Enantioselectivity of the Enzymatic Hydrolysis of Cyclohexene Oxide and (±)-1-Methylcyclohexene Oxide: A Comparison between Microsomal and Cytosolic Epoxide Hydrolases

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The hydrolysis of cyclohexene oxide and (±)-1-methylcyclohexene oxide by rabbit liver microsomal and cytosolic epoxide hydrolase (mEH and cEH) has been investigated. Microsomal preparations hydrolysed the two epoxides at respective V_S of 17.0 and 3.5 nmol min^{-1} mg^{-1} protein, cytosolic preparations at V_S of 0.95 and 1.0 nmol min^{-1} mg^{-1} protein. (−)-(*R,R*)-Cyclohexane-*trans*-1,2-diol was formed in the mEH and cEH catalysed hydrolysis of cyclohexene oxide with 94% and 22% e.e. respectively. (−)-(*R,R*)-1-Methylcyclohexane-*r*-1,*t*-2-diol, whose absolute configuration was deduced by c.d. measurements of its bis(*p*-methoxybenzoate), was obtained by partial hydrolysis of (±)-1-methylcylohexene oxide by mEH. The e.e. of the enzymatically formed diol was 94% at 8% conversion and decreased to 56% around 28% conversion. Racemic 1-methylcyclohexane-*r*-1,*t*-2-diol was instead obtained in the cEH catalysed hydrolysis of (±)-1-methylcyclohexene oxide. The substrate enantioselectivity of the mEH catalysed hydrolysis of this trisubstituted epoxide is rationalized on the basis of a better stabilization of the transition state for the *anti* opening at the carbon with (*S*) configuration of the (1*R*, 2*S*)-enantiomer of the epoxide with (3,4 *M*) helicity, in agreement with the stereochemical course of the analogous reaction of unsubstituted cyclohexene oxide.

In the course of an investigation of the hepatic metabolism of 1-methylcyclohexene, a simple endocyclic olefin whose partial structure is present in a number of terpenoid compounds,[1] we observed the formation of 1-methyl-1,2-epoxycyclohexane and 1-methylcyclohexane-*r*-1,*t*-2-diol. The epoxide—diol pathway is a common feature of the biotransformation of olefinic compounds,[2] epoxides being formed by the action of the cytochrome P 450 dependent mono-oxygenases and being hydrolysed to *trans* diols by microsomal and cytosolic epoxide hydrolases (mEH and cEH).[3,4] Since epoxides are often electrophilically reactive species which can covalently bond to cellular macromolecules resulting in toxic, mutagenic, carcinogenic, and teratogenic effects,[5] the efficency of their enzymatic hydration to less reactive and more easily excreted diols can be of crucial importance. Cyclohexene oxide is considered a relatively poor substrate for mEH[6] and its 1-methyl derivative was expected to be much worse, since this enzyme is known to hydrolyze readily aryl or alkyl mono-substituted epoxides, more slowly 1,1-disubstituted and *cis*-1,2-disubstituted epoxides, while *trans*-1,2-disubstituted, tri- and tetra-substituted epoxides are reported to be virtually inactive as substrates.[6] On the other hand, cEH is known to exhibit a different and complementary substrate selectivity.[4,7]

In order to clarify the role of the two different EH in the hydrolysis of 1-methylcyclohexene oxide to its *trans* diol and to assess the effect of the third substituent on the susceptibility of this epoxide to enzymatic hydration, we carried out a comparative investigation of the mEH and cEH catalysed hydrolysis of this epoxide and of cyclohexene oxide. Since the former enzyme is known to exhibit a remarkable enantioselectivity towards epoxycyclohexane derivatives,[8-11] we focused our attention on this feature and attempted to differentiate the two enzymes from this point of view.

