Pergamon

Tetrahedron: *Asymmetry* 9 (1998) 341–350

TETRAHEDRON: *ASYMMETRY*

Deracemization of (±)-*cis*-dialkyl substituted oxides via enantioconvergent hydrolysis catalysed by microsomal epoxide hydrolase

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Received 24 November 1997; accepted 11 December 1997

Abstract

Both enantiomers of *cis*-(±)-2,3-epoxyheptane **1a**, *cis*-3,4-epoxyheptane **1b**, *cis*-3,4-epoxynonane **1c**, *cis*-3,4-epoxynonane-1-ol **1d**, and *cis*-1-methoxy-3,4-epoxynonane **1e** undergo a highly stereoselective microsomal epoxide hydrolase catalysed hydration at the (S) carbon to give the corresponding *threo* (R,R)-diol at complete conversion. A total kinetic resolution of racemic epoxides is also obtained with **1a** and **1e**.

1. Introduction

Epoxide hydrolases (EH) are important enzymes involved in the metabolism of a broad variety of epoxides,[1] many of which are mutagenic and/or carcinogenic, generally formed in vivo from the Cytochrome P-450 catalysed biooxidation of compounds containing olefinic or aromatic functionalities.[2] Widely studied for their biological implications[1] and for the determination of their intimate mechanism of action,[3] these enzymes have been more recently investigated for their use as asymmetric biocatalysts.[4] Stereochemical investigations, carried out extensively with mammalian microsomal epoxide hydrolase (mEH), have demonstrated that the addition of water promoted by these enzymes, and occuring through the formation of a diol monoester intermediate,[3] often exhibits a remarkable substrate enantioselectivity towards racemic epoxides,[5] or product enantioselectivity with meso epoxides.[6] In the latter case the mEH catalysed oxirane ring opening generally occurs at an (S) oxirane carbon to give (R,R) diols with high enantiomeric excesses.[6] Furthermore, even if the reaction is often endowed with a very high regioselectivity, the nucleophilic attack occuring at the less substituted or less hindered oxirane carbon,[7] it has been recently demonstrated that mEH catalyses the enantioconvergent transformation

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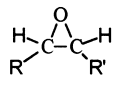
PII: S0957-4166(97)00630-7

of racemic *cis*-β-alkyl substituted styrene oxides[8] and long chain *cis*-dialkyl substituted epoxides[9] to give, at complete conversion, the (R,R) *threo* diols, often with >90% enantiomeric excesses (e.e.). It is noteworthy that a similar behaviour has been observed in the hydrolysis of (±)-disparlure by EH in gypsy moth antennae,[10] of *cis*-(±)-9,10-epoxystearic acid by soybean fatty acid EH,[11] and, more recently, of *cis*-(±)-2,3-epoxyheptane by Nocardia EH1-epoxide hydrolase.[12]

In order to verify the generality of this behaviour, the biotransformation of five *cis*-disubstituted epoxides, and in particular of *cis*-(±)-2,3-epoxyheptane **1a**, *cis*-3,4-epoxyheptane **1b**, *cis*-3,4-epoxynonane **1c**, *cis*-3,4-epoxynonane-1-ol **1d**, and *cis*-1-methoxy-3,4-epoxynonane **1e**, has been investigated using a rabbit liver microsomal preparation as the source of mEH.

2. Results and discussion

The ability of a rabbit liver microsomal preparation containing mEH to catalyse the hydrolysis of the differently substituted epoxides **1a**–**e** was preliminarly checked measuring the saturation velocities. The epoxides (10–30 mM) were incubated with the microsomal preparation,[13] diluted to a protein concentration of 10–20 mg of protein/ml, 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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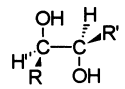


threo-**2**

a: R = CH_3, R' = $(CH_2)_3CH_3$; **b**: R = CH_3CH_2, R' = $(CH_2)_2CH_3$; **c**: R = CH_3CH_2, R' = $(CH_2)_4CH_3$;

d: R = $HOCH_2CH_2$, R' = $(CH_2)_4CH_3$; **e**: R = $CH_3OCH_2CH_2$, R' = $(CH_2)_4CH_3$

The diol formation was linear with time and with the microsomal protein amount, and was independent of the substrate concentration, indicating enzyme saturation. The saturation rates, expressed in nmol/(mg protein×min), were: **1a**, 3.1; **1b**, 0.5; **1c**, 0.3; **1d**, 5.5 and **1e**, 4.4.

