Biochemical Pharmacology, Vol. 37, No. 14, pp. 2717–2722, 1988.
Printed in Great Britain.

0006-2952/88 $3.00 + 0.00

1,2-EPOXYCYCLOALKANES: SUBSTRATES AND INHIBITORS OF MICROSOMAL AND CYTOSOLIC EPOXIDE HYDROLASES IN MOUSE LIVER*

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(*Received* 4 *June* 1987; *accepted* 23 *October* 1987)

Abstract—Six different 1,2-epoxycycloalkanes, whose rings were constituted of 5 to 12 carbon atoms, were tested as possible inhibitors of epoxide-metabolizing enzymes and substrates for the microsomal and cytosolic epoxide hydrolases (mEH, cEH) in mouse liver. The geometric configurations and the relative steric hindrances of these epoxides were estimated from their ease of hydrolysis in acidic conditions to the corresponding diols, their abilities to react with nitrobenzylpyridine, and the chemical shifts of the groups associated with the oxirane rings measured by proton and ^{13}C-NMR. The cyclopentene, -hexene, -heptene, -octene and -decene oxides adopted mainly a *cis*-configuration. By contrast, cyclododecene oxide presented a *trans*-configuration. Steric hindrance increased with the size of the ring and was particularly strong when cyclooctene, -decene and -dodecene oxides were considered. With the exception of cyclohexene oxide, all the compounds were weak inhibitors of EH and glutathione *S*-transferase (GST) activities. Cyclohexene oxide exhibited a selective inhibition of the mEH with an I_{50} of $4.0 \cdot 10^{-6}$ M. As the size of the ring increased, inhibitory potency was gradually lost. The cEH and the GST activities were less sensitive to the inhibitory effects of these epoxides (I_{50}, 1 mM or above). A marked difference between the substrate selectivities of mEH and cEH for these epoxides was observed. The mEH hydrated all of the cyclic epoxides, although some of them at a very low rate; the best substrate was the cycloheptene oxide (2.3 nmol/min/mg protein). On the other hand, cyclodecene oxide was a substrate of cEH, but no diol formation was detected when cyclopentene, -hexene and -dodecene oxides were incubated with cytosolic enzyme.

Epoxide hydrolases (EH§, EC 3.3.2.3) constitute a family of enzymes involved in the hydration of various oxiranes [1]. The microsomal and cytosolic EHs (mEH, cEH) correspond to two distinct protein forms, which can be differentiated on the basis of subcellular localization [2, 3], immunological properties [4], optimum pH [5] or molecular weight [6, 7]. They also respond differently to known inducers. For instance, phenobarbital and Aroclor 1254 induce the microsomal form [8, 9], whereas clofibrate, di-(2-ethylhexyl)phthalate and other peroxisome proliferators increase preferentially the activity of the cEH [8, 10, 11].

The enzymes also exhibit distinct substrate specificities. The mEH rapidly hydrolyzes cyclic epoxides such as benzo[*a*]pyrene-4,5-oxide [12] as well as mono- and *cis*-1,2-disubstituted epoxides [13]. By contrast, the hydration of cyclic epoxides by the cEH is much slower [14]. On the other hand, this enzyme rapidly hydrates aliphatic epoxides such as fatty acid epoxides [15]. Even the inhibition responses of the two proteins are different. Cyclohexene 1,2-oxide decreases markedly the activity of the mEH, but not that of the cEH [16, 17].

Some cyclic epoxides are naturally occurring molecules. Structures related to cyclohexene oxide have been found in plants, a large variety of such compounds having already been described [18]. Some of them have pharmacological activity. For example, crotepoxide has been shown to prevent Lewis lung carcinoma in mice [19]. In plants, these compounds are hydrated into their corresponding diols, but a putative EH has not yet been implicated in this transformation.

Cyclic epoxides correspond to a class of structures which has been studied in terms of conformation and chemical reactivity [20–22]. However, their use as potential substrates for the EHs has not been investigated extensively [23, 24].