Results

Measurements of hydrolysis rates of cyclohexene oxide (**1a**) and 1-methylcyclohexene oxide (**1b**) were carried out at 37 °C and

a; R = H
b; R = Me

(1) (2)

pH 7.4, using both the microsomal and cytosolic fraction obtained from rabbit liver, at 1.2×10^{-2}M and 1.4×10^{-1}M concentration of the epoxides and at several protein concentrations. The reactions were stopped by addition of NaCl and extraction with ethyl acetate containing cycloheptane-*trans*-1,2-diol as a standard, and the extracts were analysed by g.l.c. for the quantification of the formed diols. Only cyclohexane-*trans*-1,2-diol (**2a**) was detected in the hydrolyses of (**1a**). In the case of (**1b**) the main product, 1-methylcyclohexane-*r*-1,*t*-2-diol (**2b**), was accompanied by a very small amount of the *cis*-diol [about 1% relative to (**2b**)], presumably formed by non-enzymatic hydrolysis of the epoxide, and by a small, roughly constant amount (around 15% of the products) of a third unidentified compound. The amounts of diols (**2**) were corrected for the non-enzymatic hydrolysis of the substrates, evaluated by experiments performed under identical conditions but using unactivated microsomal and cytosolic preparations. The enzymatic diol formation was linear with the amount of microsomal or cytosolic protein as well as with time, consistent with enzyme saturation. The respective saturation velocities are reported in Table 1. Reproducible values of V_S were obtained, within the limits of experimental error, working at 1.2×10^{-2}M epoxide and protein concentration ranging between 0.8 and 3.2 mg ml^{-1}, and at 1.4×10^{-1}M epoxide and 16 mg protein ml^{-1}.

Also the non-enzymatic hydrolysis of the two substrates proceeded linearly, amounting to less than 10% during the investigated time. The respective first order rate constants,

Table 1. Rates of enzymatic and non-enzymatic hydration of cyclohexene oxide (**1a**) and (±)-1-methylcyclohexene oxide (**1b**)

	Enzymatic hydration V_S (nmol/min per mg protein)		Non-enzymatic hydration
Substrate	Microsomes	Cytosol	$k \times 10^4$ (min^{-1})
(**1a**)	17.0(1)	0.95(0.1)	2.4(0.1)[a]
(**1b**)	3.5(0.5)	1.0(0.1)	6.5(0.5)[a]

[a] In unactivated microsomal preparations containing 16 mg protein ml^{-1} this rate constant was $k = 1 \times 10^{-4}$ min^{-1} for (**1a**) and 3×10^{-4} min^{-1} for (**1b**).

calculated on the basis of these initial rates, are also reported in Table 1.

Both epoxides underwent significant non-enzymatic hydrolysis. However, the rate of hydrolysis of (**1b**) was about twice as large as that of (**1a**); as expected for a trisubstituted as compared to a 1,2-disubstituted epoxide. For each compound similar rate constants were obtained in 5×10^{-2}M Tris–HCl buffer, pH 7.4, or in boiled microsomal or cytosolic preparations containing the same buffer and low protein amounts (0.8 to 3 mg ml^{-1}). In the presence of protein amounts as large as 16 mg ml^{-1}, however, the same k values were again found in boiled cytosol, but k values about half these were found in boiled microsomes. Protein, therefore, when present in large amounts tends to protect the epoxides against non-enzymatic hydrolysis, perhaps by a sequestration effect.

Both epoxides were hydrolysed both by mEH and cEH, but with different V_S. The V_S for the mEH catalysed reaction of cyclohexene oxide was comparable with those reported for typical good substrates, like styrene oxide[12] and *cis*-stilbene oxide.[13] In the case of 1-methylcyclohexene oxide this V_S was only about fivefold slower, in contrast to the generally accepted belief that trisubstituted epoxides are not substrates for mEH.[6] The two epoxides exhibited equal V_S in the cEH catalysed hydrolyses. However, these rates were respectively 18 and 3.5 times lower than those found for (**1a**) and (**1b**) with the microsomal enzyme.

The product enantioselectivity of the mEH catalysed hydrolysis of cyclohexene oxide had already been investigated,[8] and a 70 ± 2% optical purity was reported for samples of (−)-(*R*,*R*)-cyclohexane-*trans*-1,2-diol obtained in this reaction on the basis of optical rotation measurements after purification of the diol by crystallization and sublimation. In the present investigation the enantiomeric excess of this diol was re-examined in a more accurate way by h.p.l.c. analysis of appropriate diastereoisomeric derivatives of the crude diol. In order to minimize the contribution of the non-enzymatic reaction, producing racemic diol, incubations of 0.1M cyclohexene oxide carried out with a microsomal preparation containing 16 mg protein ml^{-1} were stopped before complete hydrolysis after 7 h, when the enzyme had a considerably reduced activity. Longer incubation times increased the amount of racemic diol formed by the non-enzymatic route. The formed diol, separated by column chromatography, was then transformed into its diastereoisomeric bis (MTPA)-esters (**3a**) with (+)-(*S*)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (MTPACl). H.p.l.c. analysis showed the two peaks corresponding to the bis-esters of the (*R*,*R*)- and (*S*,*S*)-diols in a 95:5 ratio. Taking into account the small amount of racemic diol produced through the non-enzymatic pathway, determined by a blank experiment with unactivated microsomes, the e.e. of the diol produced by the mEH catalysed hydrolysis was 94%. Thus, this reaction was nearly completely enantiospecific.

