## Product Enantioselectivity of the Microsomal and Cytosolic Epoxide Hydrolase catalysed Hydrolysis of *meso* Epoxides

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1,2-Epoxycycloalkanes from C<sub>5</sub> to C<sub>8</sub> and *cis*-stilbene oxide are respectively hydrolysed to the corresponding (-)-(*R*,*R*)-*trans*-diols and to (+)-(*R*,*R*)-1,2-diphenylethane-1,2-diol by both the microsomal and the cytosolic epoxide hydrolase of rabbit liver, the former enzyme being more active and giving higher enantiomeric excesses.

Epoxide hydrolases are important enzymes involved in the metabolism of the often mutagenic and carcinogenic epoxides and arene oxides formed by oxidation of alkene and aromatic xenobiotic compounds by the cytochrome P-450 dependent mono-oxygenases.<sup>1-3</sup> Microsomal and cytosolic forms of epoxide hydrolase (mEH and cEH) have been clearly differentiated,<sup>2,3</sup> in particular with regard to their distinct substrate specificities.<sup>4</sup> mEH has also been systematically investigated for its product and substrate enantioselectivity,5 and found, with a few exceptions,6 to catalyse ring opening preferentially at (S) oxirane carbons. No information about the enantioselectivity of the cEH promoted hydrolyses has been available so far. We now report on a comparative investigation of the rates and the product enantioselectivity of the rabbit liver mEH and cEH promoted hydrolyses of meso epoxides, including 1,2-epoxycycloalkanes from  $C_5$  to  $C_8$  and cis-stilbene oxide. A report on 1,2-epoxycycloalkanes as substrates and inhibitors of mouse liver mEH and cEH, but with no mention of enantioselectivity, has appeared very recently.7

All enzymatic reactions were carried out with microsomal and cytosolic preparations<sup>6b</sup> containing 15 mg protein/ml at 37 °C and pH 7.4. None of the epoxides examined was significantly hydrolysed at the 0.1  $\bowtie$  concentrations used when inactivated preparations were used, except the C<sub>6</sub> epoxide (**1b**), for which the non-enzymatic hydrolysis was minimized by working at a 0.02  $\bowtie$  concentration. Only the *trans* diols (**2**) were obtained from all epoxides (**1**), and diol (**6**) from epoxide (**5**), both in the microsomal and in the cytosolic incubations. The saturation velocities, determined by g.l.c. [for (**2**)] or h.p.l.c. [for (**6**)] quantitation of the diols obtained in reactions carried out with the same microsomal or cytosolic preparation, are given in Table 1. All epoxycycloalkanes except C<sub>8</sub> (**1d**) were much better substrates for the rabbit than for the mouse liver mEH,<sup>7</sup> being at least as good substrates as *cis*-stilbene oxide for the former enzyme. The C<sub>5</sub>, C<sub>6</sub>, and C<sub>7</sub> epoxides (**1a**—c) were also hydrolysed by the rabbit liver cEH, although at much lower  $V_S$ , whereas the C<sub>8</sub> epoxide did not react appreciably. This is in contrast to the mouse liver cEH, which is reported to show no activity with the C<sub>5</sub>, C<sub>6</sub>, and C<sub>7</sub> and a small activity with the C<sub>8</sub> epoxide.<sup>7</sup> 10,11-Dihydro-10,11-epoxy-5H-dibenzo[*a*,*d*]cycloheptene, a poor substrate for mEH giving a 52% enantiomeric excess (e.e.) of the (*S*,*S*) diol,<sup>6c</sup> was not hydrolysed by cEH.

Diols (2) were isolated by column chromatography from larger scale incubations for times during which non-enzymatic hydrolysis was unimportant. The slowest cEH reactions of (1a) and (1c) were carried out with repeated addition of fresh cytosolic preparation. All cycloalkane-*trans*-1,2-diols obtained were laevorotatory. Their enantiomeric ratios



Table 1. Formation rates, enantiomer ratios, and absolute configurations of diols in mEH and cEH catalysed hydrolysis of meso epoxides.

	mEH					cEH		
	$V_S$ /nmol min <sup>-1</sup> mg <sup>-1</sup> protein	Enantiomer ratio	[α] <sub>D</sub> <sup>25</sup> a (MeOH)	Benzoate c.d. (MeCN) λ/nm (Δε)	Abs. config.	$V_s$ /nmol min <sup>-1</sup> mg <sup>-1</sup> protein	Enantiomer ratio	Abs. config.
( <b>1</b> a)	$23.0 \pm 0.2$	95:5	-24°	263(-22.3), 246(+8.6)	R,R	$0.45 \pm 0.1$	80:20	R,R
(1b)	$14.5 \pm 1$	88:12 <sup>ь</sup>	-28°	263(-29.9), 246(+19.5)	R,R	$1.0 \pm 0.1$	60:40	R,R
(1c)	$31.5 \pm 0.5$	70:30	-6°	263(-16.2), 246(+9.3)	R,R	$0.65 \pm 0.1$	65:35	R,R
(1d)	$1.1 \pm 0.1$	85:15	$-10^{\circ}$	263(-22.3), 246(+14.2)	R,R	c		
(5)	$17.0 \pm 1$	94:6 <sup>d</sup>	$+80^{\circ d}$	_	R,R	$1.0 \pm 0.1$	85 : 15°	R,R

<sup>a</sup> Diols (1a-d) were isolated by column chromatography and not recrystallized, in order to avoid enantiomeric enrichment; specific rotations therefore have only an indicative value. <sup>b</sup> A 70% optical purity, based on optical rotation measurements, was previously reported (G. Bellucci, G. Berti, G. Ingrosso, and E. Mastrorilli, *J. Org. Chem.*, 1980, **45**, 299. <sup>c</sup> No hydrolysis was detected. <sup>d</sup> Data taken from ref. 6c. <sup>e</sup> Optical purity (70%) determined on the basis of the reported maximum optical rotation  $[\alpha]_D^{25} + 92^\circ$  (see: T. Watabe and K. Akamatsu, *Biochim. Biophys. Acta*, 1972, **279**, 297.

(Table 1) were determined by h.p.l.c. analysis of the diastereoisomeric bis(MTPA) esters (**3a**-d) obtained by reaction with (S)-(+)- $\alpha$ -methoxy- $\alpha$ -(trifluoromethyl)phenyl-acetyl (MTPA) chloride.<sup>8</sup> As expected for *meso* substrates, the e.e. did not change with the incubation times and diol yields. The absolute configurations of these diols were checked by the c.d. spectra of their bis(*p*-methoxybenzoates) (**4a**-d) using the dibenzoate chirality rule.<sup>9</sup> All compounds (**4**) exhibited large negative Cotton effects at 263 nm and positive ones at 246 nm, indicating *gauche* dibenzoate groups with a left-handed screw, and therefore (*R*,*R*) absolute configurations for all parent diols (-)-(**2**).

The mEH reactions generally yielded fair to high e.e. of (R,R) diols, thus providing potentially useful, simple preparative routes to these chiral compounds. The cEH reactions led also to (R,R) diols, although with lower e.e., particularly for the C<sub>6</sub> substrate (**1b**). Thus, in spite of their very different activity towards 1,2-epoxycycloalkanes and *cis*-stilbene oxide, mEH and cEH exhibit in all examined cases a qualitatively similar product enantioselectivity, suggesting similar mechanisms and similar, although less strict, steric requirements of their active sites. Comparative enantioselectivity studies of the mEH and cEH catalysed hydrolysis of other, chiral substrates are in progress, in order to clarify these points.

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