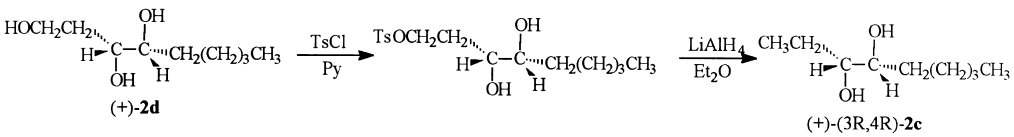
The stereochemical behaviour of the biotransformation of **1a**–**e** was determined by incubating the epoxides under saturation conditions. The reactions were stopped at different times and the residual epoxides and the formed diols were analyzed by GLC using a chiral column and/or by HPLC after transformation of the diols into the corresponding MTPA esters. The results are reported in Table 1.

The absolute stereochemistry of diols **2a**–**e** formed by enzymatic hydrolysis of the racemic substrates was established on the basis of specific rotation and chemical correlation. The relationship between specific rotation and absolute configuration was known for diols **2a** and **2b**,[12,14] and this allowed us to establish the (R,R) configuration for the dextrorotatory diols arising from the mEH catalyzed hydrolysis of **1a** and **1b**. No information was available concerning the absolute configurations of diols (+)-**2c**–**e**. The same (R,R) configuration was, therefore, attributed to diol (+)-**2c** by comparison of its retention time on the chiral GLC column with that of a sample of (3S,4S)-(−)-**2c** independently synthesized from *trans*-3-nonene by Sharpless dihydroxylation using AD-mix-α.[15] It is known that with *trans* disubstituted olefins it is possible, with reasonable certainty, to predict the face enantioselectivity by applying a simple mnemonic rule.[15] The same (R,R) absolute configuration was therefore attributed to diols (+)-**2d** and (+)-**2e** by chemical correlation with that of **2c**. Diol (+)-**2d** was transformed into diol **2c**, through a preliminary esterification of the primary hydroxy group using an equimolar amount of tosyl chloride, followed by reduction with $LiAlH_4$.

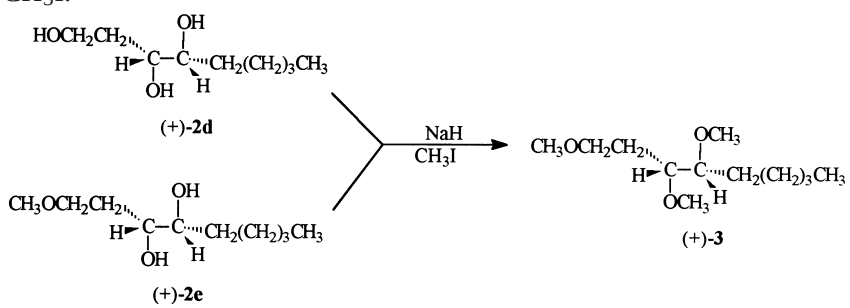
Table 1
Enantiomeric excesses and absolute configurations of epoxides and diols obtained by mEH catalysed hydrolysis of racemic substrates **1a–e**

Substrate	Hydrolysis %	Unreacted 1 e.e.[a]	Unreacted 1 Abs. Conf.	Formed 2 e.e.[a]	Formed 2 Abs. Conf.
1a	50	>98[b]	(2R,3S)	>98[b]	(2R,3R)
	100			50[b]	(2R,3R)
1b	50	14[b]	n.d.	>98[b]	(3R,4R)
	100			>98[b]	(3R,4R)
1c	50	56[b]	(3R,4S)	>98[b]	(3R,4R)
	100			>98[b]	(3R,4R)
1d	50	40[b]	(3R,4S)	90[c]	(3R,4R)
	100	-		90[c]	(3R,4R)
1e	50	>98[b]	(3R,4S)	80[c]	(3R,4R)
	100	-		80[c]	(3R,4R)

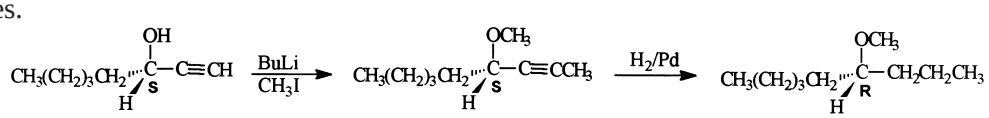
[a]Averages of three determinations: ± 2%. At the same percent of hydrolysis the e.e. values obtained with different rabbit liver microsomal preparations were reproducible ± 2%.[b] Determined by GLC using a chiral column. [c] Determined by HPLC after transfomation into the corresponding MTPA derivatives.



The chemical correlation between diols (+)-**2d** and (+)-**2e** was instead deduced from the comparison of the specific rotations of 1,3,4-trimethoxynonane **3** arising from exhaustive methylation of both diols using NaH and CH_3I.