The course of the mEH catalysed hydrolysis of 1-methylcyclohexene oxide was then examined. In this case, starting with a racemic substrate, enantiomeric ratios changing with the progress of the hydrolysis were expected. In order to isolate and analyse for its e.e. the diol formed during the course of the reaction, several incubations carried out around 0.1M epoxide and 20 mg protein ml^{-1} were stopped at different times and extracted with ethyl acetate. The yield of diol was determined by g.l.c. after addition of the standard to an aliquot of the extract and corrected for the non-enzymatic hydrolysis measured with the same but unactivated microsomal preparation. Diol (**2b**), isolated by column chromatography, was levorotatory, with the optical rotation decreasing with the progress of the reaction. This diol was finally treated with an excess of MTPACl in pyridine at room temperature, under conditions in which (±)-(**2b**) underwent only esterifcation of the secondary hydroxyl group to give the two diastereoisomeric monoesters (**5**) in a 50:50 ratio. The resulting MTPA derivatives (**5**) were analysed by h.p.l.c. to obtain the enantiomeric ratios of the starting diols.

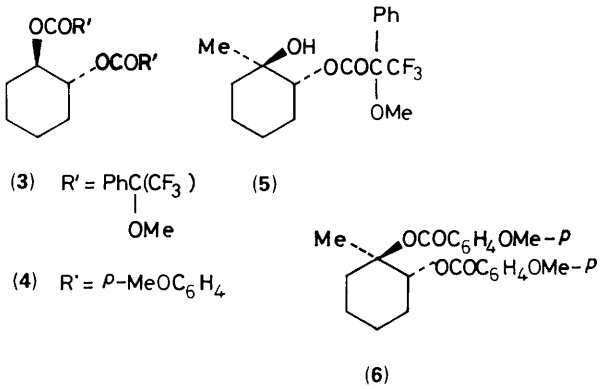


Table 2 reports the total yields of diol (**2b**) and their partition between the non-enzymatic and enzymatic routes, the optical rotations, and the enantiomeric ratios of the total diol found at different times. The values of the enantiomeric ratios of the diol produced by enzymatic hydrolysis, deduced on the basis of the amount of racemic diol formed by the non-enzymatic reaction, are also reported. These data show that the reaction was nearly enantiospecific during its early course and that a diol with a 56% e.e. was produced in around 28% conversion.

The enantioselective ability of cEH towards epoxides (**1a**) and (**1b**) was then checked. In both cases, because of the lower rate of the enzymatic hydrolysis and of the higher rate of non-enzymatic reaction in cytosolic as compared to microsomal preparations, most of the diol was produced as a racemate by the latter route when the reactions were carried out at the 0.1M substrate concentrations used for the above discussed microsomal incubations. This difficulty was overcome by reducing the substrate concentrations to values low enough to depress sufficiently the velocity of the first order non-enzymatic reaction, but still assuring enzyme saturation, so that the rate of the zero order enzymatic hydrolysis was not affected. Incubations of 2×10^{-2}M (**1a**) with cytosolic preparations containing 13 mg protein ml^{-1} gave after 5 h an 18% yield of diol (**2a**). Blank experiments showed that 2% was formed by non-enzymatic and 16% by the cEH catalysed hydrolysis. H.p.l.c. analysis of the bis(MTPA) derivatives (**3**) obtained from this diol, isolated by column chromatography, showed a 60:40 ratio of the two peaks respectively corresponding to the (*R*,*R*)- and the (*S*,*S*)-enantiomers of (**2a**). After correction for the racemic diol obtained non-enzymatically, the e.e. of the enzymatically formed (*R*,*R*)-diol was 22%.

