Enantioselectivity of the Microsomal Epoxide Hydrolase Catalyzed Hydrolysis of *trans*-4,5-Dimethyl-1,2-epoxycyclohexane

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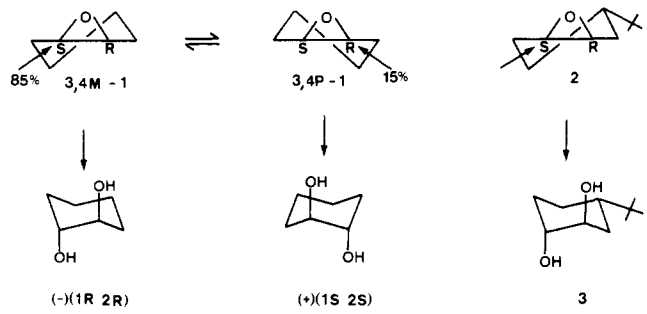
The hydrolysis of the racemic and enantiomeric forms of *trans*-4,5-dimethyl-1,2-epoxycyclohexane, catalyzed by rabbit liver microsomal epoxide hydrolase (EH), has been investigated to clarify further the mechanism of enantioselection by this enzyme. Both acid-catalyzed and enzymatic hydrolysis yielded exclusively the diol **6** derived from trans diaxial opening of the oxirane ring. Enzymatic hydrolysis of the racemic substrate under saturation conditions was moderately enantioselective, the (−)-epoxide being attacked preferentially to yield the (−)-diol. After 50% conversion an increase in the hydrolysis rate was observed. Incubations of the individual epoxide enantiomers showed that the (+)-(1*R*,2*S*,4*S*,5*S*)-epoxide is hydrolyzed at a higher rate than its antipode. These results confirm that substrates with 3,4 *M* helicity of the six-membered ring fit better into the EH active site and prove that the enantioselection must mainly be due to a lower K_m rather than to a higher V_{max} for the preferentially hydrolyzed enantiomer or conformer.

The conversion of epoxides into *trans*-diols catalyzed by the microsomal epoxide hydrolase (EH) is an important step in the metabolism of xenobiotic compounds.[1] Recent data from this laboratory have shown that this enzyme is remarkably regio-, enantio-, and diastereoselective in its action on substrates containing an oxirane function fused to a six-membered ring.

On the basis of our and other related work we have made two observations about the relation between substrate structure and stereoselectivity of EH-promoted hydrolysis.

(1) The enzymatic hydrolysis occurs with preference for the substrate conformer or enantiomer in which the six-membered ring has 3,4 *M* helicity. This can be deduced from the enantioselective formation of (*R*,*R*)-*trans*-cyclohexane-1,2-diol (**1**) in the EH-promoted hydrolysis of cyclohexene oxide[2,3] and from the fact that both enantiomers of 3,4-epoxytetrahydropyran are similarly hydrolyzed to only the optically pure (3*R*,4*R*)-diol.[4] These facts require preferential attack on the conformer of 3,4 *M* helicity of the former substrate and on the enantiomers with the equivalent 1,2 *M* helicity of the latter, based on the likely hypothesis that ring opening involves a general base-catalyzed mechanism[5] and therefore takes a diaxial (antiparallel) course. Further support comes from the hydrolysis of conformationally rigid substituted epoxycyclohexanes[3,6] and epoxytetrahydropyrans,[7] where the enzyme exhibits a preference for the enantiomer of 3,4 *M* (or 1,2 *M*) helicity. Such behavior can be explained on the basis of one of the following hypotheses: (a) preferential binding (lower K_m) of the substrate in its 3,4 *M* (or 1,2 *M*) conformation to the EH active site, or (b) more rapid formation of the (*R*,*R*)-diol from this conformer (higher V_{max}).

