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Regio- and Stereochemistry of the Acid Catalyzed and of a Highly Enantioselective Enzymatic Hydrolysis of Some Epoxytetrahydrofurans.

Pier Luigi Barili, Giancarlo Berti and Ettore Mastrorilli*.

Dipartimento di Chimica Bioorganica, Università di Pisa, Via Bonanno 33 - 56126, Pisa Italy.

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Abstract: *3,4-Epoxytetrahydrofuran is hydrolyzed by rabbit liver microsomal epoxide hydrolase (MEH) with a high preference for the attack by water at the* (S) *epoxide carbon to give the* (R,R)*-diol with an e.e. of 96.5±0.3%. In the acid catalyzed hydrolysis of* trans*-3,3a-epoxyoctahydrobenzofuran the oxirane ring is opened with inversion exclusively on the tertiary carbon atom to give the corresponding* trans*-diol, whereas hydrolysis of the* cis *isomer is less regioselective, the ratio of attack at the tertiary and secondary carbons being 81:19. The MEH catalyzed hydrolysis of the same two substrates occurs exclusively at their secondary epoxide carbons and with a very high enantioselectivity: only enantiomers of configuration (3*S*,3a*R*) of the* cis*- and* trans*-epoxide are substrates for the enzyme and give the corresponding (3*R*,3a*R*)-diols with at least 98% e.e., the corresponding (3*R*,3a*S*)-epoxides being totally resistant to enzymatic hydrolysis. These results agree well with previously formulated rules on steric requirements of MEH subtrates. Absolute configurations and optical purities of new chiral compounds were obtained by chiroptical, NMR and chiral chromatographic techniques. Conformations of the octahydrobenzofuran derivatives were derived from coupling constants and found to be in fairly good agreement with those deduced from molecular mechanics calculations.*

Microsomal epoxide hydrolase (MEH), an ubiquitous enzyme involved in many fundamental biotransformation pathways,[1] often exhibits a high discriminating capability between enantiomeric chiral centers of meso substrates and enantiomeric forms of racemic ones.[2] An accurate definition of the substrate structural features which are responsible for this enantioselectivity can be of great interest for the interpretation of the mechanism of action of MEH, in mapping its active site, and in foreseeing its activity in the detoxification or metabolic activation of xenobiotic compounds. A further point of potential practical interest is in the possibility of using MEH as a reagent for a simple access to optically active epoxides and diols, which can be useful intermediates for chiral syntheses.

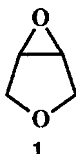
In continuation of the studies conducted in this field at our Department since several years, it was decided to extend the range of investigated substrates to compounds in which the oxirane ring is fused to positions 3,4 of a tetrahydrofuran one , since nothing was known about their behavior in the presence of epoxide hydrolases. The tetrahydrofuran ring, apart from its frequent occurrence in carbohydrates, is fairly often encountered in other classes of natural products.

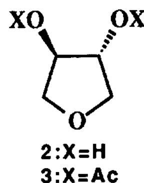
This work was started with the simplest representative of the series, 3,4-epoxytetrahydrofuran **1**, and continued with the *cis* and *trans* forms of 3,3a-epoxyoctahydrobenzofuran **5** and **6** as examples of substituted epoxides of this class, since several derivatives of 2,4,5,6,7,7a-hexahydrobenzofuran **4** are present among carotenoids, such as flavoxanthin, rubichrome, auroxanthin, etc,[3] and one of its derivatives is easily formed

from 5,6-epoxyretinoic acid.[4] Further biotransformations of these compounds may well involve the formation of the corresponding epoxides, which should be subject to easy nucleophilic opening by cell constituents.

Results

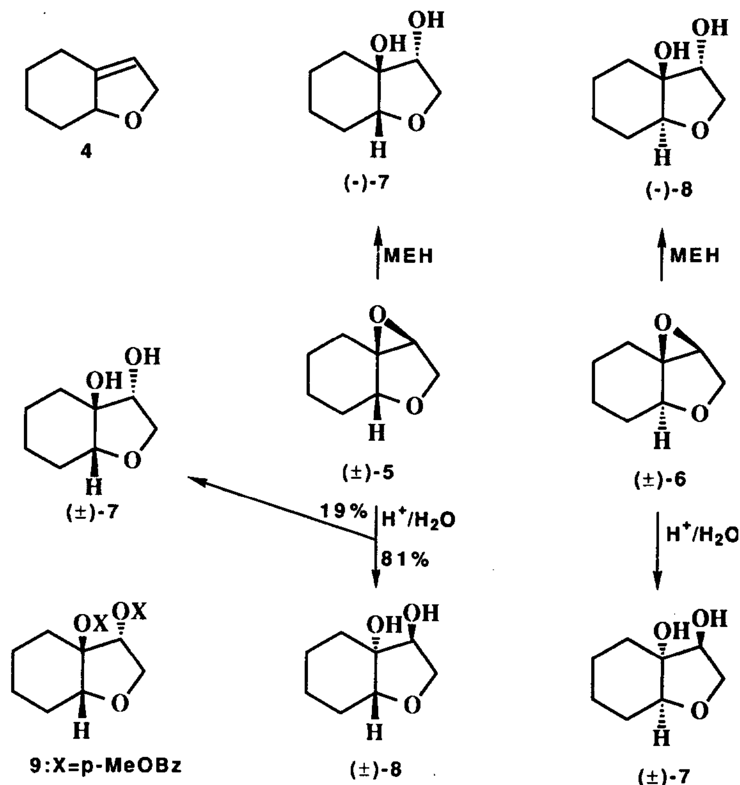
3,4-Epoxytetrahydrofuran **1** was prepared from commercial 2,5-dihydrofuran with *m*-chloroperoxybenzoic acid (MCPBA). When it was incubated with rabbit liver microsomes it was converted quantitatively into the corresponding *trans*-diol, 1,4-anhydrothreitol **2**. The rate of hydrolysis under enzyme saturation conditions (Vs) was 26±0.8 nmole min $^{-1}$ mg^{-1} protein (a value which change only within a limited range with different lots of microsomes), and remained constant up to almost complete conversion. The diol isolated from preparative runs had [α]=+4.8°, which proved that it was the (3*R*,4*R*) enantiomer of high optical purity, values ranging from -4.0° to -5.0° having be reported for 1,4-anhydro-*L*-threitol prepared from *L*-tartaric acid.[5] A more precise value for the enantiomeric excess (e.e.) of the diol **2** was obtained from the ^{1}H NMR spectrum of its diacetate **3** in the presence of the chiral shift reagent Eu(hfc)$_3$. Under the appropriate conditions, racemic **3** showed two base-line separated 6-proton singlets, corresponding to the two equivalent acetyl groups of each enantiomer. In the spectrum of the diacetate **3** obtained from the diol isolated from the enzymatic incubation one of these signals was barely perceptible, and the % e.e. was extimated to be 96.5±0.3.