Incubations of 2×10^{-2}M (±)-(**1b**) for 4 h with the same cytosolic preparation likewise yielded 18% of diol (**2b**), 15%

Table 2. Enantiomeric ratios of 1-methylcyclohexane-*r*-1,*t*-2-diol formed by mEH-catalysed hydrolysis of (±)-1-methylcyclohexene oxide

Substrate amount (mg)	Microsomal protein (mg)	Volume (ml)	Time (min)	Diol %			Enantiomer ratio[c]	$[\alpha]_D^{25}$[d]
				Total	Non-enzym[a]	Enzym[b]		
93	120	6	150	12.5	4.5	8	80:20 (97:3)	
85	120	6	360	28	11.5	16.5	75:25 (91:9)	−7.5°
71	100	5	450	36	14.5	21.5	70:30 (82:18)	
188	300[e]	11	300	37	9.5	27.5	70:30 (78:22)	−6.3°

[a] Non-enzymatic hydrolysis determined in blank experiments with unactivated microsomal preparation. [b] Enzymatic hydrolysis evaluated by difference between total and non-enzymatic hydrolysis. [c] Determined from the ratio of the diastereoisomeric monoesters (**5**). Values in parentheses are corrected for the amount of racemic diol formed by non-enzymatic hydrolysis and give an evaluation of the substrate enantioselectivity of this reaction. [d] Measured on the total produced diol, separated by column chromatography. [e] This incubation was carried out with a different, more active, microsomal preparation.

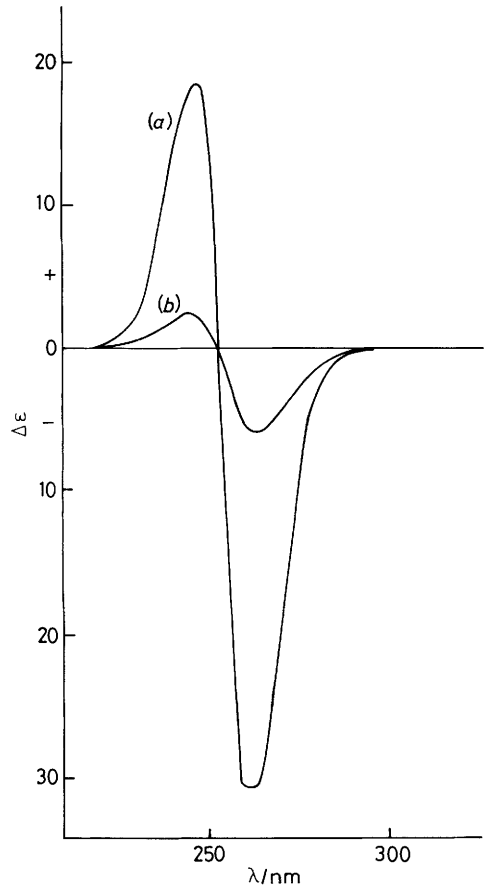


Figure 1. C.d spectra of the bis(*p*-methoxybenzoate) of (−)-(*R*,*R*)-cyclohexane-*trans*-1,2-diol (**4**) (*a*) and of the bis(*p*-methoxybenzoate) of (−)-(*R*,*R*)-1-methylcyclohexane-*r*-1,*t*-2-diol (**6**) (*b*) in acetonitrile

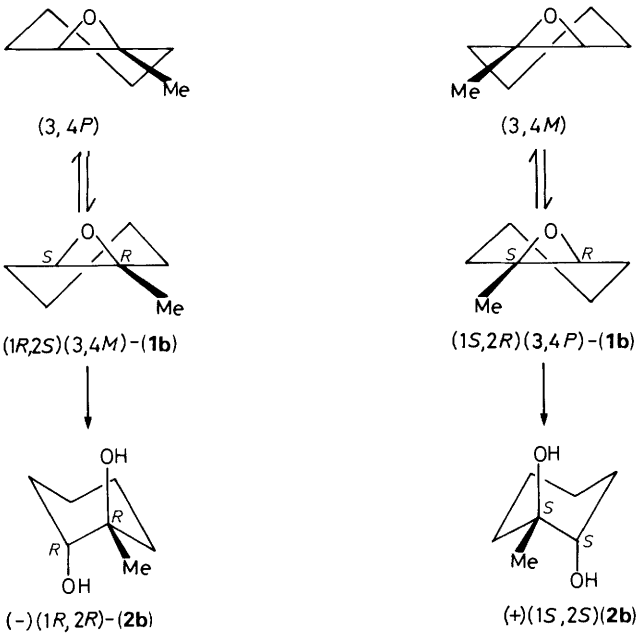
being formed by enzymatic and 3% by non-enzymatic hydrolysis. A 50:50 ratio was found by h.p.l.c. between the diastereoisomeric monoesters (**5**) obtained from diol (**2b**) formed in this experiment. Thus, (**2b**) was produced by the cEH catalysed hydrolysis of (±)-(**1b**) in a completely racemic form even at low substrate conversion, in contrast with the course of the mEH catalysed reaction. This excluded the possibility that the EH activity of the employed cytosolic preparations was actually due to a contamination by the microsomal enzyme.