(2) In substituted epoxycyclohexanes and 3,4-epoxytetrahydropyrans the enantiomer in which the lipophilic substituent is placed to the right of the oxirane ring, viewing the molecule with oxygen on the front topside, is a better substrate than its antipode. For instance, enantiomer **2** of *trans*-4-*tert*-butyl-1,2-epoxycyclohexane is hydrolyzed by EH under saturation conditions about 40 times more rapidly than its antipode to give the diaxial diol **3**.[3] This preference can be explained by a large hydrophobic pocket situated to the right backside of the epoxide oxygen-binding site of EH. The enantioselectivity of EH toward some carcinogenic polycyclic arene oxides can also be accounted for by this hypothesis.[8]



We have now investigated the EH-promoted hydrolysis of *trans*-4,5-dimethyl-1,2-epoxycyclohexane (**5**), the enantiomers of which are rigid models for the two conformers of **1**. The presence of two identical substitutents symmetrically situated with respect to the oxirane ring was expected to cancel the substituent effect discussed under **2**, thus allowing an estimate of the contribution of the helicity effect to the enantioselectivity of EH and discrimination between the two alternative hypotheses a and b.

The racemic epoxide **5** was prepared by epoxidation of 4,5-dimethylcyclohexene, obtained from 4-cyclohexene-1,2-dicarboxylic acid (**4**), through the sequence of Scheme I.[9] Resolution of (±)-**4** with quinidine gave, as reported, the (−)-(*R*,*R*)-form.[10] The (+)-(*S*,*S*)-enantiomer was obtained by further resolution with cinchonidine of the partially enriched acid recovered from the mother liquor of the first resolution. Both enantiomers of **4** were subjected to the sequence of Scheme I to give the corresponding optically active epoxides **5**. The configurations of the enantiomers of **5** as (−)-1*S*,2*R*,4*R*,5*R* and (+)-1*R*,2*S*,4*S*,5*S* were deduced from those of **4**,[11] in agreement

(1) Oesch, F. *Xenobiotica* **1973**, *3*, 305.
(2) Jerina, D. M.; Ziffer, H.; Daly, J. W. *J. Am. Chem. Soc.* **1970**, *92*, 1056.
(3) Bellucci, G.; Berti, G.; Ingrosso, G.; Mastrorilli, E. *J. Org. Chem.* **1980**, *45*, 299.
(4) Bellucci, G.; Berti, G.; Catelani, G.; Mastrorilli, E. *J. Org. Chem.* **1981**, *46*, 5148.
(5) Bellucci, G.; Berti, G.; Ferretti, M.; Marioni, F.; Re, F. *Biochem. Biophys. Res. Commun.* **1981**, *102*, 838 and references cited therein.
(6) Bellucci, G.; Berti, G.; Bianchini, R.; Cetera, P.; Mastrorilli, E. *J. Org. Chem.* **1982**, *47*, 3105.
(7) Catelani, G.; Mastrorilli, E. *J. Chem. Soc., Perkin Trans. 1* **1983**, 2717.
(8) Armstrong, R. N.; Kedzierski, B.; Levin, W.; Jerina, D. M. *J. Biol. Chem.* **1981**, *256*, 4726.
(9) Rickborn, B.; Lwo, S.-Y. *J. Org. Chem.* **1965**, *30*, 2212.
(10) Kokke, W. C. M. C.; Varkevisser, F. A. *J. Org. Chem.* **1974**, *39*, 1535.

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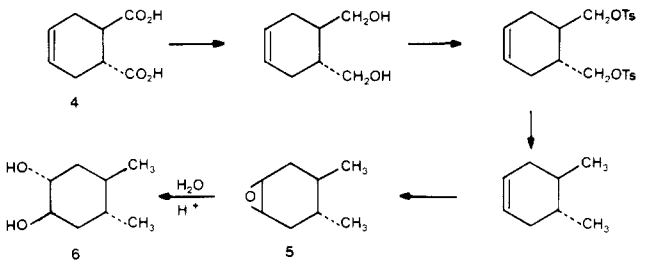
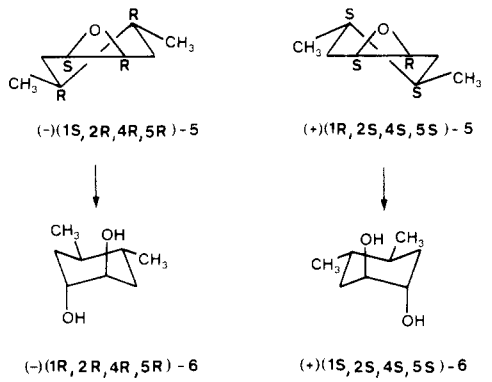
Scheme I



with a previous proposal for (−)-**5** based on chiroptical and chemical correlations.[12]