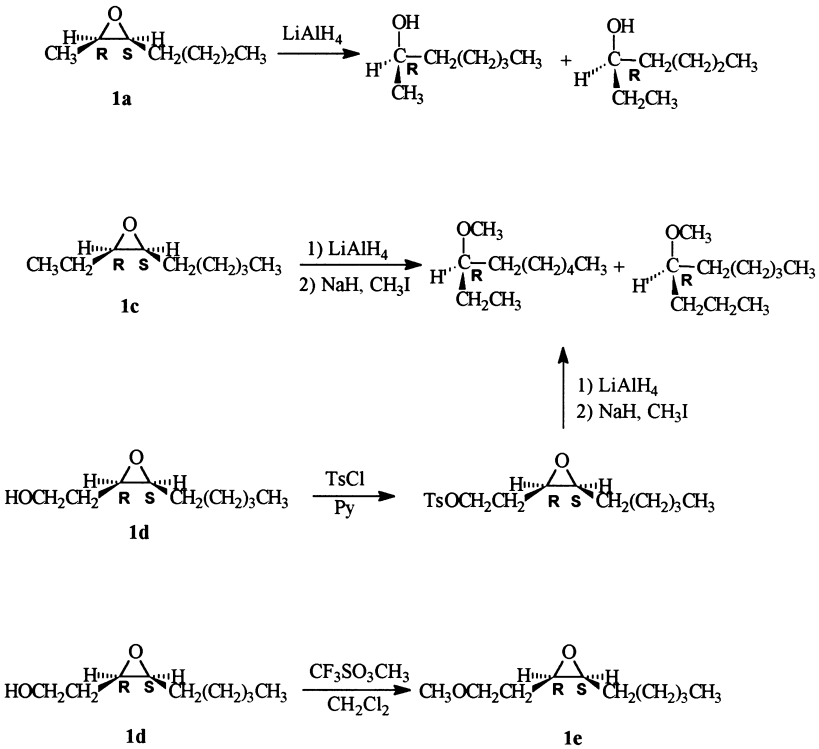
The absolute configuration of the residual enriched epoxides was determined in the cases of **1a** by reduction of the oxirane ring with $LiAlH_4$ and co-injection of the resulting mixture of 2- and 3-heptanol with a sample of commercial (R)-2-heptanol, after transformation into the corresponding trifluoroacetyl derivatives.



Similarly, the absolute configuration of **1c** was established, after reduction of the oxirane ring with $LiAlH_4$ followed by methylation of the OH group, by co-injection of the resulting mixture of 3- and 4-methoxynonane with a sample of (R)-4-methoxynonane. This optically active compound was

prepared from commercial (S)-1-octyn-3-ol by treatment with BuLi and CH_3I followed by the catalytic hydrogenation of the triple bond. Experiments carried out with racemic **1a** or **1c** showed that in both cases the four products, (+)- and (−)-2-heptanol and (+)- and (−)-3-heptanol, or (+)- and (−)-3-methoxynonane and (+)- and (−)-4-methoxynonane, were completely separated on the chiral column.

The same configuration (3R,4S) was then attributed to the unreacted epoxide **1d** by chemical correlation. The enantiomerically enriched epoxide **1d** was transformed into the corresponding *p*-toluenesulfonate and after treatment with $LiAlH_4$ and methylation of the OH group in a mixture of 3- and 4-methoxynonane. Finally, the absolute configuration of the enantiomerically pure **1e** was determined by comparison of its retention time on the chiral GLC column with that of a sample obtained from (3R,4S)-**1d** by methylation of the OH group with $CF_3SO_3CH_3$ in anhydrous methylene chloride.



All of the stereochemical results reported in Table 1 show that, in analogy to the mEH catalyzed hydrolysis of *cis* aryl alkyl and long chain dialkyl substituted alkene oxides,[8,9] and consistent with the recently reported results about the biohydrolysis of racemic epoxides catalyzed by microbial EH[12] (with the exception of **1a**) the oxirane ring opening of all the other substrates occurs stereoselectively at the (S) configured carbons of both enantiomers to give at complete conversion the corresponding (R,R) diols with very high enantiomeric excesses (80–98%). Furthermore, whereas the enzymatic hydrolysis of epoxides **1a** and **1e** proceeds through a complete kinetic resolution giving >98% ee of both (2R,3S)-**1a** and (3R,2S)-**1e** at 50% conversion, a lower substrate enantioselectivity, but still in favour of the (3R,4S) enantiomer, was found with **1c** and **1d**, and minimal selection was observed with **1b**.

