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Substrate Selectivity and Stereochemistry of Enzymatic Epoxide Hydration in the Soluble Fraction of Mouse Liver[1]

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The relative rates and geometry of hydration of several substrates and other properties of a 100,000*g* soluble subcellular fraction epoxide hydrase(s) from mouse liver were examined. Mono-, di-, tri-, and tetrasubstituted alkyl epoxides which are insect juvenile hormone mimics are rapidly hydrated in the soluble fraction. For the trisubstituted epoxides the rate of hydration decreases as one of the alkyl chains is lengthened while the opposite trend is observed with most disubstituted epoxides. For the compounds examined the initial rate of hydration of alkyl-substituted epoxides by the soluble fraction is *cis*-di- $\lesssim$ mono- $\gtrsim$ *trans*-di- > tri- > tetrasubstituted. Two trisubstituted, a disubstituted, and a monosubstituted epoxide are rapidly hydrated by a single peak of enzyme activity from a gel filtration column. *cis*-Disubstituted epoxides are hydrated stereospecifically to their *threo*-diols and *trans*-epoxides to their *erythro*-diols, indicating *trans*-opening of the oxirane ring. Trisubstituted epoxides inhibit the hydration of di- and monosubstituted epoxides and *trans*-disubstituted epoxides inhibit the hydration of *cis*-disubstituted epoxides. The apparent K_m and V_{max} for one trisubstituted epoxide compare favorably with those reported for a number of substrates hydrated by the microsomal epoxide hydrase(s). Epoxide hydrase activity in the soluble and other subcellular fractions should thus be considered when examining the role of mammalian epoxide hydrases.

INTRODUCTION

Prodigious effort has gone into the study of mammalian microsomal epoxide hydrases because of their likely involvement in the metabolism of toxic, mutagenic, and/or carcinogenic xenobiotics (1–3). Many current reports on epoxide hydrases seem to assume that all significant enzyme activity is membrane bound, largely in the microsomal fraction. This assumption is based on investigations of the subcellular distribution of epoxide hydrase activity run with only a few substrates in a limited number of species (1). It is, in fact, the rare study that actually reports a subcellular distribution of epoxide hydrase activity on a new substrate (4).

A study of the degradative metabolism of the 6,7-epoxygeranylphenyl ether A (Table 1) was warranted because it is an insect juvenile hormone mimic (juvenoid) which was under consideration for development as an insecticide, and thus it is a potential environmental contaminant (5). During a study of the metabolism of this xenobiotic in mice, significant epoxide hydration was found in the 100,000*g* soluble fraction (6, 7). A subsequent investigation established the enzymatic nature of the hydration and that the enzyme was not tightly bound to a membrane (8). The soluble epoxide hydrase is inducible, present in a number of mammalian tissues, present in all mammals so far examined, and present in varying levels in male and female mice of several ages and strains (7, 9, 10). The following study was designed to examine some of the properties of the soluble epoxide hydrase from mouse liver. This and other investigations in progress will provide the scientific basis for the future assessment of the role of the mam-

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malian soluble epoxide hydrases in the hydration of epoxide containing pesticides and other xenobiotics.

MATERIALS AND METHODS

Substrate synthesis. Most of the substrates listed in Tables 1, 2, and 3 were synthesized from *trans* or *E* pure geraniol (**A–H**) or racemic citronellol (**I–P**) by published methods (10–15). The two geometrical isomers of the 1,2-disubstituted epoxides (**E–H, K–P**) were separated by preparative high-resolution liquid chromatography (hrlc) (tapered inlet column 10 mm I.D. × 50 cm, 10 μm silica gel, eluted with 5% ether in hexane) (Fig. 1). Each compound was found to be pure by thin-layer chromatography (tlc) and gas liquid chromatography (glc) although our techniques did not resolve the geometrical isomers of the trisubstituted epoxides (**C,D**). The geometrical purity of the disubstituted epoxides was additionally verified by hrlc which could easily detect 0.5% contamination by the opposite isomer. Structural assignments of substrates and diol standards were based on spectral data including at least proton nuclear magnetic resonance (pmr) and infrared spectra (ir). Structural assignments for the *cis*- and *trans*-isomers of the propyl 1,2-disubstituted epoxides (**G,H**) were additionally based on decoupled pmr spectra and continuous-wave and broad-band decoupled ^{13}C nuclear magnetic resonance spectra (15) as well as chemical methods illustrated in part in Fig. 1.