Acid-catalyzed hydrolysis of racemic **5** and of its individual enantiomers gave exclusively the trans-diaxial diols **6**, the relative configurations of which were deduced from the narrow signal for protons α to OH in their NMR spectra. Optical purities of the enantiomeric diols **6** (and consequently of the epoxides from which they were derived) were deduced from the NMR spectra of their diacetates with the aid of an optically active shift reagent, as well as more accurately by HPLC analysis of the bis[α-methoxy-α(trifluoromethyl)phenylacetic (MTPA) ester][13] of **6**. Both methods showed that (−)-**6**, $[\alpha]_D$ −20.1°, obtained by hydrolysis of (−)-**5**, $[\alpha]_D$ −70.3°, was optically pure within the limits of sensitivity of the second method (not more than 1% enantiomer).

Enzymatic hydrolyses of the racemic epoxide **5** and of its two enantiomers were carried out at pH 9.0 with liver microsomes of phenobarbital-pretreated rabbits, at substrate concentrations exceeding K_m[14] in order to ensure active site saturation and to make V_{max} values for the three substrates comparable. As with the acid-catalyzed reaction, enzymatic hydrolysis gave the diaxial diols **6** as the only products.

The EH-promoted hydrolysis of racemic **5** was followed to over 90% conversion by determining the product diol **6** at given intervals through direct injection of the incubation mixture into the GLC column. The resulting curve (Figure 1) has a typical biphasic shape with an increase in rate after 50% conversion, which points to enantioselection by the enzyme. This was confirmed by analyzing the diol from preparative incubations by the MTPA ester method (Table I). An excess of the (−)-(*R*,*R*,*R*,*R*)-diol

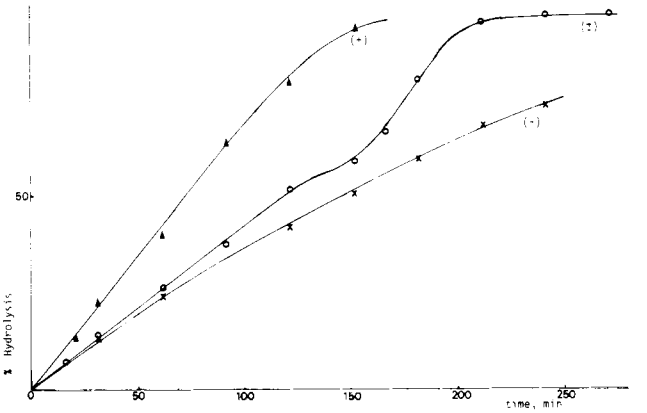


Figure 1. Time course of enzymatic hydrolysis of epoxide **5**.

Table I. EH-Catalyzed Hydrolysis of (±)-Epoxide 5

incubation time, min	% hydrolysis[a]	$[\alpha]^{20}_D$ of **6** (EtOH)	ee[a,b]
60	19		40
90	27		30
180	55	−4.8°	22
500	100	0.0°	0

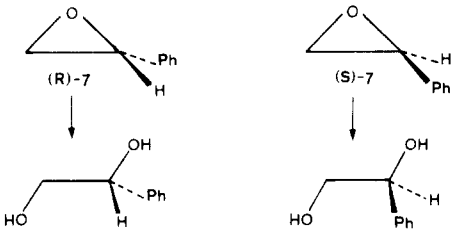
[a] Error limits ±2%. [b] Determined by HPLC of MTPA derivatives.

(40% ee) was formed after 19% conversion of the substrate, and the ee decreased with increasing conversion. After complete hydrolysis racemic **6** was obtained. Therefore, although both enantiomers of epoxide **5** are substrates for EH, (−)-(1*S*,2*R*,4*R*,5*R*)-**5** is preferentially hydrolyzed when using the racemic mixture as the substrate. Enzymatic hydrolysis of the single enantiomers of **5**, on the other hand, produces the normal hyperboloid curves shown in Figure 1. The (−)-enantiomer is hydrolyzed at a rate very close to the initial rate for the racemate, whereas the (+)-enantiomer reacts at a higher rate corresponding to that approached in the second phase of the racemate hydrolysis. In both cases the optically pure enantiomers of **6** were isolated from the incubation mixture. Although the rates of hydrolysis (V_{max}) obtained with different lots of microsomes changed within a range of ±20%, the ratio of rates for (+)- and (−)-**5** was constantly 1.8 ± 0.1%.