Compound (±)-**4** was prepared according to the method of Schweizer and Liehr [6] involving reaction of 2-hydroxycyclohexanone with triphenylvinylphosphonium bromide and was converted into a 38:62 mixture of the *cis* and *trans* epoxides **5** and **6**, which were separated by chromatography on silica gel. The attribution of relative configurations to **5** and **6** rested initially on some indirect, but not conclusive evidence, since their NMR spectra (Table 1) were not sufficiently different for a clear-cut assignment. Bicyclic alkenes with a double bond exocyclic to one of the rings usually are oxidized by peroxy acids preferentially *trans* to the vicinal tertiary hydrogen to give the *trans* epoxides.[7] For instance, the carbocyclic isosteric analogue of **4**, bicyclo[4.3.0.]non-6-ene, produces a 39:61 mixture of *cis* and *trans* epoxides, that are differentiated by the chemical shift of their epoxide ring proton: 3.25 ppm for the *cis* and 3.40 ppm for the *trans* isomer.[8] The very close correspondence of the product ratios and of the difference in chemical shifts of the oxirane ring protons of the minor and the major epoxides (3.58 and 3.74 ppm in the epoxidation products of **4**) made the assignment of the *cis* configuration to the former and of the *trans* one to the latter very likely. Further evidence came from the fact that the tertiary proton 7a signal was found at 3.87 ppm for the supposed **5** and at 3.46 ppm for the supposed **6** in accordance with a higher shielding effect by the oxirane oxygen on this proton in the *cis* than in the *trans* isomer. These attributions were fully confirmed by a correlation with the configurations of the corresponding diols **7** and **8**, which were better amenable to NMR structural analysis.

Acid catalyzed hydrolysis of the epoxides **5** and **6** proceeded exclusively in an *anti* fashion to produce the *trans* diols **7** and **8**, no trace of the diastereomeric *cis* diols being detected. Whereas the *trans* epoxide **6** was opened exclusively at the tertiary carbon to give the diol **7**, *cis*-octahydrobenzofuran-*trans*-3,3a-diol, ring opening was less regioselective for the *cis* epoxide **5**, giving a mixture of *trans*-octahydrobenzonzofuran-*trans*-3,3a-diol **8** and of **7** in a ratio of 81:19. Whereas *anti* opening is a general rule for oxirane rings lacking substituents capable of mesomeric stabilization of a positive charge at the site of nucleophilic attack, the observed high preference of this attack at the more hindered position of **5** and **6** is quite unusual.[9]



The attribution of relative configurations to **7** and **8** was based on the analysis of their NMR spectra (Table 1). The diol **8**, having its two rings fused in a *trans* fashion can only exist in the rigid conformation **8a**, whereas its *cis* fused diastereomer **7** could assume either conformation **7a**, in which the C(7a)/O(1) bond is axial to the cyclohexane ring, or **7b**, in which it is equatorial. The values found for the 7,7a proton coupling constants of **8** (4.39 and 11.85 Hz) are in good agreement with what expected for conformation **8a**, presenting an equatorial/axial and a diaxial interaction. The same constants for **7** (*J* 3.2 and 3.7 Hz), on the other hand, agree well with the equatorial/equatorial and equatorial/axial interactions present in **7a** and rule out a significant contribution from the alternative conformer **7b**. A similar preference for the conformation with an axial C(7a)/O(1) bond has been previously reported for the parent unsubstituted compound, *cis*-octahydrobenzofuran,

for which values of 3.9 Hz were found for both 7/7a proton coupling constants,[10] and for several of its 2- and 6-monosubstituted, and 2,6-disubstituted derivatives.[11]

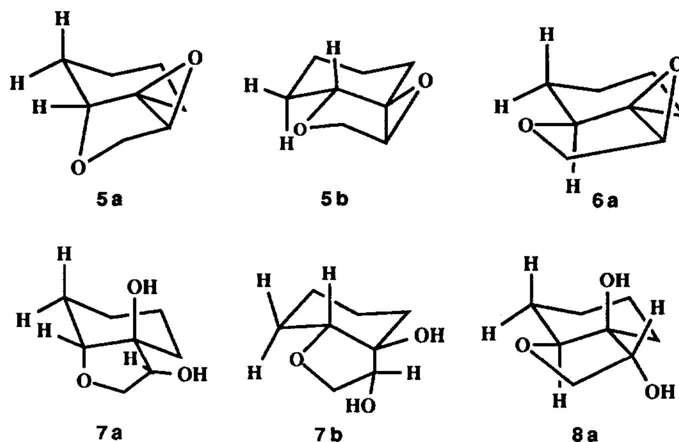
Table 1. NMR Parameters ($CDCl_3$)