As the ring size of cyclic aliphatic molecules containing an epoxide increases, freedom of rotation actually decreases, and steric hindrance for a back side attack on the epoxide increases up to about C9 for *cis*-epoxides. The influence of this increasing ring size of aliphatic epoxides was observed on the initial rates of enzymatic hydrolysis of the epoxides shown in Fig. 1. Since cyclohexene oxide is one of the best known inhibitors for mEH activity, while it is a poor cEH inhibitor, these compounds also were examined as EH inhibitors. In addition, their inhibition of selected activities of the glutathione *S*-transferase isozymes (GST, EC 2.5.1.18) which are involved in

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§ Abbreviations: mEH, microsomal epoxide hydrolase; cEH, cytosolic epoxide hydrolase; GST, glutathione *S*-transferase; CSO, *cis*-stilbene oxide; and TSO, *trans*-stilbene oxide.

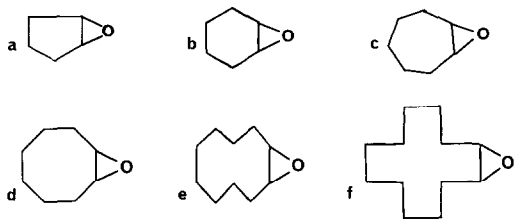

Fig. 1. Structures of the 1,2-epoxycycloalkanes. Key: (a) cyclopentene oxide; (b) cyclohexene oxide; (c) cycloheptene oxide; (d) cyclooctene oxide; (e) cyclodecene oxide; and (f) cyclododecene oxide. The geometry of all rings was *cis* except for cyclododecene oxide.

the metabolism of epoxide-containing compounds [25] was also observed.

METHODS

Chemicals. Unless otherwise mentioned, chemicals and solvents were from various suppliers; they were of the best quality commercially available and were used without further purification. Cyclopentene oxide, cyclohexene oxide, cyclododecene oxide, cycloheptene, *cis*-cyclodecene, *trans*-1,2-cyclooctanediol, *m*-chloroperbenzoic acid and 1-butaneboronic acid were provided by the Aldrich Chemical Co. (Milwaukee, WI). *N,O-bis*-(Trimethylsilyl)trifluoroacetamide (BSTFA, Regisil) was from the Regis Chemical Co. (Morton Grove, IL). Radiolabeled *trans*- and *cis*-stilbene oxides (TSO and CSO, respectively) were prepared by [^{3}H]borohydride reduction of desyl chloride followed by treatment with base [26].

Synthesis of cycloheptene oxide and cyclodecene oxide. The synthesis of these epoxides was achieved according to the procedure of Camps *et al.* [27]. Typically, cycloheptene and *cis*-cyclodecene (4 mmol) were added to a mixture of 1:1 *m*-chloroperbenzoic acid-activated potassium fluoride (12 mmol) in dichloromethane and allowed to react overnight by stirring at room temperature. The mixture was then filtered, and activated potassium fluoride (12 mmol) was added to the filtrate to ensure complete elimination of the acid. The solvent was removed by evaporation under reduced pressure. The resulting epoxide fraction was washed twice with pentane and saturated $NaHCO_3$ (5%, w/v) in water; the organic phase was washed again with brine, dried over Na_2SO_4, and finally evaporated under reduced pressure. The purities of cycloheptene oxide and cyclodecene oxide was verified by TLC using LK5DF plates (250 μm thickness, Whatman, Clifton, NJ) in hexane–ethyl acetate (3:1, v/v). The compounds were visualized by UV quenching, or exposure to iodine vapors, or spraying with 4-(4′-nitrobenzyl)pyridine [2% (w/v) in acetone] and heating the plate for 5 min at 100°, followed by spraying with tetraethylenepentamine [10% (v/v) in acetone]. This 4-(4′-nitrobenzyl)pyridine procedure yielded a blue spot characteristic of the epoxide moiety and some other alkylating agents [28]. Purities were also checked by gas–liquid chromatography according to the procedure indicated below, and retention times were recorded (see Table 1). Finally, structures were analyzed by proton NMR on a General Electric QE-300 apparatus after dissolution in deuterated chloroform (99.9% D) containing 0.03% (v/v) tetramethylsilane. The same instrument and solvent were used to monitor their ^{13}C spectra using concentrations of epoxides ranging from 3 to 5 M. The geometrical configurations of the cyclic epoxides also were estimated according to their ease of hydrolysis in acidic conditions [29]. The epoxides were hydrolyzed directly on a TLC plate by 10% (v/v) phosphoric acid for 5 min. The products of the reaction, the corresponding diols, were separated from the remaining epoxides by chromatography in hexane–ether (80:20, v/v). After exposure to iodine vapor, the diols were detected at a lower R_f than the starting epoxides.