This result can be explained by assuming that (−)-**5** fits better into the EH active site than its enantiomer and gives a more stable enzyme–substrate (ES) complex (lower K_m) that releases the product more slowly (lower V_{max}). Compound (+)-**5** would be bound less tightly to the enzyme (higher K_m) and hydrolyzed more rapidly (higher V_{max}), but its enantiomer could act as a competitive inhibitor as long as it is present, owing to its higher affinity for the active site. It has so far not been possible to confirm this hypothesis by direct determination of the K_m of (+)- and (−)-**5** because of difficulties in finding a sufficiently sensitive analytical technique for very low concentrations of **6**. However, recent observations of Watabe[15] on the hydrolysis of styrene oxide with EH are very similar to ours on **5**. (*R*)-Styrene oxide (**7**) has lower K_m and V_{max} than (*S*)-**7**, and the rate curves for (*R*)-, (*S*)-, and racemic-**7** are very similar to those shown in Figure 1 for (±)-, (+)-, and (−)-**5**. Analogous data were obtained by Hanzlik[16] for *p*-nitrostyrene oxide. Even in the absence of K_m data for

(11) Walborsky, H. M.; Barash, L.; Davis, T. C. *Tetrahedron* **1963**, *19*, 2333.
(12) Milhavet, J. C.; Salayrolles, C.; Chapat, J.-P.; Granger, R. *J. Chem. Res. Synop.* **1978**, 291.
(13) Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 2543.
(14) Values of K_m ranging between 2 μM and 0.7 mM have been reported for EH with different substrates: Bentley, P.; Oesch, F.; Tsugita, A. *FEBS Lett.* **1975**, *59*, 296. Levin, W.; Et al. *J. Biol. Chem.* **1980**, *255*, 9067. Concentrations used in the present work were in the order of 40 mM or higher.
(15) Watabe, T.; Oshikawa, N.; Yoshikava, K. *Biochem. Pharmacol.* **1981**, *30*, 1695. Watabe, T.; Ozawa, N.; Hiratsuka, A. *Ibid.* **1983**, *32*, 777.
(16) Westkaemper, R. B.; Hanzlik, R. P. *Arch. Biochem. Biophys.* **1981**, *208*, 195.

5 this similarity in the behavior of such different substrates strongly implies the same mechanistic explanation. This mechanism can probably be generally applied to account for the enantioselection in EH-promoted hydrolyses of racemic epoxides in which the absence of steric effects makes both enantiomers susceptible to the enzymatic addition of water.[17] Accordingly, hypothesis a presented under 1, which assumes a better fit of the preferentially hydrolyzed conformer into the EH active site, appears to be the valid one: the (−)-enantiomer of **5** has 3,4 *M* helicity in its more stable conformation with equatorial methyl groups and binds better to the active site (lower K_m). However, it is converted more slowly into product than its enantiomer when used as an enantiomerically pure substrate (lower V_{max}). The limited enantioselection observed with (±)-**5** is not unexpected if one considers the small size and symmetrical disposition of the substituents. The substituent effect discussed under 2 does not play a role, and enantioselection is mainly due to the ring helicity effect.



The structural hypothesis presented under 2 can be extended to epoxides derived from nonendocyclic olefins, such as styrene oxides. One may assume that the larger substituent on the epoxide ring must fit into the hydrophobic cavity situated at the right backside of the three-membered ring to give the more stable ES complex. Only (*R*)-styrene oxide (**7**) meets this requirement since in the (*S*)-enantiomer the phenyl group can only be oriented on the right frontside or on the left backside. It may therefore be worth while to extend studies of EH enantioselectivity to a wider range of racemic nonendocyclic epoxides in order to establish the generality of this working hypothesis.