	Epoxide **5**	Epoxide **6**	Diol **7**	Diol **8**	Ester **9**
δ H(2)	3.97	3.97	4.17	4.28	4.42
δ H(2')	3.80	3.59	3.60	3.56	3.91
δ H(3)	3.58	3.74	4.30	4.08	5.90
δ H(7a)	3.87	3.46	3.71	3.46	4.23
δ H(4-7)	2.10-1.19	2.07-1.29	2.12-1.38	2.05-1.20	2.17-1.54
$J_{2,2'}$	10.75	10.53	9.04	10.33	10.51
$J_{2,3}$	0.0	0.0	8.00	5.20	7.44
$J_{2',3}$	0.4	0.3	6.28	1.36	3.65
$J_{7a,7}$	10.37	11.24	3.2	11.85	3.7
$J_{7a,7'}$	6.35	4.64	3.7	4.39	3.9
δ C-2	66.20	67.12	71.05	74.33	71.97
δ C-3	61.54	60.05	79.68	76.84	78.46
δ C-3a	67.69	66.64	76.68	78.08	84.91
δ C-4	27.26	26.84	28.43	27.86	26.36
δ C-5	22.91	23.25	20.65	19.95	20.86
δ C-6	25.18	23.25	20.29	23.34	20.11
δ C-7	30.59	28.08	26.18	24.58	26.15
δ C-7a	76.51	76.23	79.41	79.45	78.00

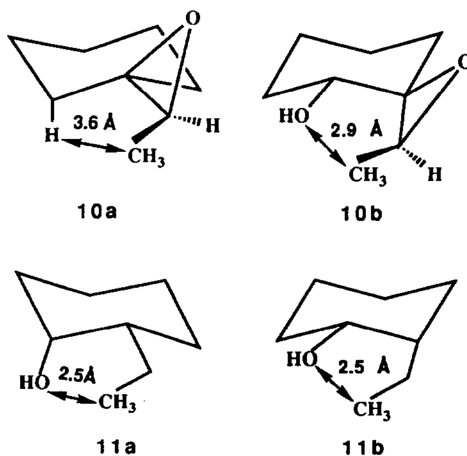
A similar analysis for the ^1H NMR spectra of the epoxides **5** and **6** revealed that they had fairly similar values of the 7/7a coupling constants (6.35 and 10.37 Hz for **5**, and 4.64 and 11.24 for **6**). Therefore, in contrast with the diol **7**, the *cis* epoxide **5** exists preferentially in the conformation **5b**, with the C(7a)/O(1) bond equatorial to the cyclohexane ring. On the other hand, in the *trans* epoxide **6** the spatial relationship between proton 7 and 7a is the same as that found in the diol **7** derived from *trans*-octahydrobenzofuran as testified by the very similar coupling constants involving these protons, in accordance with conformation **6a**.

The fact that the diol and epoxide derived from *cis*-octahydrobenzofuran have opposite conformational preferences can be intuitively and roughly explained by considering the geometries of the epoxide **10** and of *cis*-2-ethylcyclohexanol **11**, which, in a hypothetical transformation involving bond formation between hydroxyl oxygen and methyl carbon, would give rise, respectively to **5** and to the bis-dehydroxylated analogue of **7**. Inspection of Dreiding models of **10** and **11** shows that for the former the distance between oxygen and methyl is significantly higher in conformer **10a** (axial OH) than in conformer **10b** (equatorial OH), whereas for **11** this distance (in the rotameric situation of closest contact) is similar in both conformers **11a** and **11b**. A more precise evaluation of these distances come from application of the PC-MODEL program for molecular mechanics, giving respectively 3.6, 2.9, 2.5 and 2.5 A for this distance in **10a**, **10b**,**11a** and **11b**. These data, while showing that in all cases formation of the tetrahydrofuran ring requires significant deviations from normal geometry to decrease these distances in order to form the O(1)/C(2) bond, justify the high preference of epoxide **5** for

conformation **5b**, which involves less distortion. The equal values of minimum C-O distances found for **11a** and **11b**, on the other hand, show that other factors must be responsible of the preference for conformation **7a**.



The same PC-MODEL calculation gave data (Table 2) showing a 2 Kcal/mole difference in energy in favor of conformer **7a** over **7b** for the diol and 2.6 Kcal/mole in favor of conformer **5b** over **5a** for the epoxide. These computations also provided calculated angle values for the coupling constants between protons 7 and 7a on one hand and 2 and 3 on the other, which agreed in a satisfactory way with the experimental ones. Finally, calculated values of the dihedral angles relative to the ring bonds (Table 3) showed a close to normal chair conformation of the cyclohexane ring of the diol **8** and epoxide **6**. The *cis* epoxide **5b** exhibits a pronounced flattening of its cyclohexane ring in the 6,7,7a,3a,4,5 moiety, which is also present, but much less evident in the diol **7a**. It may be observed that in the disfavored conformer **5a** of the *cis* epoxide, the dihedral angle C(7)/C(7a)/C(3a)/C(4) would be close to 0°, a further confirmation of its severe distortion. The internal dihedral angles of the tetrahydrofuran rings agree fairly well with those expected for slightly distorted E_1 envelope conformations for the epoxides **5** and **6** and for twist conformations for the diols **7** and **8**.[12]



The racemic epoxides **5** and **6** were subjected to enzymatic hydrolysis with rabbit liver microsomes. In both cases the reaction proceeded at a constant initial rate which was not very different from that found for **1** (V_S of 33±1 and 17.9±1 nmole min^{-1} mg^{-1} protein, respectively, for **5** and **6**), but the reaction stopped completely around 50% conversion, hinting to a very high degree of substrate enantioselection by the enzyme. This was confirmed by preparative runs, in which unchanged epoxide and product diol were isolated and characterized. As expected, ring opening occurred in both epoxides in an entirely regiospecific and diastereospecific fashion with nucleophilic attack by water at the secondary carbon 3, the *cis* epoxide **5** giving exclusively the diol **7**, the *trans* epoxide **6** the diol **8**. Furthermore, in both cases only the enantiomers with (3*S*,3a*R*) configuration of the epoxide ring were substrates for the enzyme, so that (±)-**5** gave the (3*R*,3a*R*,7a*R*)-diol (-)-**7** and the (3*R*,3a*S*,7a*S*)-epoxide (+)-**5**, and (±)-**6** gave the (3*R*,3a*R*,7a*S*)-diol (-)-**8** and the (3*R*,3a*S*,7a*R*)-epoxide (+)-**6**.

The optical purity of the recovered epoxides was ascertained by GLC on a capillary column coated with GTA-Chiraldex^R chiral stationary phase. In the case of the *trans* epoxide **6**, where a good base-line separation of the enantiomer peaks was obtained, only one of these peaks was visible in the dextrorotatory product from the enzymatic hydrolysis. Therefore it had an e.e. of at least 98%. The separation of enantiomer peaks for the *cis* epoxide **5** was only partial, with some overlap, in order that it was only possible to set the minimum value of e.e. at about 90%.

