobiotic compounds, which promote the trans addition of water to a large variety of epoxides.[8] Stereochemical investigations, carried out extensively with mammalian EH, have demonstrated that these enzymes often exhibit a remarkable substrate enantioselectivity towards racemic epoxides,[9] or product enantioselectivity with meso epoxides.[10] In the latter case the oxirane ring opening generally occurs at an (S) oxirane carbon to give (R,R) diols with high enantiomeric excesses.[10] Furthermore, the reaction is often endowed with a very high regioselectivity, the nucleophilic attack occurring at the less substituted or less hindered oxirane carbon.[11] Finally, it has been recently demonstrated[12] that microsomal epoxide hydrolase (mEH) catalyses the enantioconvergent transformation of racemic *cis*-β-alkyl substituted styrene oxides to give, at complete conversion, the (R,R) *threo*-diols with >90% enantiomeric excesses. It is noteworthy that a similar behavior has been observed in the opening of (±)-disparlure (*cis*-(±)-7,8-epoxy-2-methyloctadecane) by EH in Gypsy moth antennae,[6] and of *cis*-(±)-9,10 epoxystearic acid by Soybean fatty acid EH.[5]

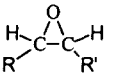
The knowledge of the regio- and stereochemical course of the mEH catalysed hydrolysis can therefore be useful not only to understand the biological effects associated with the metabolism of certain xenobiotics, but also for the small scale kinetic resolution of epoxides and preparation of chiral vicinal diols.

As a further contribution to this field, in this communication we are reporting the data related to the mEH catalyzed hydrolysis of racemic long chain dialkyl substituted cis epoxides, and in particular of *cis*-9,10-

‡Dedicated to the memory of Professor Giuseppe Bellucci (d. March 3, 1996).

epoxyoctadecane (**1a**), *cis*-(±)-9,10-epoxystearic acid (**1b**), *cis*-(±)-5,6-epoxyhexadecane (**1c**), *cis*-(±)-11,12-epoxyhexadecan-1-ol (**1d**),[13] which occurs through a selective attack at the (S) oxirane carbon of both enantiomers to give, at complete hydrolysis, (R,R) threo diols with a > 90% e.e..

The ability of a rabbit liver microsomal preparation containing EH to catalyse the hydrolysis of the differently substituted epoxides **1a-c** was preliminary checked by measuring the saturation velocities. The epoxides (5-30 mM) were incubated with a microsomal preparation,[9] diluted to a protein concentration of 3-6 mg of protein at 37 °C and pH 7.4. The reactions were stopped by extraction of the products and the extracts were analysed by GLC.



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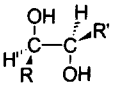


threo-**2**

a: R = R' = $CH_3(CH_2)_7$, **b**: R = $CH_3(CH_2)_7$; R' = $(CH_2)_7COOH$, **c**: R = $CH_3(CH_2)_3$; R' = $(CH_2)_9CH_3$ **d**: R = $CH_3(CH_2)_3$; R' = $(CH_2)_{10}OH$

The diol formation was linear with time and with the microsomal protein amount, and was independent of the substrate concentration, indicating substrate saturation. The saturation rates, expressed in nmol/ (mg protein x min), were: **1a**, 0.6; **1b**; 0.8; **1c**, 0.6; **1d**, 1.

The product enantioselectivities were determined by incubating epoxides **1a-d** under saturation conditions (100-150 mg of epoxide in 50 ml of microsomal preparation containing 10 mg of protein/ml). In order to achieve a complete conversion of epoxides **1a-d** into the corresponding diols **2a-d**[14] the incubations were protracted for 20 h by adding a fresh microsomal preparation after 8h. The formed diols, extracted with ethyl acetate and quantified by GLC, were analyzed by HPLC (Nitrile S5, hexane/2-propanol, 99.8:0.2, flow 1 ml/min) after transformation into the bis-(MTPA) esters.[15] In the case of **1b** the formed diol was previously transformed to the corresponding methyl ester by treatment with an ethereal solution of diazomethane. The results are reported in Table 1.

Table 1. Enantiomeric excesses and absolute configurations of diols obtained by complete mEH catalysed hydrolysis of epoxides **1a-d**.

Substrate	**Hydrolysis**	**Formed 2**	
	%	**e.e.**[a]	**Abs. Conf.**
1a	90	90	(9R,10R)[b]
1b	90	>93	(9R,10R)[c]
1c	90	>93	(5R,6R)[d]
1d	100	>93	(11R,12R)[d]

[a] Averages of three determinations: ± 2%. [b]Based on literature reports (ref. 17a). [c]After transformation in methyl ester. Based on literature reports (ref. 17b). [d]Assumed by comparison of specific rotation with **2a** and **2b**.

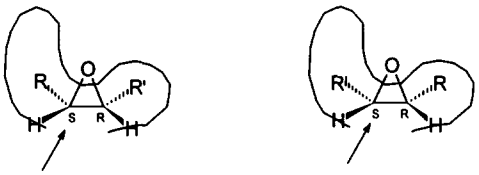
The (R,R) configuration for dextrorotatory diols **2a** and **2b**, arising from the enzymatic hydrolysis of **1a** and **1b**,[16] was established by comparison of the optical rotation values with literature data.[17] No information was instead available on the absolute configurations of diols (+)-**2c** and (+)-**2d**. The same (R,R) configuration

was, however, tentatively assumed by comparison of $[\alpha]_D$ with those of the homologous compounds **2a** and **2b**.