Experimental Section

Melting points were taken on a Kofler block and are uncorrected. IR spectra were recorded on a Perkin-Elmer 257 spectrophotometer. NMR spectra were taken in $CDCl_3$ on a Varian CFT-20 spectrometer. Optical rotations were measured on a Perkin-Elmer 241 photoelectric polarimeter. HPLC analyses were performed with a Varian 5000 instrument on a 4.5 × 250 mm, 10 μm silica column, with 98:2 hexane–THF, monitoring at 254 nm. GLC analyses were carried out with a Dani 3800 instrument on a 1.80 m × 3 mm glass column packed with 10% Carbowax 20M on silanized 80–100 mesh Chromosorb W, programmed temperature 120–200 °C, 8 °C min, injector temperature 250 °C, and N_2 flow 30 mL/min.

Optical Resolution of *trans*-4-Cyclohexene-1,2-dicarboxylic Acid (4). The method of Kokke and Varkevisser,[10] using quinidine as resolving agent, appeared simpler than a previous one of Walborsky[11] requiring the combined use of quinidine and cinchonine to obtain (−)-**4** but did not give in our hands the reported yields. Results were erratic, more than one type of salt being apparently involved, but it was possible to obtain after three crystallizations from EtOH/H_2O of the initial bis(quinidinium salt)[10] a salt that yielded on treatment with NaOH and then with HCl (see below) (1*R*,2*R*)-**4**: $[\alpha]^{20}_D$ −147°; mp 138–140 °C. This acid, and also products of lower purity, $[\alpha]^{20}_D$ −135° to −145°, underwent a further enrichment through crystallization from acetone-benzene to give a product: $[\alpha]^{20}_D$ −157° (*c* 1.8, EtOH); mp 143–145 °C (lit.[11] $[\alpha]_D$ −160° (*c* 2.7, EtOH); mp 144–146 °C). Yields of pure product ranged between 30 and 40% of one antipode.

Recovery of the acid from the combined mother liquors of the crystallizations of the quinidine salts, followed by one crystallization from acetone–benzene, gave an acid $[\alpha]_D$ +94°. This product (6.5 g, 38.2 mmol) and cinchonidine (22.5 g, 76.4 mmol) were dissolved in boiling ethanol (100 mL). On cooling the salt slowly crystallized to give 14.1 g of product, $[\alpha]^{20}_D$ −58.8°; optical rotation did not change on recrystallization from ethanol. The salt was stirred for 3.5 h in a biphasic system of $CHCl_3$ (40 mL) and 20% NaOH (15 mL). The aqueous phase was acidified with 15% HCl and repeatedly extracted with ethyl acetate. The dried ($MgSO_4$) extract was evaporated to give 3.2 g of pure (*S*,*S*)-**4**: $[\alpha]^{20}_D$ +157° (*c* 1.17, EtOH); mp 143–145 °C (65% recovery of the excess enantiomer present in the starting material).

***trans*-4,5-Dimethyl-1,2-epoxycyclohexane (5).** The racemic epoxide was prepared from the dimethyl ester of **4**, through reduction with $LiAlH_4$ in ether to *trans*-4,5-bis(hydroxymethyl)cyclohexene[18] (82% yield), conversion into the ditosylate[18] (80% yield), and reduction with $LiAlH_4$ in *N*-methylmorpholine[10] to give 4,5-dimethylcyclohexene (65% yield). The latter compound (2 g, 18 mmol) in CH_2Cl_2 (20 mL) was treated with 86% *m*-chloroperbenzoic acid (4.1 g, 20 mmol), the solution was left 12 h at room temperature, washed with 10% NaOH, dried ($MgSO_4$), and evaporated through a short Vigreux comumn, and the residue was distilled to give (±)-**5** (2.05 g, 90% yield): bp 58 °C (18 mmHg); ^1H NMR δ 0.81 (m, 6 H, CH_3), 1.20–2.30 (m, 6 H), 3.12 (m, 2 H).

The enantiomeric epoxides were similarly prepared from (−)- and (+)-**4** except that, in order to avoid an esterification step, the free acids were directly reduced by 4 h reflux in THF with $LiAlH_4$ (2 equiv). The diols, obtained in 90% yield, were converted into the epoxides as reported above for the racemic compound. (−)-(1*S*,2*R*,4*R*,5*R*)-*trans*-4,5-Dimethyl-1,2-epoxycyclohexane: $[\alpha]^{22}_D$ −70.3° (*c* 1.0, EtOH). (+)-(1*R*,2*S*,4*S*,5*S*)-enantiomer: $[\alpha]^{22}_D$ +69.5° (*c* 0.65, EtOH); (lit.[12] $[\alpha]^{22}_D$ −65° (*c* 2.5, EtOH).