Table 2. PC MODEL Calculations of Energies and Coupling Constants

	Epoxide **5**			Epoxide **6**		Diol **7**			Diol **8**	
	calcd		exptl	calcd	exptl	calcd		exptl	calcd	exptl
	5a	**5b**				**7a**	**7b**			
ΔE	18.0	15.5		15.5		14.0	16.0		15.3	
$J_{2,3}$ *(cis)*						9.30	8.43	8.00	6.65	5.20
$J_{2,3}$ *(trans)*						6.97	3.98	6.28	2.21	1.36
$J_{7,7a}$ *(cis)*	5.65	7.22	6.35	4.90	4.64	2.88	5.47	3.2	4.43	4.39
$J_{7,7a}$ *(trans)*	1.45	9.23	10.37	11.16	11.24	3.25	10.76	3.7	11.35	11.85

Since no simple way was found for direct determination of the absolute configurations of the epoxides **5** and **6**, the problem was solved by correlation with the configurations of their hydrolysis products **7** and **8**. In the case of the *cis*-octahydrobenzofuran-*trans* diol in conformation **7a**, the PC-MODEL calculations mentioned above give an O(3)/C(3)/C(3a)/O(3a) dihedral angle of -88°, which made it well suited for applying the exciton chirality method[13] for the determination of the absolute configuration of vicinal diol diesters. The optically active diol was therefore converted into the bis-(4-methoxybenzoyl) ester **9**, the CD spectrum of which exhibited a well pronounced exciton splitting, with a negative Cotton effect at 262 nm and a positive one at 247 nm, in accordance with a negative dihedral angle relative to the two C-O bonds and with the (3*R*,3a*R*,7a*R*) absolute configuration of **9** and of (-)-**7**, from which it derives. It can further be deduced that (+)-**5** recovered from the enzymatic hydrolysis has the (3*R*,3a*S*,7a*S*) configuration.

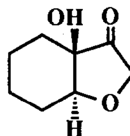
The same ester **9** was also found to be highly suitable for the determination of the optical purity of the diol **7** by the chiral shift reagent methodology. In the ^1H NMR spectrum of racemic **9** the two non-equivalent

Table 3. Computed Ring Internal Dihedral Angles (PC MODEL)

	5a	**5b**	**6**	**7a**	**7b**	**8**
Angle						
1,2,3,3a	23.1	17.8	-18.5	-15.4	0.5	13.4
2,3,3a,7a	-0.8	-3.7	-1.5	34.9	-21.6	-34.8
3,3a,7a,1	-21.7	-11.6	20.9	-44.2	36.1	46.7
3a,7a,1,2	38.0	23.8	-32.1	36.6	-37.4	-39.6
7a,1,2,3	-38.6	-26.3	32.7	-13.0	23.2	16.0
3a,4,5,6	-40.9	49.2	-50.8	-56.1	48.2	-53.3
4,5,6,7	66.8	-64.5	56.4	59.3	-57.8	52.8
5,6,7,7a	-59.6	59.6	-56.7	-54.9	60.6	-54.1
6,7,7a,3a	28.4	-39.9	54.1	48.3	-53.7	59.4
7,7a,3a,4	-4.4	28.4	-57.8	-49.8	43.5	-62.7
7a,3a,4,5	10.8	-33.2	54.6	57.1	-40.8	57.5
3,3a,7a,7	-142.1	129.7	143.0	-166.1	-85.1	170.7
4,3a,7a,1	115.9	-146.5	-179.9	76.5	164.6	173.2

methoxy groups give rise to two closely spaced singlets. Addition of increasing amounts of $Eu(hfc)_3$ causes down-field shift and splitting of these signals, so that when the weight ratio between the ester and the shift reagent reaches about 2:3, four base-line separated peaks appear in a spectral region free from overlap with other signals. In the corresponding spectrum of the diester of the diol (-)-**7**, obtained at 50% conversion in the enzymatic hydrolysis, only two of these peaks are visible so that an e.e. higher than 98% can confidently be assumed for this product (Figure 1). This also proves that the corresponding epoxide **5**, recovered from the same enzymatic reaction, for which, as said above, poor peak separation on the chiral GLC chromatographic column allowed only the evaluation of a minimum e.e. of 90%, actually had an optical purity of at least 98%.

Difficulties in preparing the bis(4-methoxybenzoate) of the diol **8** and the result of the PC-MODEL computations which indicated a value of -162° for the O(3)/C(3)/C(3a)/O(3a) dihedral angle, too close to planarity for an unambiguous application of the exciton chirality method, precluded a direct determination of the absolute configuration and optical purity of the diol **8** by this method. The optically active diol **8** was therefore converted into the ketol **12** by oxidation with the Fetizon reagent (Ag_2CO_3 on Celite[R]). Its CD spectrum in



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methanol exhibited a positive Cotton effect for the $n \longrightarrow \pi^*$ transition at 318.4 nm. Application of the ketone octant rule strongly points to an (*R*) configuration at the C(3a) of **12**, since in this enantiomer all the cyclohexane moiety falls in a positive back octant, and only the hydroxy group in a negative one, which may account for the

relatively small amplitude of the observed Cotton effect ($\Delta\epsilon$ = +0.47). It may be pointed out that the (3a*R*,7a*S*) enantiomer of **12** has a formal configurational correspondence with rings C and D of 17-oxosteroids, all of which present a positive Cotton effect in the n $\rightarrow\pi^*$ region.[14] Although an α-OH group can in some cases exhibit an "anti-octant" behavior,[15] this usually occurs when the hydroxyl is equatorial, and not axial as in **12**. Furthermore these effects are usually small and not likely to offset the sign of the Cotton effects when other much bulkier groups are present in the molecule. It can therefore be confidently assumed that **12** has the (3a*R*,7a*S*) configuration, and, consequently epoxide (-)-**6** and the diol (-)-**8** from which **12** derives, respectively, the (3*S*,3a*R*,7a*S*) and the (3*R*,3a*R*,7a*S*) configurations. Finally, although the e.e. of (-)-**8** was not directly ascertained, the value of at least 98% found for (+)-**6** at 50% conversion of the racemate implies a similar optical purity of the product diol (-)-**8**.