Synthesis of the diols. The diols of the corresponding cyclic epoxides were obtained by acid hydrolysis. Typically, a 2-mmol sample of each epoxide, except cyclododecene oxide, was dissolved in 40% (v/v) aqueous tetrahydrofuran containing 0.05 N H_2SO_4; the mixture was stirred at room temperature overnight. The formation of cyclododecene diol was achieved by refluxing 2 mmol of the epoxide in 40% (v/v) aqueous dioxane containing 0.05 N H_2SO_4 at 100° overnight. After addition of NaCl to saturation and NaOH (pellets) for neutralization of the aqueous phase, the aqueous phase was extracted twice with ethyl ether. The combined ether phases were then dried over Na_2SO_4 and evaporated under reduced pressure. The purities of the cyclic diols were verified by their migration after TLC in hexane–ethyl acetate (3:1, v/v) and by gas–liquid chromatography under the conditions described below. The retention times are indicated in Table 1.

Preparation of the enzyme fractions. Male C57 black mice, 25–30 g (Simonsen Laboratories, Gilroy, CA) were housed in steel cages with kiln-dried pine shavings as bedding in an environmentally controlled room (12 hr light cycle, 22.5 to 24.0°, constant humidity). They were fed a Purina rodent chow and had tap water *ad lib.* After 12 hr of fasting the mice were killed by cervical dislocation at approximately 7:00 a.m. Their livers were removed, freed from gall bladders, perfused with ice-cold 1.15% (v/v) KCl, and finally weighed. The livers were homogenized in 100 mM phosphate buffer, pH 7.4, with a Polytron apparatus (Brinkmann Instruments, Westbury, NY) for 20 sec to give a 10% (of the original liver weight) homogenate, and the fraction was centrifuged for 10 min at 10,000 g_{av} (Sorvall Instruments, Du Pont Co., Wilmington, DE). The supernatant fraction was centrifuged at 100,000 g_{av} for 60 min (Beckman Instruments, Palo Alto, CA). The corresponding supernatant constituted the cytosolic fraction that was used without further treatment since dialysis had no effect on hydrolysis of TSO in the mouse [30]. The remaining microsomal pellet was washed in the phosphate buffer and centrifuged again for 60 min at 100,000 g_{av}. The microsomal fraction was homogenized in this buffer and stored with the cytosol as aliquots at −80°. The aliquots were used as the enzyme sources for the microsomal and cytosolic EHs. Their protein contents were determined by the method of Bradford [31] modified for an ELISA reader with computer readout.

Enzyme assays and inhibition of the microsomal and cytosolic EH. The enzyme activities toward CSO and TSO were measured using the radiometric partition assay already described [1, 32]. The activity of GST was determined using radioactive CSO and TSO as substrates and glutathione (5 mM) as co-substrate in a partition assay using hexanol as the hyperphase that is similar to a published assay [1, 32]. Activities were expressed as specific activity (nmol/min/mg protein). For the inhibition study, the cyclic epoxides (10^{-7} to 10^{-3} M) dissolved in 1 μl ethanol were added to the microsomes and the cytosol. After preincubation of the enzyme with the inhibitor for 2 min at 37°, the activities of the EHs and GST were determined as previously indicated. Controls without inhibitors were always run and were used in calculations to represent 100% activity. I_{50} values were calculated using the linear portion of the inhibition curve for least-squares regression analysis. I_{50} values are a function of both the substrate used and the inhibitor.