Once more, the observed substrate and product enantioselectivity can be therefore rationalized on the basis of the previously proposed topology of the active site, for which two hydrophobic pockets of different shapes and sizes, situated at the right and left rear side of the epoxide binding site, able to accommodate the two lipophilic syn substituents have been postulated.[13] In agreement with this model, both enantiomers of each epoxide can fit into the active site with the same orientation of the oxirane ring

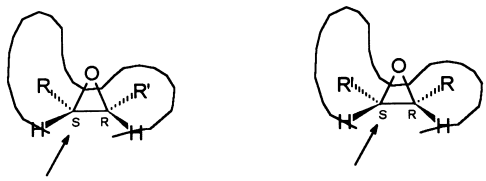


Fig. 1.

and with the two alkyl groups occupying opposite positions (Fig. 1). Furthermore, whereas in the case of mono- 1,1-disubstituted or trisubstituted 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 catalytic mechanism,[3] with *cis*-1,2-disubstituted epoxides in a large number of cases no steric or electronic effects are able to differentiate the two oxirane centres. In these cases, therefore, only the preference for the nucleophilic attack at the (S) configured chiral centre, exhibited in the hydrolysis of meso compounds,[6] determines the regio- and stereochemistry of the ring opening.

The high degree of recognition of the enantiotopic ring carbons therefore determines the product enantioselectivity observed in the hydrolysis of epoxides **1b**–**e**, while the stereochemical behaviour in the hydrolysis of **1a** can be rationalized by taking into account that the mEH catalyzed hydrolysis of the (2S,3R) enantiomer occurs with complete regioselectivity, through the attack at the S oxirane carbon bearing the less hindered substituent (methyl group), whereas that of the (2R,3S) enantiomer is completely non-regioselective. In the oxirane ring opening of this enantiomer there is probably competition between the stereocontrolled attack at the more hindered S configured carbon and the attack at the methyl substituted (R) centre. Since the mEH hydrolysis of *cis*-β-methyl styrene oxide gave, at complete conversion, a racemic diol by a regioselective oxirane ring opening at the methyl substituted carbon,[8] the data obtained in this work for **1a** seems to indicate that, at variance with the phenyl group, the *n*-butyl sustituent is not able to avoid *ca*. 50% of the reaction occurring through nucleophilic attack at the adjacent carbon.

Finally, as far as the substrate enantioselectivity is concerned, the results obtained in this work are consistent with those reported for other racemic epoxides, and point to the formation of a more stable complex of the enzyme with the enantiomer bearing the bulkier lipophilic group on the right side when the oxirane ring is oriented as reported in Figure 1. In agreement with this model, in the cases of **1a** the *n*-butyl group, but not the small methyl substituent, is able to give rise to a stronger binding into the lipophilic pocket on the right side, determining the formation of a more stable enzyme substrate complex for the enantiomer having an (R) configuration at the C(3) carbon. This determines an effect of competitive inhibition by the 2S,3R epoxide on the hydrolysis of its enantiomer, which is responsible for the observed kinetic resolution. On the other hand, in the case of **1b** probably both the ethyl group of one enantiomer and the *n*-propyl substituent of the other can bind to this pocket giving two enzyme–substrate complexes of comparable stability. No competitive inhibition between enantiomers therefore operates in this substrate, and no relevant kinetic resolution is observed. The ability of the enzyme to discriminate between the two enantiomers arises again in the hydrolysis of **1c**, where the sizes of the two substituents are sufficiently different, and in those of **1d** and **1e**. Although in these latter substrates the ethyl group presents a substituent, they probably have the more polar nature, due to the presence of a hydroxy and methoxy group, respectively, which makes the interaction of the *n*-pentyl chain of the (3S,4R) enantiomer with the lipophilic pocket on the right side relatively more important.

In conclusion, the results of this investigation not only give further information about the mechanism of hydrolysis of mEH but also confirm that *cis*-disubstituted oxides are hydrolysed by microsomal epoxide hydrolase to diols in an anti stereospecific way and, with the exception of the methyl derivatives,

in a stereoconvergent way to give practically optically pure *threo*-(R,R) diols at 100% hydrolysis. Furthermore, depending on the relative sizes of the two substituents the reaction can occur with high substrate enantioselection to give at 50% hydrolysis an enantiomerically pure residual epoxide and an enantiomerically pure diol. This once again indicates that microsomal epoxide hydrolase can be useful for small scale kinetic resolutions of epoxides and for the preparation of chiral vicinal diols, and that it may became of much greater preparative utility when biotechnologically produced enzyme becomes available in large amounts.

