chilled to −78 °C. Complex 13 was collected, washed with cold Et_2O (10 mL), and finally dried under reduced pressure to yield 0.080 g (53%) of product. Spectroscopic data was identical with literature values.[13]

Acknowledgment. M.E.W. wishes to thank the Office of the Vice President for Research at Utah State University for generously funding this research. M.E.W. wishes to thank Prof. Vernon D. Parker for several meaningful and helpful discussions of this work prior to publication.

Supplementary Material Available: Full experimental details concerning the synthesis of 3 (4 pages). Ordering information is given on any current masthead page.

Notes

Product Enantioselectivity in the Microsomal Epoxide Hydrolase Catalyzed Hydrolysis of 10,11-Dihydro-10,11-epoxy-5*H*-dibenzo[*a*,*d*]-cycloheptene

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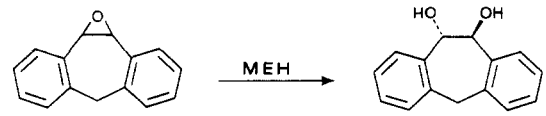
Received July 12, 1988

10,11-Dihydro-10,11-epoxy-5*H*-dibenzo[*a*,*d*]cycloheptene (1) is a product of considerable biological interest, its parent structure being present in the metabolites formed by the cytochrome P-450 catalyzed oxidation of the 10,11 double bond of several important tricyclic drugs used against central nervous system (CNS) diseases.[1-4]

Epoxides are usually biotransformed into the corresponding vicinal diols by a trans addition of water catalyzed by epoxide hydrolases, key enzymes of the xenobiotics detoxifying system.[5] The microsomal epoxide hydrolase (MEH) is endowed with a low substrate specificity, as required for an enzyme involved in the metabolism of a very broad range of exogenous compounds, but often exhibits a remarkable capability of chiral recognition, enabling it to discriminate between enantiomers of racemic epoxides[6-15] and between enantiomeric carbons of meso epoxides.[16-19] In most reported cases MEH catalyzes ring opening preferentially at *S* oxirane carbons to give the *R* or *R,R* enantiomers of the corresponding diols.[20] In particular, *cis*-stilbene oxide (5), a meso epoxide that is a close analogue of 1, was reported to be hydrolyzed to nearly optically pure (*R,R*)-(+)-1,2-diphenyl-1,2-ethanediol.[17]

In the course of an investigation of its MEH-catalyzed hydrolysis,[21] epoxide 1 was found to be a much worse substrate for this enzyme than 5, its lower reaction rate being due to a much lower V_S rather than to a higher K_m with respect to 5, thus pointing to a difficulty in the nucleophilic attack by water at the oxirane carbons of 1. It appeared therefore interesting to check if this large decrease in the rate of nucleophilic attack by water produced by the closure of a seven-membered ring through a methylene bridge between the ortho positions of the two phenyl rings of 5 was also accompanied by a change in the steric course of the reaction.



1 2

The enzymatic hydrolysis of 1 was carried out with rabbit liver microsomes at 37 °C and pH 7.4. Owing to the slow rate, it was necessary to incubate 50-mg samples of 1 for at least 24 h, with repeated addition of microsomal preparation, in order to achieve an about 25% conversion into the diol. No spontaneous hydrolysis of 1 occurred during this time, as shown in a blank experiment with inactivated enzyme. HPLC analysis revealed, by comparison with authentic samples of the (±)-*trans*-dihydrodiol 2 and of the diastereomeric *cis*-dihydrodiol, the

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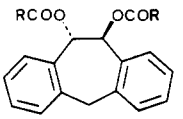
sole presence of the former, besides the unreacted epoxide 1. No trace of the *cis*-diol could be detected, in agreement with the exclusive anti ring opening of both alkyl- and aryl-substituted oxiranes observed in MEH-catalyzed hydrolyses. Diol **2** was separated from the unreacted epoxide by column chromatography. Recrystallization was avoided before the measurement of the optical rotation. Different samples obtained in several experiments had $[\alpha]^{20}_D$ −69 ± 2°.