Initial rates for the formation of the diol by the EHs. The microsomal and cytosolic fractions were diluted with 100 mM Tris–HCl buffer (pH 9.0) or 76 mM Na^+/K^+ phosphate buffer (pH 7.4), respectively, to 1 mg protein/ml, and the cyclic epoxides were added (1 mM in 1 μl ethanol). The mixture was vortexed gently and incubated for 30–60 min at 37°. When the formation of the diols from the cyclopentene, -hexene or -heptene oxide was considered, incubation was in sealed tubes to prevent any evaporation of the epoxides during the incubation time. Controls were run simultaneously and consisted of enzyme exposed to boiling water for 10 min. An internal standard, 10 nmol, was added in 10 μl of ethanol just prior to the end of the incubation. The internal standards that were used were (1) 1,2-octane diol when the formation of the diol from C5 to C10-ring epoxides was followed, and (2) stilbene glycol to quantitate the production of cyclododecene diol. The reaction was immediately stopped by addition of about 250 mg NaCl and placement of the tube in an ice bath. The sample was extracted twice with 2 ml of ether, and the organic phase was dried over Na_2SO_4. The ether was evaporated under a gentle stream of nitrogen. The residue was derivatized with 50 μl of BSTFA for 60 min at 70°, and 1 μl was injected into the gas chromatograph. Derivatization of these diols, except cyclododecene diol, by 1-butaneboronic acid was not successful, as expected of *trans*-diols on cyclic systems. Only cyclododecene diol was able to form a diester with this reagent after 30 min at 60°. As the size of the ring increases, the structure of the cyclic epoxides becomes more flexible [33], thus allowing the reaction of cyclododecene diol with 1-butaneboronic acid.

Gas–liquid chromatography. A Varian Aerograph (series 1400) gas–liquid chromatograph with a flame ionization detector was used. The column was silanized glass spirals (1.5 m × 1.5 mm, internal diameter) containing 3.0% OV 225 (Supelco Inc., Bellefonte, PA) on Supelcoport (100/120 mesh). The temperatures of the injection port and detector were 230° and 270° respectively. Nitrogen (carrier gas), hydrogen and air flow rates were 15, 20 and 300 ml/min respectively. For the detection of cyclopentene diol and cyclohexene diol, the temperature of the column was set at 90° for 2 min and then increased gradually to 150° at a rate of 6°/min. The formation of the other cyclic diols was followed using a column temperature of 110° for 2 min and then a temperature gradient (6°/min) to 160°. The peaks corresponding to the diols were identified using the retention times of the synthetic diols and were quantitated by a Hewlett–Packard 3390a integrator by comparing peak area of the diols with that of the internal standard.

RESULTS

Inhibition of the epoxide-metabolizing enzymes by the cyclic epoxides. The I_{50} values for the inhibition of the EH and GST activities by the series of cyclic epoxides are reported in Table 1. The inhibitory

Table 1. Gas–liquid chromatography conditions for separation of the cyclic epoxides and their inhibition potencies on epoxide-metabolizing enzymes

Carbon number	Retention time (min)		I_{50}‡ (M)			
			mEH	cEH	GST	
	Epoxide*	Diol†	CSO	TSO	CSO	TSO
5	0.50	1.98	$5.5 \cdot 10^{-4}$	$>10^{-3}$	$>10^{-3}$	$>10^{-3}$
6	0.58	2.23	$4.0 \cdot 10^{-6}$	$>10^{-3}$	$>10^{-3}$	$>10^{-3}$
7	1.20	3.50	$1.0 \cdot 10^{-4}$	$>10^{-3}$	$>10^{-3}$	$>10^{-3}$
8	2.21	4.50	$>10^{-3}$	$>10^{-3}$	$>10^{-3}$	$>10^{-3}$
10	5.71	6.17	$5.0 \cdot 10^{-5}$	$1.0 \cdot 10^{-3}$	$1.0 \cdot 10^{-3}$	$8.5 \cdot 10^{-4}$
12	12.72	8.34	$6.5 \cdot 10^{-4}$	$3.5 \cdot 10^{-4}$	$1.0 \cdot 10^{-3}$	$5.5 \cdot 10^{-4}$

* The temperature of the column was isothermal at 130°.