Two diol standards were prepared by acid hydration of the corresponding epoxide (**A,B**) and identified based on their pmr, ir, ultraviolet, and mass spectra as well as their chemical properties (13) while all other diol standards were prepared on a

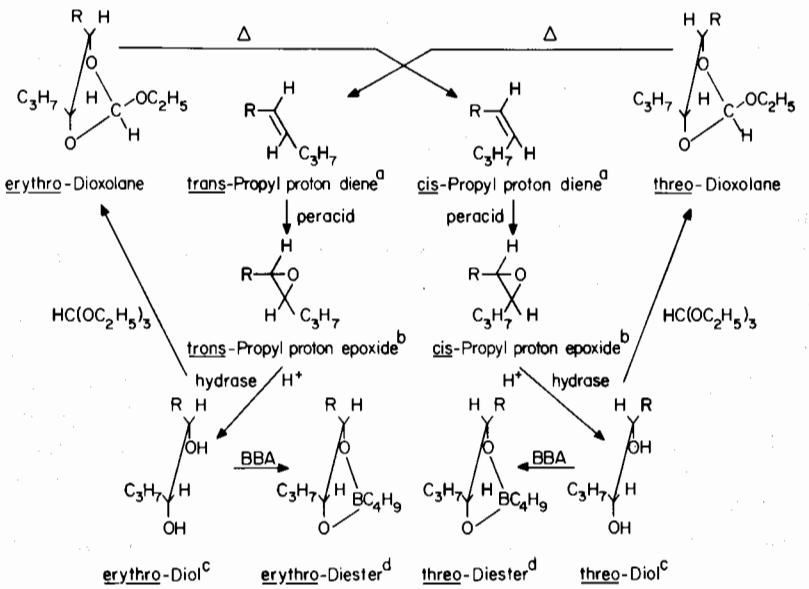
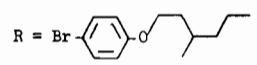


FIG. 1. *Proof of the stereochemistry of the epoxide hydration occurring in the soluble fraction of mouse liver homogenates.*



The dienes[a] were identified by glc. Stereospecific epoxidation with m-*chloroperbenzoic acid gives the* cis- *and* trans-*epoxides[b] which were isolated by hrlc with a stereochemical purity of >99%. Acid- or enzyme-catalyzed hydration yielded the* threo- *and* erythro-*diols, respectively,[c] which gave separate peaks on glc as their boronic diesters.[d] For confirmation the diols were converted through their dioxolane derivatives to the opposite dienes. Chiral compounds were equal mixtures of enantiomers.*

microscale as the sole detectable product from the weak acid hydration of the corresponding epoxide (15). The *threo-* and *erythro*-diols were prepared from the *cis-* and *trans*-epoxides, respectively. The structure of two of these diols was further confirmed chemically (Fig. 1).

For an estimation of the lipophilicity of the substrates the linear free energy parameter log P was used (P = [compound in octanol]/[compound in water]) (16). The log P values (Table 1) estimated from retention volumes from a reversed-phase hrlc column (2 mm × 250-mm column, 5 μm ODS silica, 25% water in methanol, v/v) (17) agreed closely with two values determined by the classical partition method (15) or calculated using π values determined from the partitioning of substituted phenoxyacetic acids (16).