The absolute configuration of diol (−)-(**2b**) was determined using the exciton coupling method. To this purpose a sample of (**2b**), $[\alpha]_D^{25}$ −7.5°, 50% e.e., was converted into its bis-(*p*-methoxybenzoate), (**6**), in order to apply the dibenzoate chirality rule.[14] Figure 1 shows the c.d. spectrum of (**6**) in acetonitrile, together with that of the bis(*p*-methoxybenzoate) (**4**) obtained from a sample of (−)-cyclohexane-*trans*-1,2-diol with a 70% e.e. Both spectra exhibited exciton splittings, due to conformations with equatorial *p*-methoxybenzoyloxy substituents. Negative Cotton effects at 263 nm and positive ones at 245 nm were found in both cases, indicating a right-handed screwness of the two *p*-methoxybenzoyloxy groups and therefore (*R*,*R*)-absolute configurations for both bis(*p*-methoxybenzoates) (**4**) and (**6**). This confirmed the (*R*,*R*)-configuration assigned to (−)-(**2a**) by chemical correlations[15] and established the same (*R*,*R*)-configuration for diol (−)-(**2b**) preferentially formed during the mEH catalysed hydrolysis of racemic 1-methylcyclohexene oxide.

Discussion

The results of this investigation show that 1-methylcyclohexene oxide (**1b**) is actually hydrolysed by mEH, although at V_S fivefold slower than unsubstituted cyclohexene oxide (**1a**), and that both these reactions are highly enantioselective, leading to (*R*,*R*)-enantiomers of the respective trans diols, (**2b**) and (**2a**). In the case of (**1a**), a *meso*-compound actually consisting of two rapidly equilibrating enantiomeric monoplanar conformers of (3,4 *M*)-and (3,4 *P*)-helicity,[16] this product enantioselection was explained on the basis of a higher stabilization of the transition state resulting from antiparallel attack by water at the (*S*)-carbon of the conformer of (3,4 *M*)-helicity.[8] This was confirmed by the results of studies of substrate enantioselection in the hydrolysis of racemic epoxycyclohexane[8–11] and epoxytetrahydropyran derivatives,[17,18] that further more revealed the importance of selective binding of suitably orientated lipophilic substituents at the non-oxirane carbons to a hydrophobic area present in the enzyme active site.

The course of the mEH promoted hydrolysis of the 1-substituted epoxycyclohexane (**1b**) appears to be conditioned by the same factors determining that of the unsubstituted compound (**1a**). This enzyme is known to hydrate epoxides by a general base-catalysed mechanism[19] and water attack occurs with inversion of configuration at the less substituted or less hindered oxirane carbon.[20] On this basis opening of the oxirane ring in (**1b**) can safely be assumed to occur with inversion of configuration at C-2. Furthermore, water attack normally occurs in an antiparallel way, leading to the diol in its diaxial conformation.[8–11] Thus, (−)-(*R*,*R*)-(**2b**) must arise from the reaction of (1*R*,2*S*)-(**1b**) in its (3,4 *M*)-conformation and (+)-(*S*,*S*)-(**2b**) from that of (1*S*,2*R*)-(**1b**) in its (3,4 *P*)-conformation. If the transition state for the *anti* opening at the (*S*)-carbon of the (1*R*,2*S*)(3,4 *M*)-form of (**1b**) is better stabilized by the enzyme with respect to that for opening at the (*R*)-carbon of the (1*S*,2*R*)(3,4 *P*)-form, as anticipated on the basis of the



hydrolysis course of cyclohexene oxide, then (1*R*,2*S*)-(**1b**) is expected to exert a competitive inhibition on the hydrolysis of its antipode. This must cause a preferential formation of diol (−)-(*R*,*R*)-(**2b**) in the partial hydrolysis of (±)-(**1b**), as actually observed. Thus, the introduction of the third substituent on the oxirane ring of 1,2-epoxycyclohexane appears to exert a modest retarding effect on the mEH-catalysed hydrolysis, without altering in any way the substrate enantioselection.