† The diols were derivatized by *N,O-bis*-(trimethylsilyl)fluoroacetamide) before injection. The temperature of the column was set at 90° for 2 min and then increased at a rate of 6°/min to 150° for the detection of cyclopentene and -hexene diols. The temperature of the column was 110° for 2 min and then increased at a rate of 6°/min to 160° for the separation of the other cyclic diols.

‡ Inhibition of the three enzyme systems was measured with the substrate shown under conditions described in the text.

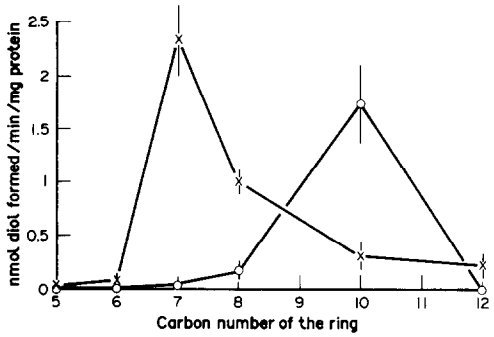

Fig. 2. Rates of the formation of the diols by the microsomal and the cytosolic epoxide hydrolases. Results are expressed as specific activity (nmol diol formed/min/mg protein) and are the means ± SD of at least three independent experiments. Key: (×) microsomal epoxide hydrolase; and (○) cytosolic epoxide hydrolase. Except for cyclodecene oxide, all the cyclic epoxides presented a *cis*-configuration. The number of carbons of the ring refers to the various 1,2-epoxycycloalkanes tested as substrates for the epoxide hydrolases.

potencies of these compounds varied according to the size of the ring and the enzyme system studied. They were generally quite weak inhibitors. Only the mEH was sensitive to inhibition by the cyclic epoxides. The most powerful and selective inhibitor was cyclohexene oxide (I_{50} $4.0 \cdot 10^{-6}$ M). Cyclopentene oxide, cycloheptene oxide, cyclodecene oxide and cyclododecene oxide also inhibited the mEH to some extent. By contrast, neither cycloheptene oxide nor cyclooctene oxide exerted any effect on cEH or GST activities using the current substrates (Table 1).

Measurement of the hydration rate of the cyclic epoxides by the EHs. The microsomal EH was able to hydrate all the various cyclic epoxides although at a low rate (Fig. 2). The specific activities toward cyclopentene- and cyclohexene oxides were very low but increased with the size of the ring and presented a maximum for cycloheptene oxide (2.3 nmol/min/mg protein). Cyclooctene oxide was also hydrated to some extent (1.0 nmol/min/mg protein). For larger epoxides the activity was decreased gradually. The cEH only hydrated the medium size rings, especially the cyclodecene oxide (1.7 nmol/min/mg protein). No activity could be detected when cyclopentene, -hexene and -dodecene oxides were used as substrates (Fig. 2). It should be noted that the cyclododecene oxide has *trans*-geometry.

Estimation of the chemical reactivities of the cyclic epoxides. The accessibility of the epoxide moiety was estimated by its reactivity with nitrobenzylpyridine. Table 2 shows the comparative formation of a blue complex in the presence of nitrobenzylpyridine and tetraethylenepentamine. A light blue spot could be seen with the smaller size ring epoxides (5 to 7 carbon atoms). No reactivity was observed, in our conditions, with the cyclooctene, -decene and -dodecene oxides, suggesting that these epoxides were highly hindered. This observation was supported by examination of molecular models. In contrast, the standard substrates of the EHs, TSO and CSO, at the same concentration as that of the cyclic epoxides, presented bright spots upon exposure to the reagents (Table 2).