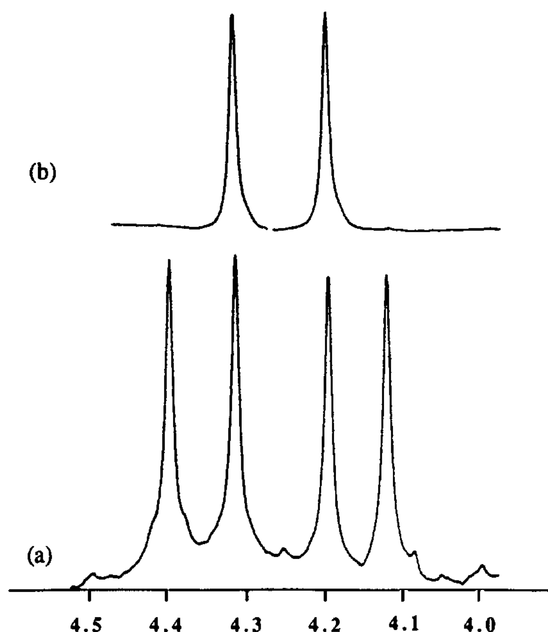
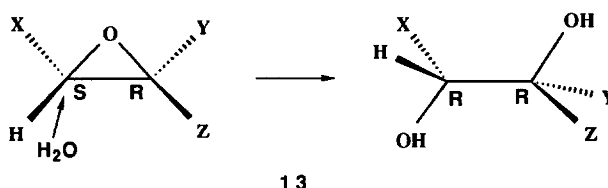


Figure 1. Methoxyl proton signals in the NMR spectra of 8.5 mM (±)-**9** (a) and (-)-**9** (b) in the presence of 7.5 mM Eu(hfc)3.

Discussion

The results described above fit well into the picture emerging from the by now very extensive documentation from this and other laboratories on the structural and configurational factors affecting the tendency of epoxides to be hydrolytically opened by mammalian epoxide hydrolase. The regio- and stereoselectivities of these reactions obey some rules,[2] with only very few known exceptions,[16] that can be schematized as shown in **13**, where **X** and **Z** are H or small groups, and **Y** larger lipophilic substituents. **X**, **Y** and **Y**, **Z** can be linked to form a ring. The epoxide ring opening always occurs through nucleophilic attack by water *anti* to the oxirane oxygen, usually at the less hindered (primary or secondary, never tertiary) carbon. In the case of meso or chiral

epoxides, if viewed as shown in **13** with the oxirane ring in the plane and oxygen on top, a preferential attack is often observed at the left hand carbon. In the case of terminal epoxides (X=H) this produces an excess of the (*R*)-diol. When the epoxide is meso (X=Y; Z=H) the (*R*,*R*)-diol is usually formed preferentially by attack on the (*S*) carbon. Finally, when both oxirane carbons are chiral, the enantiomer which has the larger lipophilic moiety (Y) on the back right side is a better substrate than its antipode, a fact that implies the presence of a large lipophilic pocket in the active site of the enzyme situated in such a way that it can offer the best accommodation for the larger lipophilic substituent.[17] As a consequence good kinetic resolutions can often achieved in which the (*S*,*R*) enantiomer of a racemic epoxide usually gives the (*R*,*R*)-diol of medium to high optical purity.



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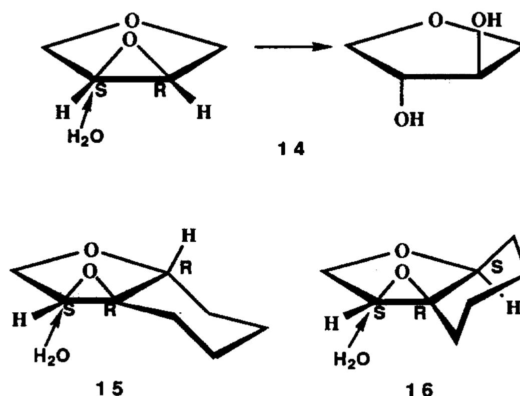
Epoxides **1**, **5**, and **6** behave in perfect agreement with the above rules and provide further support for them. 3,4-Epoxytetrahydrofuran **1**, a meso epoxide, is opened with high preference at the (*S*) carbon to give the corresponding (*R*,*R*)-diol (1,5-anhydro-*D*-threitol, **2**), as shown in **14**. Similar results had been obtained with its carbocyclic analogue, 1,2-epoxy-cyclopentane, that was converted by MEH into the corresponding (*R*,*R*)-diol, having an e.e. of 90%, with a V_S value (23 nmol min^{-1} mg^{-1} protein),[18] very close to that found for **1**, which shows that replacement of a methylene group by an oxygen atom has little influence on the susceptibility of an epoxide to MEH catalyzed hydrolysis.

In a different enzymatic approach to the diol **2**, a lipase-catalyzed selective hydrolysis of the diacetate **3**, the (*R*,*R*)-monoacetate was obtained with an e.e. of only 51%.[19] It may also be mentioned that in the case of a higher ring homologue of **1**, 3,4-epoxytetrahydropyran, its two enantiomers are good substrates for MEH, but react with opposite regiospecificity of attack by water, which occurs in both of them at the (*S*) oxirane carbon, in order that the racemic epoxide is converted entirely into the (3*R*,4*R*)-diol.[20] Also in several racemic 3,4-anhydropyranosides the steric course sketched in **13** was strictly followed, which allowed to achieve highly enantioselective kinetic resolutions.[21]

The results obtained with the racemic **5** and **6** also fit very well into the picture and are among the best examples of very high enatioselectivity and complete regiospecificity so far observed in this type of reaction. MEH catalyzed attack by water occurs exclusively on the secondary oxirane carbon of configuration (*S*) of the (3*S*,3a*R*,7a*R*) enantiomer of **5** and (3*S*,3a*R*,7a*S*) enantiomer of **6**.