3. Experimental section

Optical rotations were measured with a Perkin–Elmer 241 polarimeter. The ^{1}H and ^{13}C NMR spectra were registered in $CDCl_3$ with a Bruker AC 200 instrument using TMS as the internal reference. HPLC analyses were carried out with a Waters 600E apparatus equipped with a diode array detector. The e.e.s of the diols **2d** and **2e** were determined after conversion into the diastereoisomeric MTPA esters by HPLC using a Nitrile S5 column with hexane/2-propanol (99.5:0.5) as the eluant at a flow rate of 1 ml/min. The yields of recovered epoxides and formed diols and the e.e.s of epoxides **1a**–**e** and of diols **2a**–**c** were obtained by GLC analysis using a Carlo Erba HRGC 5300 instrument equipped with a 20 m Chiraldex G-TA (ASTEC) column, evaporator and detector 245°C, helium flow 1 ml/min, at the following temperatures: **1a**–**c** 80°C; **1d** 130°C; **1e** 120°C. **2a**, as bis(trifluoroacetyl)derivative 80°C; **2b** 110°C; **2c** 130°C; **2d**, as trifluoroacetyl derivative 130°C.

3.1. Materials

Commercial *cis*-2-heptene (Aldrich, 97%), *cis*-3-heptene (Aldrich, 96%), and *cis*-3-nonen-1-ol (Aldrich, 95%) were used without further purification. *cis*-3-Nonene was prepared from *cis*-3-nonen-1-ol by tosylation of the hydroxy group followed by reduction with lithium aluminium hydride. *cis*-1-Methoxy-3-nonene, was obtained by methylation according to the general alkylation procedure.[16]

cis-2,3-Epoxyheptane **1a**, *cis*-3,4-epoxyheptane **1b**, *cis*-3,4-epoxynonane **1c**, *cis*-3,4-epoxynonane-1-ol **1d**, and *cis*-1-methoxy-3,4-epoxynonane **1e** were synthesized by epoxidation of the corresponding olefins with *m*-chloroperbenzoic acid and KF in dichloromethane for 24 h, followed by distillation. **1a** ^{1}H NMR δ ppm: 0.97 (t, 3H, J=7.30 Hz, CH_3); 1.27 (d, 3H, J=5.60 Hz, CH_3); 1.30–1.60 (m, 6H, CH_2); 2.93 (m, 1H, CH); 3.09 (m, 1H, CH). ^{13}C NMR δ ppm: 13.04 (CH_3); 13.89 (CH_3); 22.49, 27.10, 28.50 ($3CH_2$); 52.60 (CH); 57.12 (CH). **1b**: oil, ^{1}H NMR δ ppm: 0.97 (t, 3H, CH_3); 1.05 (t, 3H, CH_3); 1.40–1.60 (m, 6H, CH_2); 2.90 (m, 2H, CH). ^{13}C NMR δ ppm: 11.22 (CH_3); 14.69 (CH_3); 20.55, 21.70, 30.34 ($3CH_2$); 57.95 (CH); 59.10 (CH). **1c**: ^{1}H NMR δ ppm: 0.82 (t, 3H, J=6.74 Hz, CH_3); 0.96 (t, 3H, J=7.50 Hz, CH_3); 1.30 (m, 4H, CH_2); 1.48 (m, 4H, CH_2); 2.80 (m, 2H, CH). ^{13}C NMR δ ppm: 10.40 (CH_3); 13.80 (CH_3); 20.92, 22.44, 26.10, 27.47, 31.58 ($5CH_2$); 57.30 (CH); 58.30 (CH). **1d**: ^{1}H NMR δ ppm: 0.80 (t, 3H, J=6.20 Hz, CH_3); 1.20–1.50 (m, 6H, CH_2); 1.57 (m, 2H, CH_2); 1.74 (m, 2H, CH_2); 2.83 (m, 1H, CH); 2.98 (m, 1H, CH); 3.69 (t, 2H, J=6.2 Hz, CH_2OH). ^{13}C NMR δ ppm: 14.40 (CH_3); 23.00, 26.58, 28.27, 31.13, 32.10 ($5CH_2$); 55.46 (CH); 57.55 (CH); 60.58 (CH_2). **1e**: ^{1}H NMR δ ppm: 0.84 (t, 3H, J=6.80 Hz, CH_3); 1.30 (m, 4H, CH_2); 1.45 (m, 4H, CH_2); 1.75 (m, 2H, CH_2); 2.95 (m, 2H, CH); 3.30 (s, 3H, OCH_3); 3.47 (m, 2H, CH_2OCH_3). ^{13}C NMR δ ppm: 14.50 (CH_3); 23.12, 26.71, 28.40, 28.98, 32.24 ($5CH_2$); 55.28 (CH); 57.66 (CH); 59.35 (CH_2); 70.62 (OCH_3).