The enantiomeric excess of this diol was determined through conversion into the diastereomeric bis(MTPA) esters **3** with (*S*)-(+)-α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride[22] followed by HPLC analysis. A (50 ± 1):(50 ± 1) ratio of the peaks corresponding to the two diastereomers was measured for a sample obtained from synthetic (±)-**2**, whereas a (24 ± 1):(76 ± 1) ratio was found with a sample of (−)-**2**, $[\alpha]^{20}_D$ −71°. This corresponded to a 52 ± 2% ee and a maximum optical rotation $[\alpha]^{20}_D$ 137 ± 5°.

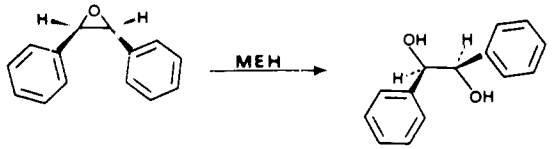


3 R = PhC(CF$_3$)(OCH$_3$)

4 R = p-CH$_3$O-C$_6$H$_4$

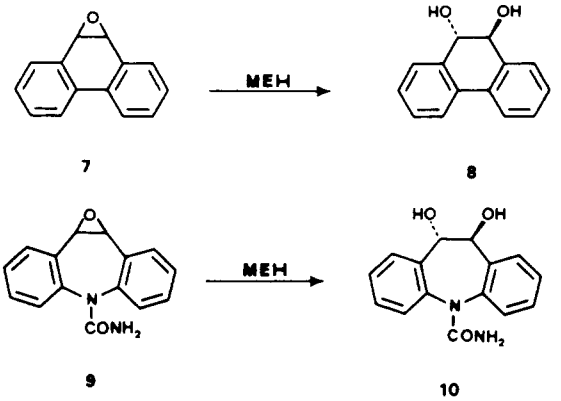
The exciton coupling method was used to determine the absolute configuration of (−)-**2**. A sample of diol with $[\alpha]^{20}_D$ −68° was converted into its bis(*p*-methoxybenzoate) **4** in order to apply the reliable dibenzoate chirality rule.[23] The expected exciton splitting was observed, with a positive Cotton effect at 267 nm ($\Delta\epsilon$ = +13.2) and a negative one at 249 nm ($\Delta\epsilon$ = −13.4). Although the relative orientation of the two 10,11 substituents could not be deduced from the NMR spectrum of **4** by ^3J measurements owing to the equivalence of the two α protons, the large $\Delta\epsilon$ values clearly pointed to a high preference for a gauche disposition, and their signs showed a right-handed screwness of the *p*-methoxybenzoyloxy groups. This corresponds to a 10*S*,11*S* absolute configuration for the bis(*p*-methoxybenzoate) of (−)-**2** and consequently for (−)-**2** itself. Water attack in the MEH-catalyzed hydration had occurred therefore with a 76% preference at the *R* carbon of the meso epoxide **1**.

For comparison purposes, the enzymatic hydrolysis of *cis*-stilbene oxide, **5**, with the same lot of rabbit liver microsomes after 1:1 dilution with buffer was also reexamined. Complete hydrolysis of the substrate occurred within 4 h, and the produced *R*,*R*-1,2-diphenyl-1,2-ethanediol, **6**, had $[\alpha]^{20}_D$ +80°, corresponding to 87% optical purity.[24]



5 **6**

These results show that in the MEH-catalyzed hydrolysis of **1** not only is nucleophilic attack by water much slower than in the case of the acyclic analogue *cis*-stilbene oxide, but it occurs with a lower and opposite product enantioselectivity. This unusual type of enantioselection is not due to the seven-membered ring, since the parent cycloheptene oxide is easily hydrolyzed by the rabbit liver MEH to (*R*,*R*)-*trans*-1,2-cycloheptanediol in an about 40% ee.[18] Two other examples of preference for opening at an *R* carbon during the MEH-catalyzed hydration of meso epoxides have been reported: phenanthrene 9,10-oxide (**7**), giving the 9*S*,10*S* diol **8** in about 25% ee,[13] and a close analogue of **1** deriving from the 5*H*-dibenz[*b*,*f*]azepine ring system, carbamazepine 10,11-oxide, **9**, yielding an 80% ee of the 10*S*,11*S* diol **10**.[19] All three epoxides **1**, **7**, and **9**