***c*-4,*t*-5-Dimethylcyclohexane-*r*-1,*t*-2-diol (6).** A suspension of (±)-epoxide **5** (50 mg) in 0.1 N $HClO_4$ was stirred 24 h at room temperature. The homogeneous solution was extracted with ether (3 × 10 mL) and the dried ($MgSO_4$) extract was evaporated to give a quantitative yield of the diol **6**: single peak in GLC; mp 87–89 °C, after crystallization from hexane; ^1H NMR δ 0.96 (m, 6 H, CH_3), 1.60 (m, 6 H, CH_2), 1.70 (s, 2 H, OH), 3.73 (m, 2 H, $W_{1/2}$ = 8 Hz, CHO). Anal. Calcd for $C_8H_{16}O_2$: C, 66.7; H, 11.1. Found: C, 66.9; H, 11.3.

The same reaction, carried out on (−)-**5**, gave (−)-(1*R*,2*R*,4*R*,5*R*)-**6**: $[\alpha]^{25}_D$ −20.1° (*c* 1.6, EtOH).

Determination of Enantiomeric Excess of 6. (a) The sample of **6** was converted into the corresponding diacetyl ester by dissolving it in acetic anhydride and leaving it 24 h at room temperature. Treatment with 2 N HCl, extraction with ethyl acetate, washing with saturated $NaHCO_3$, drying ($MgSO_4$), and evaporation gave the pure diacetate (GLC). In the ^1H NMR spectrum of racemic diacetate the acetyl proton signal appeared as a singlet δ 2.05; after addition of Eu(facam)$_3$ (10 mg for 3 mg of diacetate) this signal was shifted and split into two singlets of equal intensities at δ 2.36 and 2.39. The analogous spectrum of the diacetate obtained from partially resolved **6**, $[\alpha]^{20}_D$ −5.0°, corresponding to an optical purity of 25%, showed the same peaks but in a ratio of about 65:35, indicating an ee of about 30%. The small separation of the AcO singlets limits the accuracy of this method.

(b) The sample of (±)-**6** (20 mg, 0.14 mmol) and MTPA chloride (70 mg, 0.28 mmol) in pyridine (1 mL) was stored at room temperature for 12 h, diluted with water, and extracted with ether; the extract was washed with 2 N HCl and saturated Na_2CO_3, dried ($MgSO_4$), and evaporated. The residue of bis-MTPA ester (complete esterification checked by IR) showed two well-separated HPLC peaks of equal areas (retention times 10.45 and 11.04 min).

(17) A similar enantioselection involving a biphasic reaction curve has recently been observed for (±)-*cis*-3-bromo-1,2-epoxycyclohexane: Bellucci, G.; Ferretti, M.; Lippi, A.; Marioni, F.; Palla, A. "IV Convegno Nazionale della Divisione di Chimica Farmaceutica della S.C.I.", Palermo, Oct 18–22, 1983; Abstracts p 88.

(18) Casadevall, E.; Largeau, C.; Moreau, P. *Bull. Soc. Chim. Fr.* **1968**, 1514.

The same procedure, when applied to the (-)-diol, $[\alpha]_D$ -20.1°, gave exclusively the peak with 11.04-min retention time; from a diol, $[\alpha]^{20}_D$ -4.8° (optical purity 23%), the two peaks were present in a ratio of 61:39, corresponding to an ee of 22%.

Microsomal Preparations. Liver microsomes were prepared from phenobarbital-pretreated male New Zealand white rabbits as previously described,[3] suspended in 0.1 M Tris-HCl buffer (pH 9.0) to a final protein concentration of ca. 15 mg/mL, and stored at -40 °C. A single lot of microsomes was used as far as possible for incubation with the different substrates. Enzymatic activity was checked before every series of runs and whenever a new lot of microsomes had to be used, in order to compensate for any loss of activity during storage or for differences in the activity of different lots. Compound (-)-**5** was used as the standard substrate for these activity tests.