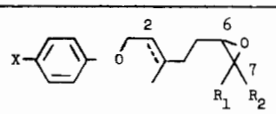
Enzyme preparation. Male adult specific pathogen-free Swiss–Webster (ICR strain, ~40 g) mice obtained from Hilltop Laboratories (Chatsworth, Calif.) were killed by cervical dislocation. The gallbladders were removed intact and discarded before removal of the livers which were subsequently immersed in ice-cold sodium phosphate buffer (pH 6.8, I = 0.2 M). The livers were dabbed dry with tissue before their weight was determined (~1.2 g each). The livers were homogenized in enough ice-cold phosphate buffer to give a 10% weight to volume suspension using a Potter Elvehjem tissue grinder and subsequently centrifuged for 20 min at 10,000g and 4°C to remove cellular debris, nuclei, and mitochondria. The 10,000g supernatant was filtered through glass wool to remove lipid and centrifuged at 100,000g for 60 min at 4°C. The "microsomal" pellet was resuspended in a volume of buffer equal to the volume of the 100,000g supernatant (soluble fraction). This procedure results in a microsomal solution equivalent to 10% liver weight to buffer volume and both the soluble and microsomal preparations were further diluted with the same buffer before use. The protein concentration of the microsomal and soluble fractions of each enzyme preparation were routinely determined by the method of Lowry *et al.* (18).

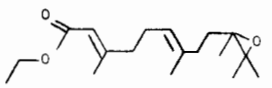
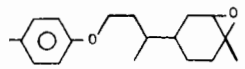
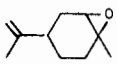
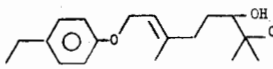
The 10% soluble fraction retained ≥80% of its epoxide hydrase activity for 10 days if stored on ice and for a month or more if frozen (−5°C), while the 10% microsomal fraction retained activity for only a few days if stored on ice and no activity if it was frozen. Gel filtration was routinely performed by applying 10% 100,000g soluble fraction to a 90 × 2.5-cm-I.D. column filled with Sephacryl S200 and eluted with sodium phosphate buffer (pH 6.8, I = 0.2 M) flowing at 17 ml/hr and monitored by its absorbance at 280 nm.

Analysis of epoxide hydrase activity. Routine analysis of hydrase activity was performed using a partition assay with radiolabeled **A** as substrate (Table 1) (19). The substrate in ethanol (1 μl) was added to the enzyme (100 μl) and incubated at 37°C for varying lengths of time. The reaction was halted by the addition of methanol (150 μl) and isooctane (250 μl), vortexed, centrifuged, and the radioactivity in the organic phase (epoxide) and methanol-aqueous phase (diol) analyzed by liquid scintillation counting. The method was verified by tlc procedures including the spotting of a tetrahydrofuran-quenched reaction mixture directly on cellulose prelayer tlc plates (Whatman LK5DF) and microchemistry (6–8). Electron-capture glc proved to be a very sensitive technique for the analysis of the bromine analogs (**B–P**). The appropriate amount of substrate was dissolved in freshly distilled ethanol (10 μl) and mixed with soluble fraction (1 ml) to yield a 3×10^{-5} M substrate concentration in a 10 × 75-mm glass culture tube (previously heated to 400°C, >6 hr). After incubation (37°C) ether-washed sodium chloride was added to saturation and the sample extracted with ether (1 × 0.75 ml and 1 × 0.5 ml, Burdick and Jackson glass-distilled grade). The ether extract was dried over ether-washed sodium sulfate, evaporated under nitrogen, and derivatized by 1-bu-

TABLE I

Designation, Structure, Lipophilicity, Initial Rate of Hydration, and Inhibition of Hydration of Mono-, Di-, Tri-, and Tetrasubstituted Epoxides