threo-2,3-Heptanediol **2a**, *threo*-3,4-heptanediol **2b**, *threo*-3,4-nonanediol **2c**, *threo*-1,3,4-nonanetriol **2d**, and *threo*-1-methoxy-3,4-nonanediol **2e**, were obtained by $HClO_4$ (0.05 M) promoted hydrolysis of

(±)-**1a–e** in 60:40 THF:H_2O for 24 h. **2a**: oil, ^{1}H NMR δ ppm: 0.90 (t, 3H, J=6.6 Hz, CH_3); 1.17 (d, 3H, J=6.30 Hz, CH_3); 1.20–1.50 (m, 6H, CH_2); 3.3 (m, 1H, CH); 3.55 (m, 1H, CH). ^{13}C NMR δ ppm: 13.85 (CH_3); 19.24 (CH_3); 22.59, 27.62, 32.79 ($3CH_2$); 70.70 (CH); 75.99 (CH). **2b**: oil, ^{1}H NMR δ ppm: 0.97 (t, 3H, CH_3); 0.9 (t, 3H, CH_3); 1.2 (m, 2H, CH_2); 1.40 (m, 4H, CH_2); 3.24 (m, 1H, CH); 3.35 (m, 1H, CH). ^{13}C NMR δ ppm: 9.95 (CH_3); 13.96 (CH_3); 18.77, 26.22, 35.56 ($3CH_2$); 73.74 (CH); 75.80 (CH). **2c**: oil, ^{1}H NMR δ ppm: 0.89 (t, 3H, J=6.4 Hz, CH_3); 0.97 (t, 3H, J=7.50 Hz, CH_3); 1.30 (m, 4H, CH_2); 1.42–1.65 (m, 6H, CH_2); 3.35 (m, 2H, CH). ^{13}C NMR δ ppm: 10.67 (CH_3); 14.63 (CH_3); 23.21, 26.00, 26.9, 32.50, 34.05 ($5CH_2$); 74.70 (CH); 76.45 (CH). **2d**: oil, ^{1}H NMR δ ppm: 0.89 (t, 3H, J=6.20 Hz, CH_3); 1.20–1.50 (m, 8H, CH_2); 1.70 (m, 2H, CH_2); 3.45 (m, 1H, CH); 3.63 (m, 1H, CH); 3.78 (t, 2H, J=5.5 Hz, C*H*$_2$OH). ^{13}C NMR δ ppm: 13.95 (CH_3); 22.55, 25.38, 31.80, 33.10, 35.15 ($5CH_2$); 59.90 (CH_2); 72.96 (CH); 74.55 (CH). **2e**: oil, ^{1}H NMR δ ppm: 0.89 (t, 3H, J=6.25 Hz, CH_3); 1.30 (m, 4H, CH_2); 1.45 (m, 4H, CH_2); 1.78 (m, 2H, CH_2); 3.35 (s, 3H, OCH_3); 3.42 (m, 2H, C*H*$_2$$OCH_3$); 3.60 (m, 2H, CH). ^{13}C NMR δ ppm: 13.96 (CH_3); 22.53, 25.36, 31.80, 33.08, 33.30 ($5CH_2$); 58.69 (CH_2); 70.58 (OCH_3); 73.00 (CH); 74.25 (CH).

(3S,4S)-(−)-3,4-nonanediol was prepared from *trans*-3-nonene, using AD-mix α, according to the standard procedure reported by Sharpless.[15] $[\alpha]_D$=−30 (c=1, MeOH), ^{1}H NMR δ ppm: 0.89 (t, 3H, J=6.4 Hz, CH_3); 0.97 (t, 3H, J=7.50 Hz, CH_3); 1.30 (m, 4H, CH_2); 1.42–1.65 (m, 6H, CH_2); 3.35 (m, 2H, CH). ^{13}C NMR δ ppm: 10.67 (CH_3); 14.63 (CH_3); 23.21, 26.00, 26.9, 32.50, 34.05 ($5CH_2$); 74.70 (CH); 76.45 (CH).