The present results also show that, as expected,[4] cyclohexene oxide is a rather worse substrate for cEH than for mEH, while the difference is only slight for 1-methylcyclohexene oxide. The practically identical V_S values for the cytosolic EH reactions of the two epoxides (**1a**) and (**1b**) show that, in contrast to mEH, increasing substitution at the oxirane carbons does not affect the activity of the cytosolic enzyme.

The two enzymes exhibit a qualitatively similar, even if quantitatively different, product enantioselectivity in favour of the (*R*,*R*)-diol in the hydrolysis of cyclohexene oxide, the e.e. being 94% in the mEH-catalysed and only 22% in the cEH-catalysed reaction. Unfortunately, no conclusive information concerning the enantioselectivity can be presently inferred from the obtainment of racemic (**2b**) in the cEH-promoted hydrolysis of (±)-1-methylcyclohexene oxide. This finding could be due to a complete absence of substrate enantioselection by the enzyme, but could also be the result of a complete lack of regioselectivity in the hydrolysis of both enantiomers of the epoxide. In fact, nucleophilic attack at C-1 and C-2 in a 1:1 ratio must lead to racemic (**2b**), independent of the relative hydrolysis rates of the two enantiomers of (**1b**) and of the extent of conversion. Although less probable, this second possibility cannot be excluded at present, since no data are available concerning the regioselectivity of the cEH-catalysed hydrolysis of epoxides. The regio-and enantioselectivity of these reactions are presently being investigated in this laboratory, with the aim at clarifying the reaction mechanism and the features of the active site of cEH.

Experimental

I.r. and u.v. spectra were respectively obtained with a Pye-Unicam SP3-300 and a Pye-Unicam SP4-800 spectrophotometer. ^{1}H N.m.r. spectra were recorded at 60 MHz using Me_4Si as internal standard. The optical rotations were measured on a Perkin-Elmer 241 photoelectric polarimeter. C.d. spectra were recorded with a Jasco J 500 C spectropolarimeter. G.l.c. analyses were carried out with a Dani 3 800 instrument equipped with a 2 m glass column (2.5 mm i.d.) packed with 10% NPGS on 80—100 mesh sylanized Chromosorb: column 150 °C, evaporator and detector 245 °C, nitrogen flow 60 ml min^{-1}. H.p.l.c. analyses were carried out with a Pye-Unicam PU 4010 apparatus equipped with an u.v. detector and a normal column, 25 cm, 5 μm, spherisorb (pS Phase Sep) in the following conditions: (*a*) hexane–ethyl acetate (97.5:2.5), 1.5 ml min^{-1}, λ 254 nm; (*b*) hexane–ethyl acetate (96:4), 1.5 ml min^{-1}, λ 254 nm.

Materials.—Commercial cyclohexene oxide (**1a**) (Merck, 98%) was distilled before use. 1-Methylcyclohexene oxide, (**1b**), b.p. 138 °C (lit.[21] 140—142 °C) was obtained by epoxidation of commercial 1-methylcyclohexene (Aldrich, >97%) with *m*-chloroperoxybenzoic acid in dichloromethane. 1-Methylcyclohexane-*r*-1,*t*-2-diol, (**2b**), m.p. 84—85 °C (lit.[21] 84—85 °C) was obtained by $HClO_4$ catalysed hydrolysis of (**1b**) in aqueous tetrahydrofuran. 1-Methylcyclohexane-*r*-1,*c*-2-diol was obtained by dihydroxylation of 1-methylcyclohexene with OsO_4, m.p. 64—65 °C (lit.[21] 65—66.5 °C). (+)-(*S*)-α-Methoxy-α-(trifluoromethyl)phenylacetyl chloride (MTPACl), b.p. 54 °C/1 mm Hg, was prepared from the (+)-(*R*)-acid and thionyl chloride.[22] *p*-Methoxybenzoyl chloride (Aldrich 99%) was distilled before use (b.p. 123—125 °C/25 mm Hg). Kieselgel (150—230 mesh, ASTM, Merck) was used for column chromatography. Preparative t.l.c. were run on PSC-Fertigplatten Kieselgel 60 F_{254} (Merck) 1 mm thickness. Solvents were reagent grade.