The interesting fact that the acid catalyzed ring opening of **5** and **6** shows an opposite regioselectivity with respect to the MEH catalyzed one, with preferential attack at the tertiary rather than at the secondary carbon, is in accordance with the operation of an S_N2-type mechanism involving general base catalysis, without an electrophilic component, in the enzymatic reaction,[22] and a borderline S_N1-type mechanism with oxygen protonation and a significant degree of positive charge development before the attack by water in the acid-promoted reaction.[9]



The high regio- and enantioselectivity observed in the enzymatic hydrolysis of **5** and **6** can well be explained on the basis of model **13**. Only the (3*S*,3a*R*)-enantiomers of diastereomers **5** and **6** meet the requirements for optimal fitting into the enzyme active site, as shown in **15** and **16.** The total resistance of their (3*R*,3a*S*,7a*S*)- and (3*R*,3a*S*,7a*R*)-enantiomers to enzymatic hydrolysis can well be explained by an impossibility to fit into the active site, owing to non-correspondence between the bulky cyclohexane ring close to the oxirane one and the hypothesized lipophilic pocket. A similar situation had been found, for instance, in the case of 3-*tert*-butyl-1,2-epoxycyclohexane.[23]

In view of the fact that liver microsomes are prepared easily and cheaply and of the practically complete enantioselectivity of these hydrolysis reactions, they could well be of practical interest for kinetic resolutions of epoxides **5** and **6** and of their analogues, with the aim of preparing them and the corresponding *trans*-diols in high optical purity on a small to medium scale.

Experimental

General Methods. ^{1}H and ^{13}C NMR spectra were recorded in the indicated solvent on a Bruker AC 200 instrument with Me_4Si as internal standard. Chemical shift values were confirmed by COSY and HETCOR experiments. *J* couplings were obtained by first-order analysis whenever possible or by simulation with the PANIC (Bruker) computer program. The overlapping cyclohexane proton signals in the spectra of **5, 6, 7** and **8** prevented a complete analysis; the $J_{7a,7}$ and $J_{7a,7'}$ were obtained as splittings from H-7a multiplets. Optical rotations were measured at 20°C with a Perkin-Elmer Model 241 photoelectric polarimeter. CD spectra were measured with a JASCO J-500 C spectropolarimeter. GLC analyses were carried out with a Dani 3800 instrument equipped with a glass column (2 mm i.d. x 2.0 m) packed with 3% OV-225 on 80/100 Supelcoport under the following conditions: A) column temp. 135°-185°C (5°C/min), high isotherm 185°C, N_2 flow 30 ml/min; B) column temp. 170°-190°C (4°C/min), high isotherm 190°C, N_2 flow 30 ml/min; C) column temp. 180°C, N_2 flow 30 ml/min. Enantiomeric purities were measured on a Carlo Erba HRGC 5300 instrument equipped with a GTA-Chiraldex^{R} capillary column. Preparative chromatography was carried out on glass columns (25 mm i.d. x 60 cm) packed with Kieselgel 60 (70-230 mesh ASTM) (Merk).

Commercial reagent grade and anhydrous solvents were used without further purification. Acetic anhydride and *p*-methoxybenzoyl chloride were distilled before use. $MgSO_4$ was used as the drying agent.

3,4-Epoxytetrahydrofuran (1). It was prepared by oxidation of the corresponding olefin with MCPBA in CH_2Cl_2; b.p. 53-54°C/20 mm Hg (lit.[25] 53-54°C/20 mm Hg); δ_H ($CDCl_3$) 3.63 (1 H, bd, *J* 10.52, < 0.5 Hz, H-2), 3.78 (1 H, d, *J* 10.52 , 0.0 Hz, H-2'), 4.00 (1 H, bs, $J < 0.5$, 0.0 Hz, H-3); δ_C ($CDCl_3$) 55.59 (C-3), 67.22 (C-2).

(±)-*trans*-Tetrahydrofuran-3,4-diol (2). It was prepared as reported by Hawkins[24]. δ_H ($CDCl_3$) 3.72 (1 H, *J* 10.13, 1.85 Hz, H-2*cis*), 4.07 (1 H, *J* 0.13, 4.04 Hz, H-2*trans*), 4.23 (1 H, *J* 1.85, 1.92 Hz H-3). It was converted into the corresponding *diacetate* **3** by treatment with excess acetic anhydride in pyridine at room temp. (24 h), followed by dilution with cold 10% HCl and extraction with EtOAc. The organic layer was washed with saturated $NaHCO_3$ aq., dried, evaporated *in vacuo* and purified by Kugelrohr distillation (bath temp. 100°C/1 mm Hg); δ_H ($CDCl_3$) 3.766 (2 H, *J* 10.65, 2.38 Hz, H-2*cis*), 4.095 (2 H, *J* 10.65, 4.68 Hz, H-2*trans*), 5.167 (2 H, *J* 2.38, 1.89 Hz, H-3); δ_C ($CDCl_3$) 20.80 (Me), 71.88 (C-2), 77.22 (C-3), 170.00 (C=O).

2,4,5,6,7,7a-Hexahydrobenzofuran (4). It was prepared as previously reported[6]; δ_H ($CDCl_3$) 0.51 (m, 3 H, H-5ax, H-6ax and H-7ax), 1.03 (m, 2 H, H-5eq and H-6eq), 1.23 (m, 1 H, H-4ax), 1.37 (m, 1 H, H-7eq), 1.80 (m, 1 H, H-4eq), 3.71 (m, 1 H, H-7a), 3.84 (m, 2 H, H-2 and H-2'), 4.61 (m, 1 H, H-3). δ_C ($CDCl_3$) 23.15 (C-6), 26.46 (C-5), 26.96 (C-4), 35.12 (C-7), 74.54 (C-2), 84.57 (C-7a), 115.30 (C-3), 141.57 (C-3a).