The data of Table 1 show that the mEH catalyzed hydrolysis of the long chain alkyl disubstituted meso compound **1a** proceeds with a high product enantioselectivity, the water attack occuring stereoselectively at the (S) oxirane carbon, in agreement with the generally observed enzymatic hydrolysis of meso epoxides.[5] Furthermore, in analogy to the mEH catalyzed hydrolysis of cis-β-alkyl substituted styrene oxides,[12] and consistent with the previously reported results of disparlure hydration by the *L. dispar* EH and *cis*-(±)-9,10-epoxystearic acid by Soybean EH, the oxirane ring opening of epoxides **1b-d** occurs at the (S) configurated carbons of both enantiomers to give stereoconvergently the corresponding (R,R) diols with > 90% e.e.

The product enantioselectivity observed within this class of compounds can be therefore rationalized on the basis of the previously proposed topology of the 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, able to accommodate the two lipophilic syn substituents have been postulated.[18] Indeed, in agreement with this model, both enantiomers of each epoxide can fit into the active site with the same orientation of the oxirane ring, with the two alkyl groups occupying opposite positions (Figure 1). Furthermore, whilst in most cases

Figure 1



related to racemic epoxides, the observed regioselectivity can be explained by a preferential nucleophilic attack on the least substituted, or sterically hindered carbon of the oxirane ring, according to the general base-catalyzed mechanism,[8] in this case no steric or electronic effect is able to differentiate the two epoxide reaction centres. Therefore, only the preference for the nucleophilic attack at the (S) oxirane carbon, exhibited in the hydrolysis of meso compounds,[4] determines the regio- and stereochemistry of the ring opening. A similar model of the active site and an analogous explaination for the observed stereoselectivity has been recently reported even for the biohydrolysis of substituted styrene oxide derivatives by *Beauveria sulfurescens*.[4]

In conclusion the results of this investigation show that also cis long chain alkyl disubstituted epoxides are hydrolyzed by mEH in an anti stereospecific and stereoconvergent way to give practically optically pure *threo*-(1R,2R) diols at 100% hydrolysis. These findings, once again indicate that microsomal epoxide hydrolase can be useful not only for small scale kinetic resolutions of epoxides, but also for the preparation of chiral vicinal diols in larger amounts, in particular considering that mEH has been already cloned and expressed in Saccharomyces Cerevisiae.[19]

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13. Compounds **1a-d** were synthesized by epoxidation of the corresponding olefins with *m*-chloroperbenzoic acid in dichloromethane for 24h, followed by purification on column chromatography. **1a**: ^{1}H NMR δ ppm: 0.9 (t, 6H, CH_3), 1.27 (m, 24H, CH_2), 1.47 (m, 4H, CH_2), 2.90 (m, 2H, CH). **1c**: ^{1}H NMR δ ppm: 0.9 (t, 3H, J = 6.8 Hz, CH_3), 0.93 (t, 3H, J = 6.8 Hz, CH_3), 1.27 (m, 20H, CH_2), 1.5 (m, 4H, CH_2), 2.9 (m, 2H, CH). **1d**: ^{1}H NMR δ ppm: 0.90 (t, 3H, J = 6.7 Hz, CH_3), 1.27 (m, 20H, CH_2), 1.48 (m, 4H, CH_2), 2.90 (m, 2H, CH), 3.60 (t, 2H, 6.5 Hz, $C\underline{H}_2OH$).
14. *threo*-Diols **2a-d**, were obtained by $HClO_4$ (0.05 M) promoted hydrolysis of **1a-d** in 60:40 THF/H_2O for 12 h. **1a**: ^{1}H NMR δ ppm: 0.9 (t, 6H, J = 6.7 Hz, CH_3), 1.27 (m, 24H, CH_2), 1.50 (m, 4H, CH_2), 3.40 (m, 2H, CH). **1c**: ^{1}H NMR δ ppm: 0.9 (m, 6H, CH_3), 1.27 (m, 20H, CH_2), 1.5 (m, 4H, CH_2), 3.4 (m, 2H, CH). **1d**: ^{1}H NMR δ ppm: 0.90 (t, 3H, J = 7.3 Hz, CH_3), 1.30 (m, 20H, CH_2), 1.5 (m, 4H, CH_2), 3.4 (m, 2H, CH), 3.64 (t, 2H, 6.5 Hz, $C\underline{H}_2OH$).
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16. A lower product enantioselectivity as been reported (Watabe, T.; Akamatsu, K. *Biochim. Biophys. Acta* **1972**, *279*, 297) for the mEH catalyzed hydrolysis of **1b** determined on the basis of the optical rotation of the methyl ester of the product diol. However, optical rotation values for vicinal diols should be regarded with caution because they are very sensitive to traces of moisture.
17. a) **2a**, $[\alpha]_D$ = 21.5 (methanol). Reported value (Mc Ghie, J. F.; Ross, W. A.; Spence, J. W.; James, F. J. *Chem. Ind.* **1972**, 536) for (R,R)-**2a** 25.42. b) Methyl ester of **2b**, $[\alpha]_D$ = 21.0 (methanol). Reported value (McGhie, J. F.; Ross, N. A.; Polton D. J. *Chem. Ind.* **1956**, 353) for the methyl ester of (R,R)-**2b**, 22.52.
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