Compound Designation	Structure: X	Saturation 2,3	R_1	R_2	Geometry 6,7	Log P[a]	Initial Rate of Epoxide Hydration nmoles/min/mg Protein[b]	% Inhibition of Hydration of Compound A: Inhibitor 10X Substrate	Inhibitor 1X Substrate
A	C_2H_5	unsat	CH_3	CH_3	-	3.66	4.0	78	24
B	Br	unsat	CH_3	CH_3	-	3.50	2.8	81	26
C	Br	unsat	CH_3	C_2H_5	mix	3.97	1.9	93	33
D	Br	unsat	CH_3	n-C_3H_7	mix	4.46	1.0	90	39
E	Br	unsat	C_2H_5	H	cis	3.71	13.0	16	4
F	Br	unsat	H	C_2H_5	trans	3.71	12.6	28	9
G	Br	unsat	C_3H_7	H	cis	4.25	25.8	8	3
H	Br	unsat	H	C_3H_7	trans	4.25	12.6	30	10
I	Br	sat	CH_3	CH_3	-	4.00	1.3	100	35
J	Br	sat	H	H	-	3.48	17.4	12	8
K	Br	sat	CH_3	H	cis	3.82	26.0	56	11
L	Br	sat	H	CH_3	trans	3.82	11.3	64	13
M	Br	sat	C_2H_5	H	cis	4.28	33.1	71	15
N	Br	sat	H	C_2H_5	trans	4.28	12.3	83	17
O	Br	sat	n-C_3H_7	H	cis	4.73	53.4	62	14
P	Br	sat	H	C_3H_7	trans	4.73	17.5	66	16
Q					-	3.44	1.0	29	10
R					mix	-	-	80	5
S					-	-	-	2	0
T					OH (diol of A)	2.83	-	8	2

[a] *P* = [octanol]/[water] of respective compounds (16) estimated by reversed-phase hrlc (17, 19).

[b] Assays routinely performed with 0.25 mg/ml 100,000*g* soluble protein for the trisubstituted epoxides (B–D, I), 0.1 mg/ml for trisubstituted epoxide (A), and 0.05 mg/ml for the di- and monosubstituted epoxides (E–H, J–P) with all substrates at 3×10^{-5} *M*. B–P assayed based on relative peak area on a Hewlett–Packard 5710A electron-capture glc with a 3380A integrator. Column 1.5 mm i.d. × 6-ft, 2% OV101 on gas Chrom Q, methane/argon carrier 14 ml/min, oven 250°C, detector and injector 300°C. The relative initial rates of hydration of all compounds were the same on at least three separate mouse liver preparations. Compound A (^{14}C-ring-labeled, 17 mCi/mmol) was a gift of Stauffer Chemical Company, and it was assayed by the partition method (19) while Q (1-^{14}C-labeled, 11 mCi/mmol) was a gift of Hoffman–LaRoche & Company, and it was assayed by tlc.

taneboronic acid (BBA) added in isooctane (20 μg in 50 μl). After 30 min 1 μl of the sample in isooctane was analyzed by glc (Table 1) (15). This procedure recovered 50% of the epoxide and diol and the epoxide/diol ratio did not change after the first extraction. Quantative recovery (~98%) could be achieved by repeated ex-

traction. Preliminary studies indicate that identical procedures are applicable to the investigation of the substrate selectivity of insect epoxide hydrases.

Optimization of assay conditions. Assay conditions for 100,000*g* soluble and microsomal hydrases were established using **A** as substrate. Optimum pH and ionic strengths were determined by preparing the initial homogenate in 0.05 *M* KCl followed by dilution with buffers of varying pHs (5.8–9.8), ionic strengths (I = 0.05–0.4 *M*), and ions (sodium phosphate, ammonium chloride, and Tris·HCl). To determine the apparent K_m and V_{max} of the hydrases acting on **A** by the Lineweaver–Burke method (20) three replicates of six substrate concentrations from 2.4×10^{-6} to 2.2×10^{-5} *M* were incubated with 0.05% soluble fraction (50 μg protein/ml) and 5% microsomal fraction (500 μg protein/ml) from three separate enzyme preparations.

Time courses were run at 2×10^{-5}, and 4×10^{-4} *M* in addition to lower substrate concentrations to determine whether substrate solubility would affect the initial rate of epoxide hydration by the soluble fraction at higher substrate concentrations. A correspondingly higher enzyme concentration of 0.5% soluble fraction (500 μg protein/ml) was used.