Incubations. The following standard procedure was used to follow the conversion of the racemic epoxide **5** and of its enantiomers into the diol **6**: samples of epoxide (7.5 mg in 120 μL of CH_3CN), drawn from a stock solution, were added to a series of flasks containing the microsomal preparation (1.5 mL) preheated at 37 °C, and the suspension was incubated with shaking. At given times single flasks were withdrawn and immediately cooled in acetone–dry ice at -40 °C. Analysis was carried out by adding *trans*-1,2-cyclohexanediol (3.85 mg in 100 μL of H_2O) as internal standard and injecting into the GLC column immediately after thawing. The amount of diol **6** was deduced from a comparison of the areas of the corresponding peak with that of the standard, after applying the correction factor obtained from known artificial mixtures of **5**, **6**, and *trans*-1,2-cyclohexanediol; the values should be accurate within ±2%.

The amounts of formed diol (averages of two determinations) for each of the epoxides (±)-**5**, (+)-**5**, and (-)-**5** are represented by the curves in Figure 1. Similar though slightly less accurate data were obtained from the integration of GLC peaks corresponding to the remaining epoxide **5**. No peaks other than those for the epoxide **5**, diol **6**, and standard were visible in the GLC tracings, a fact that pointed to the absence of reactions other than the hydrolytic trans opening of the epoxide ring.

Larger scale runs were carried out on 50–70 mg of (±)-**5** in 5 mL of microsomal suspension under the same conditions. After GLC determination of the ratio of **5**:**6** on a small sample of the incubated mixture, the remainder was saturated with NaCl and extracted with ethyl acetate (4 × 6 mL). The residue of the evaporated extract was subjected to complete sublimation under reduced pressure, converted into the bis-MTPA ester, and analyzed for diastereoisomeric ratio as described above. The results are shown in Table I. Optical rotation of the diol was determined on a sample that had been incubated for 180 min.

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Effect of Poly(methacrylic acid) Hypercoils on the Neutral and Acid-Catalyzed Hydrolyses of 1-Acyl-1,2,4-triazoles in Aqueous Solution

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Rates and thermodynamic activation parameters have been measured for the neutral and acid-catalyzed hydrolyses of the 1-acyl-1,2,4-triazoles **1**–**3** in the presence of atactic poly(methacrylic acid) (at-PMAA). Under the employed reaction conditions at-PMAA resides in a coiled, compact conformation. The rates of hydrolysis of the relatively hydrophilic 1-acetyl- (**1**) and 1-benzoyl-1,2,4-triazole (**2**) are only little affected by the presence of the polymer. By contrast, the hydrolysis of the hydrophobic 1-benzoyl-3-phenyl-1,2,4-triazole (**3**) is effectively inhibited as a result of binding of **3** to hydrophobic microdomains within the at-PMAA hypercoil. Only small rate retardations are found for the hydrolysis of **3** in the presence of poly(acrylic acid). The effect of at-PMAA concentration on the rates of hydrolysis of **3** can be described in terms of a kinetic scheme that is essentially a variant of Michaelis–Menten enzyme kinetic formalism. In the presence of at-PMAA, $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ for the neutral hydrolysis of **3** undergo large and partly compensatory changes, the retardations being dominated by the increase of $\Delta H^{\ddagger}$. These findings are interpreted by assuming reduced hydration of the dipolar transition state for hydrolysis in the relatively "dry" hydrophobic microdomains. Comparable results were obtained for the HCl-catalyzed hydrolysis of **3**. The inhibitory effect of at-PMAA on this reaction is attenuated in the presence of urea, presumably because of destabilization of the compact conformation of the polymer.

In recent years there has been considerable interest in hydrophobic microdomains within compact conformations of water-soluble polymers. These microdomains provide binding sites for sufficiently hydrophobic solutes and presumably mimic aspects of hydrophobic active sites in enzymes.[1,2] If catalytic functional groups are also present in the polymer, efficient enzyme-like catalysis, involving Michaelis–Menten kinetics, may be observed.[1] Polyelectrolytes carrying hydrophobic side chains (charged polysoaps) exhibit similar behavior.[3,4]

Relatively little attention has been paid to un-ionized polymers with no catalytic groups. These systems allow the study of purely microenvironmental effects, reflecting the hydrophobicity of the reaction site in the microdomain.[5] Examples include poly(methacrylic acid)[6]

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