To determine if the carbons of the ring are *cis*- or *trans*- to one another across the oxirane moiety, the formation of the corresponding diol after reaction for 5 min with 10% phosphoric acid was followed. In these conditions, according to Bierl *et al.* [29], *cis*-epoxides are hydrolyzed more easily than *trans*-epoxides. Table 2 shows the result of this experiment. After 5 min of acid hydrolysis, the cyclopentene and cyclohexene oxides were totally transformed into diols of lower R_f, thus suggesting that these epoxides were in *cis*-configurations. Cycloheptene oxide and cyclodecene oxide synthesized from the corresponding *cis*-decene also were hydrated easily, although not completely. By contrast, no detectable acid hydrolysis was observed with cyclododecene

Table 2. Chemical reactivities of the cyclic epoxides and standard epoxides

	Number of carbons of the ring							
Reactivity to	5	6	7	8	10	12	CSO*	TSO*
Nitrobenzylpyridine†	++	++	+	ND	ND	ND	+++	+++
Acid hydrolysis‡	+++	+++	++	+	++	ND	+++	+++

* *cis*-Stilbene oxide (CSO) and *trans*-stilbene oxide (TSO) correspond to the standard substrates used to monitor the activities of the microsomal and cytosolic epoxide hydrolases, respectively.

† Twenty nanomoles of each epoxide in ethanol was loaded onto a LK5DF TLC silica gel plate and developed. After evaporation of the solvent, nitrobenzylpyridine [2% (w/v) in acetone] was sprayed on the plate which was then heated for 5 min at 100°. Tetraethylenepentamine [10% (v/v) in acetone] was then sprayed, and the formation of a blue spot was followed: (+++), bright spot; (++), weak spot; (+), barely visible spot; and ND, no reaction detectable.

‡ On a LK5DF TLC plate, 10 μl of 10% aqueous phosphoric acid was spotted at each position where the epoxide was thereafter loaded. The acid was allowed to dry for 20 min, and then the various epoxides (5 μmol) were spotted on the phosphoric acid. After 5 min of acid hydrolysis, the plate was developed in hexane–ethyl ether (80:20, v/v), and the products of the reaction were revealed by iodine vapor exposure. When the epoxide was hydrolyzed, the corresponding diol could be seen at a lower R_f. Simultaneously, a control experiment was run under the same conditions but without phosphoric acid. Key: (+++) complete acid hydrolysis; (++) incomplete acid hydrolysis; (+) weak acid hydrolysis; and (ND) no detectable acid hydrolysis.

epoxide, thus strongly suggesting that this epoxide is in the *trans*-configuration. Using these hydrolysis conditions, cyclooctene oxide exhibited an intermediary behavior. Some diol could be formed after acid hydrolysis, but the amount obtained after 5 min was less than that observed with the other cyclic epoxides, except with the cyclododecene epoxide. By comparison, the standard substrates of the EHs, CSO and TSO, were easily hydrated by 10% phosphoric acid (Table 2).

DISCUSSION

From the cyclic epoxides tested as potential inhibitors of epoxide-metabolizing enzymes, only cyclohexene oxide exerted a strong inhibitory effect. The other compounds exhibited generally an I_{50} value of 1 mM or even above; this was particularly true for the smallest size ring epoxides. Only mEH activity was sensitive to inhibition by the cyclic epoxides, especially cyclohexene oxide. The selective inhibition of mEH activity by this epoxide allows its use as a diagnostic inhibitor. Oesch *et al.* [13] reported a similar finding concerning the inhibition of mEH from guinea pig by cyclohexene oxide with styrene-oxide as substrate. This inhibitor has been shown to inhibit competitively the hydration of octene oxide but was reported to act as a non-competitive inhibitor toward the transformation of benzopyrene-11,12-oxide [16]. On the other hand, a slight (10%) activation of the mEH with cyclooctene oxide and cyclododecene oxide was obtained at a concentration of $2.0 \cdot 10^{-3}$ M, and no inhibition was found with cyclopentene oxide [13]. It is intriguing to notice that the inhibition of the mEH by the cyclic epoxides decreased markedly when the size of ring was smaller or larger than 6 carbons. As the mEH catalyzes mainly the hydration of large lipophilic epoxides [34], we postulate that cyclopentene oxide is too water soluble to reach the active site of the enzyme within the membrane and, on the other hand, the larger epoxides by their conformation or steric hindrance exhibit less affinity for the protein.