7 **8**

9 **10**

have in common two benzo groups condensed to a central ring, which is fused to the oxirane ring. Although they are all closely related to *cis*-stilbene oxide, **5**, they lack its conformational flexibility, owing to their rigid tricyclic structures. Therefore **5** can better fit in the MEH active site in a suitable conformation and with the right orientation with respect to the histidine imidazole ring that is known[25] to provide nucleophilic assistance to the back-side attack by the water, normally occurring efficiently at the *S* oxirane carbon. It is reasonable to assume that compounds **1**, **7**, and **9** also fit into the not highly structure discriminating active site, as proven by the low K_m value[21] of **1**, but that their rigidity does not allow them to assume the optimal orientation for an efficient water attack at the *S* carbon so that an anomalous attack at the *R* carbon occurs.

Experimental Section

Melting points were determined on a Kofler block and are uncorrected. NMR spectra were recorded at 60 MHz. CD spectra were recorded with a JASCO J500C spectropolarimeter.

Kieselgel (150–230 mesh, ASTM, Merck) was used for column chromatography. HPLC analyses were carried out with a Pye-Unicam PU4010 apparatus equipped with an UV PU4020 detector under the following conditions: (a) reverse phase, 25 cm, 10 μm C_{18} Techopack, 70:30 MeOH/H_2O, 1 mL min^{-1}, 260 nm; (b) direct phase, 25 cm, 5 μm, Lichrospher Si 100 (Merck), 97.5:2.5 hexane/EtOAc, 1.5 mL min^{-1}, 260 nm.

Materials. 5*H*-Dibenzo[*a*,*d*]cycloheptene, mp 133–134 °C (lit.[26] mp 132 °C), was prepared from commercial 5*H*-dibenzo[*a*,*d*]-cyclohepten-5-one (Jannsen, >97%) as reported.[26] 10,11-Dihydro-10,11-epoxy-5*H*-dibenzo[*a*,*d*]cycloheptene (**1**), mp 143–145 °C (lit.[27] mp 144–145 °C), was obtained in 80% yield by epoxidation of 5*H*-dibenzo[*a*,*d*]cycloheptene with *m*-chloroperoxybenzoic acid in $CHCl_3$ for 8 h, followed by crystallization from EtOH. (±)-*trans*-10,11-Dihydro-10,11-dihydroxy-5*H*-dibenzo-

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[a,d]cycloheptene (2), mp 178–180 °C, was prepared by reacting 5H-dibenzo[a,d]cycloheptene with I_2 and silver benzoate in anhydrous benzene, followed by hydrolysis with ethanolic KOH and crystallization from $CHCl_3$, as reported elsewhere.[28] *cis*-10,11-Dihydro-10,11-dihydroxy-5H-dibenzo[a,d]cycloheptene, mp 180–181 °C (from EtOH), was obtained from 5H-dibenzo[a,d]-cycloheptene and OsO_4, as reported elsewhere.[28] *p*-Methoxybenzoyl chloride (Aldrich, 99%) was distilled before use (bp 123–125 °C/25 mm). (*S*)-(+)-α-Methoxy-α-(trifluoromethyl)-phenylacetyl chloride, bp 54 °C/1 mm, was prepared from the *R*-(+) acid and thionyl chloride.[22] Solvents were reagent grade.