Initial rate comparisons. The initial rates of hydrolysis of the epoxide substrates (3×10^{-5} *M*) to their respective diols by the 100,000*g* soluble fraction were determined either by the glc (**B–P**) or the partition method (**A**). The initial rates for the various substrates were not all obtained from the same enzyme preparation. Each enzyme preparation and each previously frozen sample of any preparation were assayed for activity with A using 0.1% soluble fraction and **B** using 0.25% soluble fraction. This procedure standardized the preparations before the rates for other substrates were determined.

Initial rates were calculated from plots of percentage diol formed vs time with at least three points in the linear region and each point determined from the average of three separate incubations. Substrates yielding similar initial rates were compared by reanalyzing them using the same enzyme preparation.

Inhibition of hydration. All of the compounds in Table 1 at 3×10^{-5} and 3×10^{-6} *M* were incubated with radiolabeled **A** at 3×10^{-6} *M* in 0.05% soluble fraction to determine whether they would inhibit hydration of this trisubstituted epoxide (**A**). The samples were incubated for 20 min and assayed by the partition method. Conversely, **A** was incubated as inhibitor using the trisubstituted dimethyl (**B**), the disubstituted *cis*-propyl (**G**), and the monosubstituted (**J**) epoxides as substrates. The substrates were incubated at 1.5×10^{-5} *M* with **A** added simultaneously at several concentrations and assayed by the glc method to determine the I_{50} by plotting the log of the concentration of **A** vs percentage inhibition of diol formation.

Geometry of epoxide hydration. The geometry of hydration was determined by incubating the soluble hydrase with the pure 1,2-disubstituted geometrical isomers (**E–H, K–P**) and comparing the glc retention times of product diols with the *threo*- and *erythro*-diols from acid cleavage of the *cis*- and *trans*-epoxides. For further confirmation of the geometry the *threo*-diol from the *cis*-epoxide was converted to its 2-ethoxy-1,3-dioxolane and subsequently thermally decomposed to the *trans*-diene; similarly the *erythro*-diol from the *trans*-epoxide was converted to the *cis*-diene (Fig. 1) (15, 21).

RESULTS AND DISCUSSION

The 10% mouse liver soluble and microsomal fractions prepared as described varied only slightly in protein concentration from preparation to preparation, averaging 10 mg/ml for soluble and 1 mg/ml for the microsomal. The rates of hydration of the substrates used were proportional to the concentration of protein and constant during the entire incubation periods used unless noted otherwise.

Among the buffer systems tested, the optimum for epoxide hydration of **A** was

found to be sodium phosphate at pH 6.8 for both the unwashed microsomal and soluble fractions. Variation in ionic strength resulted in very little change in epoxide hydrase activity in the case of either the microsomal or soluble fractions. Each fraction gave similar patterns of activation with a slight increase in activity at I = 0.067 and 0.15 *M* for the microsomal and I = 0.075 and 0.2 *M* for the soluble fraction. All further studies were done using pH 6.8 sodium phosphate buffer with I = 0.2.

A Lineweaver–Burke plot (20) for 0.05% soluble fraction with **A** as substrate had a linear correlation coefficient of 0.91 and indicated an apparent K_m of 2.0×10^{-6} *M* and a V_{max} of 2.4 nmol/min/mg protein (for unpurified 100,000*g* soluble fraction). The apparent K_m and V_{max} for the washed microsomal fraction was 8.7×10^{-6} *M* and 0.36 nmol/min/mg protein, respectively, with a linear correlation coefficient of 0.98. Although **A** is not the optimum substrate for either the microsomal (1) or the soluble fraction (Table 1) the above comparison supports our previous observation that expressed either on a tissue equivalent basis or as specific activity the soluble fraction hydrates this trisubstituted epoxide much faster (>50×) than the microsomal fraction. When compared to literature values for K_m and V_{max} of mammalian microsomal epoxide hydrases, the soluble epoxide hydrase consistently demonstrates a higher apparent affinity and much higher velocity per tissue unit (1, 3, 4, 22–25).