There is a question as to the isomeric composition of some epoxides on cyclic systems. On gas–liquid chromatography there was only one peak for each epoxide, suggesting the presence of a single geometrical isomer. Chemical hydrolysis [29] clearly supports our observation that cyclododecene oxide was pure *trans* while all others were clearly *cis*, except possibly the cyclooctene oxide which gave intermediate behavior with these conditions. Similar conclusions have been drawn by Mihailovic *et al.* [33] when determining the ease of reduction by lithium aluminium hydride of 1,2-epoxycycloalkanes. The broad band, decoupled ^{13}C spectra of the cyclic epoxides used in this study were identical to those reported in the Sadler index with carbons alpha to the epoxide, giving a sharp symmetrical peak between 24 and 26 ppm for the *cis*-compounds, with the *trans* carbon peak being at 31.0 ppm. However, a small amount of the opposite geometrical isomer may have been obscured under these conditions. The proton on the epoxide carbon showed peaks between 2.95 and 3.0 ppm for the cyclopentene, -hexene, -heptene, -octene and decene oxides with no signals in the region expected for the *trans*-isomer. The proton chemical shifts of the groups associated with the oxirane were in good accordance with those reported by Kas'yan *et al.* [35]. By contrast, the *trans*-cyclododecene oxide showed a clear doublet at 2.53 ppm. A similar doublet at 2.70 ppm indicated less than 5% contamination with the corresponding *cis*-epoxide.

The mEH was able to catalyze the hydration of all the cyclic epoxides used in this study, although at a very low rate, especially when cyclopentene, -decene and -dodecene oxides were considered. Even cyclohexene oxide which inhibited markedly the mEH, thus suggesting some affinity for the protein, was slowly hydrated. With the exception of the cyclododecene oxide, all the other compounds were *cis*-epoxides. The enzyme is known to hydrate a large variety of oxiranes of aromatic systems, presenting a *cis*-configuration, such as benzo[*a*]pyrene 4,5-oxide or phenanthrene 9,10-oxide [16, 36, 37]. However, sterically hindered epoxides are more resistant to hydration. This and the absence of benzylic carbons probably explains why some of the aliphatic cyclic epoxides were very poor substrates of the mEH. When compared with the mEH, the cEH exhibited a radically different specificity toward the cyclic epoxides. This was particularly true for cyclodecene oxide which was hydrated at a reasonable rate by the cEH but was a poor substrate for the mEH. Molecular models indicate that this is the first compound in the series for which there is the possibility of a relatively unhindered back side attack on the epoxide by water. A large nucleophile-like nitrobenzylpyridine would be hindered from approaching the epoxide carbon. The two EHs are known to differ mainly in their substrate specificities [38]. The cEH which can catalyze the formation of diol from *trans*-epoxides such as TSO [1] was unable to hydrate the *trans*-cyclododecene oxide isomer. The strong steric hindrance of the oxirane group in this large ring could account for such results.

In conclusion to this work, 1,2-epoxycycloalkanes with rings of 5 to 12 carbon atoms were, at the same time, poor inhibitors and poor substrates for the microsomal and cytosolic EHs. Despite this low reactivity, the two enzyme systems exhibited radically different behaviors in the presence of these chemical structures.

Acknowledgements—Ken Musker and Barbara Wagner of the Department of Chemistry at Davis provided NMR spectral data. J.M. received partial support for his sabbatical leave from C.N.R.S.; B.D.H. is a Burroughs Wellcome Scholar in Toxicology. The expert secretarial assistance of Peggy Kaplan and Kathleen Dooley is also gratefully acknowledged.

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