Microsomal and Cytosolic Preparations.—Livers of New Zealand white rabbits (2.5—3 kg) were removed and homogenized in four volumes of 5 × 10^{-2}M Tris–HCl buffer (pH 7.4) containing KCl (1.15% w/v), and the resulting suspension was centrifuged at 9 000 g for 30 min. The supernatant was further centrifuged at 125 000 g for 1.5 h leading to the cytosolic and microsomal fractions. The protein concentrations of cytosolic fractions were 13—16 mg ml^{-1}. The microsomal pellets were resuspended in the same buffer to final protein concentrations of 16—27 mg ml^{-1}. Both cytosolic and microsomal fractions were used directly or stored at −40 °C.

Rates of Enzymatic Hydrolysis.—Samples of 0.1 ml of a 1.5M or of a 1.3 × 10^{-1}M ethanolic stock solution of (**1a**) or (**1b**) were respectively added to 1 ml of cytosolic or microsomal preparation containing 16 mg protein ml^{-1} or to 1 ml of the same preparations previously diluted with 5 × 10^{-2}M Tris–HCl buffer to final protein concentrations of 0.8, or 1.6, or 3.2 mg ml^{-1}. All mixtures were incubated at 37 °C with shaking for 1 h or for 3 h. The reactions were stopped by addition of NaCl and ethyl acetate (2 ml) containing an appropriate amount of cycloheptane-*trans*-1,2-diol as a standard for g.l.c. analysis, and vigorously shaken. The organic phase was separated and the aqueous phase re-extracted with ethyl acetate (2 × 2 ml). The combined ethyl acetate phases were directly analyzed by g.l.c. for the quantification of diols (**2a**) or (**2b**). Each experiment was accompanied by a blank carried out under identical conditions but using cytosolic and microsomal preparations deactivated by boiling, or using the sole Tris–HCl buffer, in order to evaluate the amount of non-enzymatic hydrolysis of both epoxides (**1a**) and (**1b**). All experiments were carried out in duplicate. In all cases the diol formation was linear with time. Average rate constants for the non-enzymatic hydrolysis of (**1a**) and (**1b**), determined from the initial rates measured at 1 and 3 h, and average saturation velocities for the enzymatic hydrolysis, determined from the amounts of detected diol corrected for the non-enzymatically formed quantities, are reported in Table 1.

Isolation of the Hydrolysis Products.—Enzymatic incubations were carried out as described above. 0.1M Cyclohexene oxide (**1a**) was hydrolysed in 5 ml of a microsomal preparation containing 16 mg protein ml^{-1} for 7 h. Hydrolyses of 1-methylcyclohexene oxide (**1b**) with microsomal preparations were performed under the conditions reported in Table 2. CEH-catalysed hydrolyses of (**1a**) and (**1b**) were carried out at 2×10^{-2}M substrate in 40 ml of cytosolic preparation containing 13 mg protein ml^{-1} for 5 and 4 h, respectively.

In all cases the reactions were stopped by addition of NaCl and extraction with ethyl acetate (3 × 15 ml). The combined extracts were diluted to 50 ml in a volumetric flask, a proper amount of an ethyl acetate stock solution of cycloheptane-*trans*-1,2-diol was added to 5 ml of the extract and the yield of diol (**2a**) and (**2b**) determined by g.l.c. The remaining part of the extract was evaporated under reduced pressure and the residue chromatographed on a silica gel column using hexane–acetone (75:25) as eluant and monitoring the eluate by g.l.c. The diol containing fractions were combined and evaporated to give the pure diols (**2a**) and (**2b**), which were subjected to the measurement of the optical rotation.

All hydrolyses were also carried out with an identical procedure but using boiled cytosolic or microsomal preparations in order to evaluate the amounts of diols formed non-enzymatically.