Kinetic studies with microsomal epoxide hydrases have often resulted in nonlinear inverse plots (25) and such was the case with early studies on the soluble epoxide hydrase (8). With a trisubstituted epoxide (**A**) as substrate the initial rate of hydrolysis increases as the substrate concentration increases until the critical micelle concentration (CMC) of the substrate is exceeded (CMC $\cong 1.2 \times 10^{-5}$ *M* in dHOH by surface tension measurements). Further additions of substrate fail to change the initial rate of the enzyme reaction even when more concentrated enzyme solutions are used; however, the additional substrate does cause an increase in the linear portion of the velocity vs time plots. Such data indicate that the epoxide hydrase acts only on the monomeric form of the substrate, but that it is not significantly inhibited by the micelle form of the substrate. A similar observation has been made for microsomal epoxide hydrases when phosphatidylcholine is added to the enzyme incubation (25).

The similar structure and lipophilicity (log *P*, Table 1) of **A** and the brominated analogs (**B**–**P**) indicated that high substrate levels could not be used for kinetic studies while the sensitivity of the glc method precluded determination of reliable initial rates at substrate concentrations below 1×10^{-5} *M*. Thus, classical kinetic studies could not be done with the brominated substrates and so they were compared using initial rates of hydrolysis by the soluble fraction (Table 1) and inhibition of hydration of four substrates (Tables 1 and 2).

Since a purified enzyme was not used for the initial rate or inhibition studies (Tables 1, 2, and 3) it is possible that the soluble epoxide hydrase activity observed is due to multiple enzymes. However, enzyme(s) hydrating epoxides eluted in a single sharp peak from a gel filtration column when the column fractions were monitored with trisubstituted epoxides (**A**,**B**), a disubstituted epoxide (**G**), and a monosubstituted epoxide (**J**). The elution volume was consis-

TABLE 2

Inhibition of Hydration of a Mono-, Di-, and Trisubstituted Epoxide by the Trisubstituted Epoxide, R-20458

Substrate	I_{50} (*M*)
Monosubstituted-proton proton (J)	9×10^{-6}[a]
Disubstituted-*cis*-propyl proton (G)	5×10^{-6}
Trisubstituted-methyl methyl (B)	1×10^{-4}

[a] All substrates were incubated with 0.05% soluble fraction at 1.5×10^{-5} *M* and exposed to three replicates of at least five different concentrations of the epoxygeranylphenyl ether **A**.

TABLE 3

Hydrolysis Rates for Pure cis- *and Pure* trans-*Propyl Proton-Substituted Epoxide (G,H) and a 1:1 Mixture of the Geometrical Isomers*

Composition	Hydrolysis rates (nmol/min/mg)			
	1.5×10^{-5} *M* substrate		3.0×10^{-5} *M* substrate	
100% *trans*	8.5		12.6	
100% *cis*	21.5		25.8	
50% *trans*	7.1	10.9	8.3	10.9
50% *cis*	3.8		2.6	

tent with a molecular weight of 130,000 (9). These studies indicate that the mono-, di-, and trisubstituted epoxides tested are hydrated by enzymes of similar or identical Stokes' radii. The competitive inhibition of hydration observed among the epoxide-containing substrates is further indication that similar or identical enzymes are involved.