***cis*- and *trans*-3,3a-Epoxyoctahydrobenzofurans (5) and (6).** To a stirred and cooled (0°C) solution of the olefin **4** (2.0 g, 1.6 mmol) in CH_2Cl_2 (30 ml) was added portionwise 70% MCPBA (4.4 g, 1.8 mmol). After standing at 0°C overnight the suspension was filtered, washed with saturated Na_2CO_3, dried and evaporated *in vacuo*. The residue was distilled (Kugelrohr, 130°C/15 mmHg) to give 1.8 g (80% yield) of a 38:62 mixture of **5** and **6** (GLC cond. A). After column chromatography on silica gel with hexane-ethyl acetate (9:1 v/v) pure **5** (0.6 g) and **6** (0.9 g) were obtained (NMR, see Table 1).

***trans* and *cis*-Octahydrobenzofuran-*trans*-3,3a-diols (7) and (8).** The pure racemic epoxide **5** or **6** (50 mg, 0.36 mmol) was stirred in 0.3 *M* H_2SO_4 (2 ml) at room temperature for 12 h; after neutralization with saturated $NaHCO_3$, the solution was extracted with EtOAc (2 x 30 ml) and the organic layer was dried and evaporated *in vacuo*. GLC analyses (condition B) showed that only the diol **7** was obtained from **6** and an 81:19 mixture of the diols **8** and **7** was obtained from **5**. Purification by column chromatography on silica gel (3:7 v/v EtOAc-hexane) gave pure **8**, m.p. 102-104°C (plates from EtOAc-hexane) (Found: C, 60.6; H, 8.8. $C_8H_{14}O_3$ requires C, 60.7; H, 8.9) and pure **7**, m.p. 99-101°C (plates from EtOAc-hexane) (Found: C, 60.6; H, 8.7. $C_8H_{14}O_3$ requires C, 60.7; H, 8.9.) (NMR, see Table 1).

(±)- and (-)-*cis*-Octahydrobenzofuran-*trans*-3,3a-diol bis(*p*-methoxybenzoate) (9). A solution of racemic or optically active **7** (45 mg, 0.28 mmol), *p*-methoxybenzoyl chloride (485 mg, 2.8 mmol) and DMAP (5 mg, 0.04 mmol) in pyridine (1.5 ml) was refluxed for 3.5 h, then cooled and diluted with aq. 10% HCl and extracted with EtOAc (3 x 10 ml). The washed (aq. satd. $NaHCO_3$) and dried extract was evaporated *in vacuo* and purified by preparative TLC (8:2 v/v hexane-EtOAc) to obtain as a colorless oil (±)-**9** or (-)-**9** $[\alpha]_D$= -150° ($CHCl_3$, *c* 1.0). (NMR, see Table 1).

***trans*-3a-Hydroxyoctahydrobenzofuran-3-one (12).** To a suspension of freshly prepared Ag_2CO_3 on Celite[R] (50%) (5g, 9.0 mmol) in benzene (70 ml) was added (-)-**8** (70 mg, 0.44 mmol). Some of the benzene was distilled azeotropically and then the suspension was refluxed . The very slow reaction was monitorized by GLC and after 26 h, when the conversion had reached 60%, the reaction mixture was cooled, the solid was filtered, the solution was concentrated and pure **12** was obtained by column chromatography on silica gel (7:3 v/v hexane-EtOAc) as a solid, m. p. 117°-121°C, $[\alpha]_D$= +75.4° (EtOAc, *c* 1.0) δ_H ($CDCl_3$) from 1.22 to 1.38 (m, 2 H) and from 1.62 to 2.07 (m, 6 H, cyclohexane protons), 3.52 (m, 1 H, $J_{7a,7ax}$ 11.0 Hz, $J_{7a,7eq}$ 5.1 Hz, H-7a), 3.83 (dd, 1 H, $J_{2,2'}$ 17.26 Hz, $J_{2,7a}$ 0.82 Hz, H-2), 4.33 (dd, 1 H, $J_{2',7a}$ 1.09 Hz, H-2'). δ_C ($CDCl_3$) 19.75 (C-5), 23.38 (C-6), 24.29 (C-7), 27.18 (C-4), 69.98 (C-7a), 72.60 (C-3a), 83.64 (C-2), 211.18 (C-3).

Microsomal Preparations. Liver microsomes, prepared from male New-Zealand white rabbits as previously described,[17] were suspended in 0.1 *M* Tris-HCl buffer (pH 9.0) to a final protein concentration of 11-15 mg/ml and were used directly or stored at -80°C.

Analytical runs. Analytical experiments were carried out with the following standard procedure: solutions of concentrations ranging between 0.3 and 2 mg of epoxides in 100 μl of acetonitrile were added to 1 ml of microsome suspension (protein concentration ranging between 2 and 11 mg/ml) and the mixtures were incubated with shaking at 37°C. After the required times, the reactions were stopped by cooling at 0°C.

In the case of **1**, the diol **2**, very soluble in water, was determined by immediate direct injection of the incubation mixture into the GLC column (cond. A) after addition of an appropriate amount of pure cyclohexane-*trans*-1,2-diol as internal standard. In the case of **5** and **6**, to the cooled mixtures were added solid NaCl and EtOAc (2 ml) containing an appropriate amount of pure *t*-3-*tert*-butylcyclohexane-*r*-1,*t*-2-diol as internal standard. After shaking and further extraction with EtOAc (2 x 5 ml) the combined organic layers were analyzed by GLC (cond. B) for the quantitation of optically active diol products **7** and **8**. These analyses showed that only diol **2** from **1**, only diol **7** from **5** and only diol **8** from **6** were formed. Each value is the average of at least three determinations, corrected by using a calibration curve obtained with standard solutions of the pure reference compounds in the appropriate solvents.

Blank experiments carried out with pure compounds **1**, **5** and **6**, and inactivated microsomes or using only Tris-HCl buffer (pH 9.0) showed that no spontaneous hydrolysis of the epoxides occurred even at the longest incubation times. Other tests with pure racemic **2**, **7** and **8** and active microsomes, showed that they were recovered from incubations in 70-85% yield. In the case of epoxide **1**, hydrolysis to diol **2** occurred up to almost complete conversion linearly with time, as well as with the amount of microsomal proteins, whereas for epoxides **5** and **6** the rate of hydrolysis decreased after about 40% conversion and the reaction stopped when 50% of the epoxide had been converted into diol. The following reproducible values of saturation velocities were obtained from duplicate experiments; for (±)-**1** Vs = 26.4±0.8 nmol min^{-1} mg^{-1} protein; for (±)-**5** Vs = 17.9±0.9 nmol min^{-1} mg^{-1} protein and for (±)-**6** Vs = 33.1±0.8 nmol min^{-1} mg^{-1} protein.