Determination of Enantiomeric Excesses.—Samples of diols (**2a**) or (**2b**) (5—30 mg), obtained by enzymatic hydrolysis of epoxides (**1a**) or (**1b**), were dissolved in pyridine (0.5—2 ml) with a fivefold molar excess of (+)-(*S*)-MTPACl and left at room temperature for 2 days. The reaction mixtures were then diluted with 10% HCl and extracted with ethyl acetate. The extracts, washed with saturated $NaHCO_3$ and dried ($MgSO_4$), were evaporated under reduced pressure and the residues analyzed by h.p.l.c. [conditions (*a*) for (**3**), conditions (*b*) for (**5**)]. Two peaks, corresponding to the diastereoisomeric bis(MTPA)-esters (**3**) or the mono(MTPA)-esters (**5**), were detected. Retention times: (**3**), 8.5 and 10.2 min; (**5**), 19.6 and 20.7 min. The ratios of these peaks are reported in Table 2 or given in the text. The identification of (**3**) and (**5**) were based on the n.m.r. and i.r. spectra of the 1:1 mixtures of diastereoisomeric products obtained from racemic samples of (**2a**) and (**2b**) and (+)-(*S*)-MTPACl. (**3**); δ_H (CCl_4) 1.4—2.0 [8 H, $(CH_2)_4$], 3.5 (6 H, s, 2 OMe), 5.3 (2 H, m, CHO), and 7.5 (10 H, m, ArH). (**5**); $\nu_{max.}$(nujol) 3 480 and 1 735 cm^{-1}; δ_H (CCl_4) 0.95 and 1.05 (3 H, 2 s, 2 × Me), 1.2—2.0 [8 H, $(CH_2)_4$], 2.35 (1 H, s, OH), 3.5 (3 H, s, OMe), 4.8 (1 H, m, CHO), and 7.2—7.75 (5 H, m, ArH). The same mono(MTPA)-esters (**5**) were also obtained in a 50:50 ratio (h.p.l.c.) when (±)-(**2b**) was reacted with a ten-fold excess of (+)-(*S*)-MTPACl.

Determination of Absolute Configurations.—*A. p*-Methoxybenzoyl chloride (117 mg, 0.69 mmol) was added to a solution of (−)-(**2a**) (70% e.e.; 20 mg, 0.17 mmol) in pyridine (2 ml). After 4 days at room temperature the reaction mixture was diluted with 10% HCl and extracted with ethyl acetate (3 × 10 ml). The washed (saturated aqueous $NaHCO_3$) and dried ($MgSO_4$) extract was evaporated under reduced pressure and the residue was purified by preparative t.l.c. using hexane–ethyl acetate (70:30) as eluant to give the bis (*p*-methoxy)benzoate (**4**); $\nu_{max.}$(nujol) 1 720 cm^{-1}; $\lambda_{max.}$(CH_3CN) 257 nm (ε 36 150); $\Delta\varepsilon_{263}$ −29.9 and $\Delta\varepsilon_{246}$ +19.5 (CH_3CN); δ_H (CCl_4) 1.5—2.1 [8 H, $(CH_2)_4$], 3.8 (6 H, s, 2 × OMe), 5.4 (2 H, m, 2 × CHO), and 6.9 and 8.0 (8 H AA′BB′ system, ArH).

B. A solution of (**2b**) ($[\alpha]_D^{25}$ −7.5°; 50% e.e.; 21 mg, 0.16 mmol) and *p*-methoxybenzoyl chloride (200 mg, 1.15 mmol) in 0.3 ml of pyridine was refluxed for 3 h and worked up as described above. Preparative t.l.c. of the reaction product (hexane–acetone, 75:25) gave the pure bis (*p*-methoxy)benzoate (**6**); $\nu_{max.}$(Nujol) 1 700 cm^{-1}; $\lambda_{max.}$(CH_3CN) 256 nm (ε 36 300); $\Delta\varepsilon_{263}$ −6.6 and $\Delta\varepsilon_{245}$ +2.8 (CH_3CN); δ_H ($CDCl_3$) 1.45—2.5 [8 H, $(CH_2)_4$], 1.7 (3 H, s, Me), 3.95 (6 H, s, 2 × OMe), 5.6 (1 H, m, CHO), and 6.85—7.15 and 7.9—8.25 (8 H, 2 overlapping AA′BB′ systems, ArH).

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