3.1.1. (R)-(+)-4-Methoxynonane

(S)-(−)-octyn-3-ol (530 mg, 4.2 mmol) was dissolved in anhydrous THF (8 ml) and a solution of BuLi (5 ml, 1.6 M in hexane) was added at 0°C. The reaction mixture was stirred for 3 h and then iodomethane (0.7 ml, 8.5 mmol) was added. After a night at room temperature the solution was diluted with dichloromethane, and washed with water. The organic phase was dried ($MgSO_4$) and evaporated to give a residue (630 mg) which was subjected to hydrogenation in ethyl acetate (30 ml) using Pd–C (100 mg) as catalyst. After 24 h at room temperature the reaction mixture was filtered on a Celite bed and the solution was dried ($MgSO_4$) and evaporated to give 870 mg of a crude product identified by NMR as (R)-(+)-4-methoxynonane. $[\alpha]_D$=+2.57 (c=1, $CHCl_3$). ^{1}H NMR δ ppm: 0.85 (t, 3H, CH_3); 0.89 (t, 3H, CH_3); 1.25–1.55 (m, 12H, CH_2); 3.10 (m, 1H, C*H*OCH_3); 3.30 (s, 3H, OCH_3). ^{13}C NMR δ ppm: 14.02 (CH_3); 14.25 (CH_3); 18.50; 22.65, 25.00, 32.10, 33.40, 35.70 ($6CH_2$); 56.30 (OCH_3); 80.78 (OCH_3).

3.2. Enzymatic hydrolysis

*3.2.1. Rates of mEH catalyzed hydrolysis of epoxides **1a–e***

Aliquots (50 μl) of ethanolic stock solutions of (±)-**1a–e** were added to 2 ml of diluted microsomal preparation[13] containing 10 or 20 mg of protein/ml, in a such way as to obtain a 10, 20 or 30 mM final substrate concentration, and the mixtures were incubated with shaking at 37°C. After 10 and 20 min (or 20 and 40 min for **1c**) the reactions were stopped by extraction with ethyl acetate (2 ml) containing a proper amount of benzaldehyde as a standard. After centrifugation, the extracts were analyzed by GLC for the quantification of the unreacted epoxides and the formed diols. The diol formation was linear with time and protein concentration, and was independent of the subtrate concentration, indicating enzyme saturation. Blank experiments, carried out under identical conditions but using boiled microsomal preparations, showed that no spontaneous hydrolysis occurred under the employed conditions. The average saturation rates in nmol/(mg protein×min) obtained for the various substrates with the microsomal preparation were: **1a**, 3.1; **1b**, 0.5; **1c**, 0.3; **1d**, 5.5; **1e**, 4.2.

3.2.2. Determination of enantiomeric excesses

Aliquots (20 μl) of 2 M ethanolic stock solutions of (±)-**1a–e** were added to 2 ml of microsomal preparation containing 20 mg of protein/ml and the mixtures were incubated with shaking at 37°C. At prefixed times the reactions were stopped by extraction with ethyl acetate containing a proper amount of benzaldehyde as a standard for the quantification of the residue epoxide, and the extracts were analyzed using the chiral column for the determination of the e.e.s of the residue epoxides and formed diols. At least three determinations were made at each time. The average results are reported in Table 1.

3.2.3. Diol isolation

In order to determine the absolute configuration of the excess enantiomer of the formed diols, epoxides **1b–e** as neat liquids (*ca*. 40–60 mg) were added to 20 ml of the microsomal preparation, containing 20 mg of protein/ml, and the reaction mixtures were incubated with shaking at 37°C for the time necessary to obtain the proper conversion. The incubation mixtures were then stopped by extraction with ethyl acetate. The organic phases were diluted to an exactly known volume and a proper amount of the standard was added to an aliquot of these extracts in order to verify by GLC the conversion. The remaining parts of the organic phases were evaporated *in vacuo* and the residues were chromatographed on silica gel columns to give pure diols **2a–e**. **2a**, $[\alpha]_D$=+13.4 (c=1, EtOH)[12]; e.e. 56%; **2b**, $[\alpha]_D$=+19.0 (c=1, $CHCl_3$)[14]; **2c**, $[\alpha]_D$=+30.0 (c=1, MeOH); **2d**, $[\alpha]_D$=+26 (c=1, MeOH); e.e. 90%; **2e**, $[\alpha]_D$=+7.8 (c=1, MeOH), e.e. 80%.[17]

3.3. Determination of the absolute configuration

The absolute configuration of the excess enantiomer of the residue epoxides was determined on the crude reaction mixtures, after evaluation of the enantiomeric excesses by GLC on a chiral colunm, as reported below:

*3.3.1. Epoxide **1a***

$LiAlH_4$ (20 mg) was added to a solution containing the enantiomerically pure (GLC) *cis*-2,3-epoxyheptane (**1a**, 20 mg, 0.17 mmol) in anhydrous ethyl ether (2 ml) and the mixture was stirred at room temperature for 3 h. After addition of ice the organic phase was washed with water and dried ($MgSO_4$) to give a mixture of 2- and 3-heptanol. The absolute configuration of 2-heptanol was shown to be (R) by comparison of its emergence order with that of a sample of commercial (R)-2-heptanol.