Initial rates of hydration of 17 epoxide-containing substrates are presented in Table 1. It was previously shown that a tetrasubstituted epoxide is hydrated more slowly than a trisubstituted epoxide (8). Among the trisubstituted epoxides chemical changes in the substrate remote from the oxirane moiety cause measurable change in the rate of hydration. The *p*-bromo analog (**B**) is hydrated at ~70% the rate of the *p*-ethyl epoxide (**A**). Saturation of the 2,3-olefin causes a 50% reduction in the hydration rate of the trisubstituted epoxide (**I**), while the rate of hydration usually increases with saturation of the disubstituted epoxides (**E**−**H** vs **K**−**P**). Saturation of the 2,3-olefin causes a surprisingly large increase in log *P* and a reduction in the rate of epoxide hydration by aqueous acid, and it was speculated that saturation permitted conformations which allow the epoxide to exist in a more hydrophobic environment (15). A myriad of other possibilities exist to explain changes in rates of enzyme-catalyzed hydration including creation of an asymmetrical center at C-3.

As one alkyl substituent of the trisubstituted epoxide is lengthened, the initial rate of enzymatic hydration decreases: methyl (**B**) > ethyl (**C**) > *n*-propyl (**D**). In both the 2,3-saturated and olefinic series of compounds the disubstituted epoxides (**E**−**H**, **K**−**P**) are hydrated faster than trisubstituted epoxides (**B**−**D**, **I**). In contrast to the trisubstituted series, the initial rate of hydration is either the same or it increases with increasing length of the alkyl chain (**E**<**G**, **F**=**H**, **K**<**M**<**O**, **L**<**N**<**P**), and increases with 2,3-saturation in both the *cis*-and *trans*-series except for **F** vs **H**. These observations imply hydrophobic interaction between the enzyme and the substrate on both sides of the epoxide moiety. In contrast, the microsomal epoxide hydrase is speculated to have a single hydrophobic site (1). However, more than simple hydrophobicity of the substrate is involved in enzyme−substrate interaction because several of the trisubstituted epoxides demonstrate log *P* values similar to those of the disubstituted epoxides. For the geometrically pure isomers (**G**, **K**, **M**, **O**, vs **H**, **L**, **N**, **P**) the *cis*-isomer is hydrated much faster than the *trans*-isomer. The monosubstituted epoxide (**J**) is hydrated more rapidly than one *cis*-disubstituted (**E**), most of the *trans*-disubstituted (**F**, **H**, **L**, **N**), and all of the tri- and tetrasubstituted epoxides studied. Within the limitations of this report, the initial rate of hydration for the soluble epoxide hydrase appears to be *cis*-di- ≲ mono- ≳ *trans*-di- > tri- > tetrasubstituted epoxides.

A comparison of the hydrolysis of many of the compounds in Table 1 in dilute aqueous acid demonstrated that for trisubstituted epoxides ease of hydrolysis decreases with the same trends observed with enzymatic hydration (methyl [**B**] > ethyl [**C**] > propyl [**D**]) (15). In contrast to the situation observed with enzymatic hydrolysis, lability to aqueous acid decreases with increasing chain length of disubstituted epoxides. A carbonium ion-like intermediate probably involved in acid-catalyzed epoxide hydration predicts that for alkyl-substituted epoxides, epoxides which can potentially form a stable tertiary carbonium ion should be hydrated much faster than those which cannot (15, 26). The relative rates of hydration of the compounds in Table 1 by the soluble epoxide hydrase are not consistent with an acid-catalyzed mechanism of hydration.