Preparative runs. Racemic **1** (200 mg, 2.3 mmol) in CH_3CN (200 μl) was added to the microsome suspension (15 ml, 11 mg/ml protein) and the mixture was incubated as above. When the complete hydrolysis was reached (GLC), the cooled mixture was shaken with aq. 15% $ZnSO_4$ (1 ml) and then with saturated aq. $Ba(OH)_2$ (1 ml). Then the suspension was evaporated to dryness *in vacuo* , extracted with EtOAc (3 x 20 ml), dried, concentrated and purified by TLC (1:1 v/v EtOAc-hexane) to obtain **(3*R*,4*R*)-tetrahydrofuran-3,4-diol (2)** as a deliquescent solid; $[\alpha]_D$= +4.8° (H_2O, *c* 4.0). The corresponding **diacetate 3**, prepared as above, had $[\alpha]_D$= -45.5° (EtOAc, *c* 1.0).

A sample of racemic **5** or **6** (200 mg, 1.3 mmol) in CH_3CN (200 μl) was added to the microsome suspension (15 ml, 11 mg/ml protein) and incubated with shaking at 37°C. When the maximum hydrolysis was reached (GLC), the cooled mixture was shaken with aq. 15% $ZnSO_4$ (1 ml) and then with saturated aq. $Ba(OH)_2$ (1 ml). The suspension was extracted immediately with *n*-pentane (3 x 10 ml). The organic layer was dried, concentrated and purified by column chromatography on silica gel (9:1 v/v hexane-EtOAc) to recover unreacted **(3*R*,3a*S*,7a*S*)-(+)-3,3a-epoxyoctahydrobenzofuran (5)** $[\alpha]_D$= +19.0° ($CHCl_3$, *c* 1.0), or **(3*R*,3a*S*,7a*R*)-(+)-3,3a-epoxyoctahydrobenzofuran (6)** $[a]_D$= +30.0° ($CHCl_3$, *c* 1.0). The aqueous suspension remaining from the pentane extraction was submitted to further extraction with EtOAc (5 x 10 ml), the organic layer was dried, concentrated *in vacuo* , and after purification by column chromatography on silica gel (7:3 v/v hexane-EtOAc), pure **(3*R*,3a*R*,7a*R*)-octahydrobenzofuran-3,3a-diol (7)** $[\alpha]_D$= -20.5° (EtOAc, *c* 1.0) and pure **(3*R*,3a*R*,7a*S*)-octahydrobenzofuran-3,3a-diol (8)** $[\alpha]_D$= -20.0° (EtOAc, *c* 1.0) were obtained, respectively, from (±)-**5** and (±)-**6**.

Enantiomeric Composition and Absolute Configurations of Products. The enantiomeric excesses (e.e.) of unreacted (+)-**5** and (+)-**6**, recovered from enzyme promoted hydrolysis, were ascertained by chiral GLC on a GTA-Chiraldex^R capillary SiO_2 column (0.25 mm i.d. x 10 m; column. temp. 99°C, N_2 flow 1 ml/min). The retention times of the enantiomers of a sample of racemic **5** were respectively 15.0 and 16.1 min; under the same GLC conditions a sample of (+)-**5** showed only the peak corresponding to the enantiomer with relative retention time 15.0 min. Nevertheless, in this case, poor separation of the peaks allowed only to set a minimum value of 90% for the e.e. of (+)-**5**. In the case of racemic **6**, chiral GLC analysis showed two enantiomeric peaks with retention times of 7.1 and 8.3 min. Under the same GLC conditions, a sample of (+)-**6** showed only the peak corresponding to the enantiomer with retention time 7.1 min. When 4% of racemic **6** was added to (+)-**6** the peak for (-)-**6** become clearly visible. Therefore an e.e. of at least 98% can be deduced for (+)-**6**.

The addition of $Eu(hfc)_3$ (9.2 mg, 7.7 μmol) to the diacetate (±)-**3** (2.1 mg, 11 μmol) in $CDCl_3$ (0.5 ml) led to a shift (δ 3.76) and a split (Δδ 7.2 Hz) of ^1H NMR methyl signal. Fourfold repeated spectra of the diacetate (-)-**3** were taken and the methyl signals were accurately integrated to give an e.e. of 96±0.3%.

When $Eu(hfc)_3$ (3.7 mg, 3.1 μmol) was added to the bis(*p*-methoxybenzoate) (±)-**9** (1.6 mg, 3.7 μmol) in $CDCl_3$ (0.4 ml) the two methoxy signals at δ 3.87 and 3.885 ppm shifted and split to give four well separated singlets at δ 4.12, 4.20, 4.32 and 4.40 ppm. The spectrum obtained from a solution of (-)-**9**, $[\alpha]_D$= -150° ($CHCl_3$, c 1.0) (1.4 mg, 3.3 μmol) and $Eu(hfc)_3$ (3.3 mg, 2.8 μmol) in $CDCl_3$ (0.4 ml) showed only the signals at δ 4.20 and 4.32 ppm. When 4% of racemic **9** was added to (-)-**9** the signals for (+)-**9** at δ 4.40 and 4.12 become clearly detectable. Therefore the e.e. was extimated to be at least 98%. A 1400 Hz spectral width for 8

K data points zero filled to 16 K and an exponential multiplication function (EM = 1.0) were used to minimize the integral error.

The CD spectrum of (-)-**9** in CH_3CN showed a double Cotton effect with $\Delta\epsilon_{262}$ -28.5 and $\Delta\epsilon_{246}$ +10.7. The CD spectrum of (+)-**12** in MeOH showed a Cotton effect with $\Delta\epsilon_{318.4}$ +0.47.

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