*3.3.2. Epoxide **1c***

$LiAlH_4$ (20 mg) was added to a solution containing the enantiomerically enriched (GLC) *cis*-3,4-epoxyheptane (**1c**, 20 mg, 0.17 mmol) in anhydrous ethyl ether (2 ml) and the mixture was stirred at room temperature for 3 h. After addition of ice the organic phase was washed with water and dried ($MgSO_4$) to give a mixture of 3- and 4-heptanol, which was subjected to methylation by treatment with NaH and CH_3I according to the general alkylation procedure.[16] The absolute configuration of 4-methoxynonane was shown to be (R) by comparison of its emergence order with that of a sample of (R)-(+)-4-methoxynonane prepared as reported above.

*3.3.3. Epoxide **1d***

Tosyl chloride (40 mg) was added to a solution containing the enantiomerically enriched (GLC) *cis*-3,4-epoxynonan-1-ol (**1d**, 30 mg) in pyridine (2 ml) and the mixture was stirred at room temperature. After 3 h the reaction mixture was diluted with ice water, acidified with conc. HCl and extracted with

ethyl ether. The organic phase was washed with saturated $NaHCO_3$, dried ($MgSO_4$), and evaporated to give a crude product which was subjected to methylation by treatment with NaH and CH_3I.[16] The absolute configuration of 4-methoxynonane was shown to be (R) by comparison of its emergence order with that of a sample of (R)-(+)-4-methoxynonane.

*3.3.4. Epoxide **1e***

To a solution of the enantiomerically enriched (GLC) (3R,4S)-*cis*-3,4-epoxynonane-1-ol (**1d**, 70 mg) in anhydrous dichloromethane (6 ml), containing 2,6-di-*t*-butyl-4-methylpyridine (1 mmol), methyl triflate (0.6 mmol) was added under an argon atmosphere, and the mixture was refluxed for 2 h, and then filtered. The solution was washed with 5% HCl and saturated $NaHCO_3$, dried ($MgSO_4$) and evaporated to give a crude product, (3R,4S)-*cis*-1-methoxy-3,4-epoxynonane **1e**, having the same retention time on the chiral GLC column as a sample of pure **1e**, arising from the partial mEH catalyzed kinetic hydrolysis of (±)-**1e**.

The absolute configuration of the excess enantiomer of the formed diols was determined on the pure product as reported below.

*3.3.5. Diol **2d***

Tosyl chloride (55 mg, 27 mmol) was added to a pyridine solution (2 ml) of the enantiomerically enriched *threo*-(+)-1,3,4-nonanetriol, (**2d**, 40 mg) and the mixture was stirred at room temperature. After 3 h the reaction mixture was diluted with ice water, acidified with HCl and extracted with ethyl ether. The organic phase was washed with saturated $NaHCO_3$, dried ($MgSO_4$), and evaporated to give a crude product which was subjected to reduction by treatment with $LiAlH_4$ (20 mg) in anhydrous ethyl ether (2 ml). After 3 h at room temperature, ice was added to the reaction mixture and the organic phase was separated, washed with water, dried ($MgSO_4$) and evaporated to give the enantiomerically enriched diol **2c**.

*3.3.6. Diol **2e***

The enantiomerically pure or enriched diols **2d** and **2e** (30 mg) were subjected to exhaustive methylation by treatment with NaH and CH_3I according to the general alkylation procedure,[16] to give in both cases a crude product which was identified as (+)-1,3,4-trimethoxynonane. $[\alpha]_D$=+7.7 (c=1, CH_3OH), 95% ee. ^1H NMR δ ppm: 0.89 (t, 3H, J=6.4 Hz, CH_3); 1.25 (m, 10H, CH_2); 3.35 (s, 3H, OCH_3); 3.40 (s, 3H, OCH_3); 3.42 (s, 3H, OCH_3); 3.46 (m, 2H, CH_2OCH_3); 3.75 (m, 2H, CH). ^{13}C NMR δ ppm: 14.05 (CH_3); 22.61, 25.66, 26.20, 29.70, 30.14; ($5CH_2$); 58.40 (OCH_3); 58.50 (OCH_3); 58.70 (OCH_3); 69.00 (CH_2); 78.00 (CH); 81.70 (CH).

Acknowledgements

This work was supported in part by grants from Consiglio Nazionale delle Ricerche (CNR, Roma) and Ministero dell'Università e della Ricerca Scientifica e Tecnologica (MURST, Roma).

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