When selected *p*-bromophenyl compounds (Table 1) were assayed as inhibitors of the hydrolysis of **A** by the soluble fraction only the trisubstituted epoxides (**B**–**D**, **I**) were found to cause significant inhibition. It was earlier shown that neither thiaranes nor the classical microsomal epoxide hydrase inhibitors trichloropropene oxide and cyclohexene oxide significantly inhibited the hydration of **A** (7). The 1,2-epoxide of limonene (**S**) and the substituted cyclohexane epoxides (**R**) (Table 1) failed to inhibit hydration of **A** although they are moderate inhibitors of some insect epoxide hydrases. The diol product (**T**) from the hydration of **A** also failed to inhibit hydration, indicating that, at least for the trisubstituted epoxides, product inhibition is negligible. The trisubstituted epoxide (**A**) was found to inhibit the hydration of tri-, di-, and monosubstituted epoxides (**B**, **G**, **J**) with the monosubstituted epoxide inhibited most strongly. When assayed as pure isomers the *cis*-disubstituted epoxides are usually hydrated much faster than are the *trans*-epoxides, but when the propyl-substituted compounds (**G**,**H**) are mixed in a 1:1 ratio, the hydration of the *cis*-epoxide (**G**) is greatly slowed while the hydration rate of the *trans*-epoxide (**H**) remains much the same (Table 3). For the other disubstituted epoxides analyzed as geometrical mixtures (Table 1) under conditions approaching substrate saturation, the preponderance of the later-eluting *erythro*-diol indicates that the *trans*-epoxide is also more rapidly hydrated. Neither the previous studies on microsomal epoxide hydrases (with the exception of some recent work on arene oxides) (1, 25, 27–30) nor this study have involved a rigorous, kinetic determination of substrate specificity as defined by k_{cat}/K_m (31) and compounds of vastly differing structures have been used. The conclusions regarding "specificity" of the microsomal epoxide hydrases are largely based on experiments which probably emphasize the affinity of the substrate for the enzyme although some comparative rate studies have been performed. Because solubility and detection problems have precluded a valid determination of the true substrate specificity, this paper compares separately the relative initial rates of hydrolysis and competitive enzyme affinity of several substrates. Only in the case of the disubstituted epoxides can one conclude that the soluble epoxide hydrase shows true specificity for the *trans*-epoxide over the *cis*-epoxide. Based on the reports from Oesch and coworkers also using a limited number of substrates (1, 27, 28) the ability of a potential substrate to inhibit the microsomal epoxide hydrase correlates with its ability to be metabolized by the enzyme with only a few exceptions such as trichloropropene oxide. Lipophilic monosubstituted epoxides, 1,1-disubstituted epoxides, and *cis*-1,2-disubstituted epoxides are hydrated by the microsomal enzyme while *trans*-1,2-disubstituted, trisubstituted, and tetrasubstituted epoxides are not. For the series of compounds examined with the soluble hydrase, the initial rate of hydration of the compound shows the opposite trend from the efficacy of the compound as an inhibitor. Possibly some of these compounds,

such as the trisubstituted epoxides, bind well at or near the enzyme active site, but they are hydrated with difficulty. Other substrates are rapidly hydrated, but are poor competitors with the trisubstituted epoxides at the active site. Such observations raise the disturbing possibility that poorly metabolized substrates could prevent the hydration of dangerous xenobiotics by the soluble enzyme(s).

The pure *cis*-disubstituted epoxides (**E, G, K, M, O**) are hydrolyzed by the soluble epoxide hydrase to give only *threo*-diols, and the *trans*-epoxides (**F, H, L, N, P**) give only the *erythro*-diols (Fig. 1). The geometry of epoxide hydration of the disubstituted epoxides by the soluble fraction is the same as the geometry of acid-catalyzed hydration which is *trans*. The stereoselectivity of the hydration of disubstituted epoxides by the soluble fraction has recently been confirmed using the epoxides of oleic and elaidic acid and their authentic diols (9). For all cases so far investigated with mammalian microsomes, pure *trans*-hydration has been reported (1).

CONCLUSION

In contrast to the situation in insects where epoxide hydrase activity on the substrates examined appears to be entirely membrane bound (10, 32–36), substantial hydrolytic activity is observed in the 100,000*g* soluble fraction of mammalian tissues (10). The mouse liver soluble epoxide hydrase is an enzyme(s) which will rapidly metabolize a variety of lipophilic substrates by a mechanism with the same geometry of hydration as the microsomal hydrase. The soluble epoxide hydrase seems to hydrate a broader range of aliphatic substrates than the microsomal enzyme(s), and the epoxide hydrase activity in the soluble fraction may be important when considering the relationship of the metabolism of drugs, pesticides, or other xenobiotics to toxicity, effectiveness, mutagenicity, or carcinogenicity.

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