Enzymatic Hydrolysis of 10,11-Dihydro-10,11-epoxy-5H-dibenzo[a,d]cycloheptene (1). A solution of epoxide 1 (50 mg, 0.24 mmol) in acetonitrile (1 mL) was added to 10 mL of microsomal preparation containing 40 mg protein/mL, obtained from male New Zealand white rabbits as previously reported,[19] preheated at 37 °C, and the mixture was incubated with shaking. After 12 h a fresh microsomal preparation (10 mL) was added, and the incubation continued for 12 more hours. The reaction was then stopped by addition of NaCl and the mixture was extracted with EtOAc (3 × 20 mL). The combined extracts were reduced to an exactly known volume by evaporation in vacuo, a proper amount of a stock solution of 9-formylanthracene in EtOAc was added to a sample as an internal standard, and the amount of *trans*-10,11-dihydro-10,11-dihydroxy-5H-dibenzo[a,d]cycloheptene (2) was determined by HPLC (conditions a) in order to evaluate the extent of hydrolysis of the substrate. This was typically around 25%. The remaining part of the extract was evaporated in vacuo and chromatographed on a column of silica gel (40 g) with 7:3 hexane/EtOAc as the eluant. Eluted fractions (5 mL) were analyzed by HPLC (conditions a). The fractions containing the *trans*-diol 2 were combined and evaporated to give 10 mg of pure (HPLC) 2: mp 147–150 °C; $[\alpha]^{20}_D$ −69° (*c* = 0.55, MeOH). This product had IR and NMR spectra identical with those of synthetic (±)-2. Samples of (−)-2 with $[\alpha]^{20}_D$ ranging between −67 and −71° were obtained in several enzymatic hydrolyses of 1 under the above described conditions.

Enzymatic Hydrolysis of *cis*-Stilbene Oxide. A solution of *cis*-stilbene oxide (50 mg) in EtOH (1 mL) was added to 5 mL of microsomal preparation and 5 mL of 50 mM Tris·HCl buffer, pH 7.4, preheated at 37 °C, and the mixture was incubated for 4 h and extracted with EtOAc (3 × 10 mL). Evaporation of the extracts gave a residue containing only diol 6 (HPLC, conditions a), which, after purification by column chromatography over silica gel, had $[\alpha]^{20}_D$ +80° (*c* = 0.9, EtOH).

Determination of the Enantiomeric Excess of Diol 2. A sample of diol 2 (6 mg, 0.026 mmol), $[\alpha]^{20}_D$ −71°, was dissolved in pyridine (2 mL) containing 3 mg (0.025 mmol) of *p*-(dimethylamino)pyridine and treated with (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (67 mg, 0.26 mmol). After 5 days at room temperature, the mixture was diluted with H_2O, acidified with 10% HCl, and extracted with EtOAc. The extract was washed with saturated $NaHCO_3$, dried ($MgSO_4$), and evaporated in vacuo, and the residue was analyzed by HPLC (conditions b). Two peaks with relative retention times of 1 and 1.3, corresponding to the diastereomeric bis(MTPA esters) 3, were detected in a ratio of 24 ± 1:76 ± 1. When racemic 2 was used as starting material, the two diastereomeric bis(MTPA esters) were present in a ratio of (50 ± 1):(50 ± 1).

Determination of the Absolute Configuration of (−)-2 via Its Bis(*p*-methoxybenzoate) (4). *p*-Methoxybenzoyl chloride (92 mg, 0.53 mmol) was added to a solution of 15 mg (0.066 mmol) of (−)-2, $[\alpha]^{20}_D$ −68°, in pyridine (1.5 mL) containing 3 mg (0.025 mmol) of *p*-(dimethylamino)pyridine. After 7 days at room temperature the reaction mixture was diluted with H_2O and extracted with AcOEt. The organic phase was washed with 10% aqueous HCl and saturated $NaHCO_3$, dried ($MgSO_4$), and evaporated in vacuo. The crude residue was crystallized from EtOH to give 8 mg of the pure bis(*p*-methoxybenzoate) 4, mp 140–141 °C. NMR ($CDCl_3$): δ 3.83 (s, 6 H, OCH_3), 4.33 (s, 2 H, CH_2), 6.90 and 8.06 (AA'BB' system, 8 H, aromatic protons ortho and meta to the methoxy groups), 7.00 (s, 2 H, CHO), 7.30 (m, 8 H, dibenzocycloheptene aromatic protons). IR (Nujol): 1690 cm^{-1} (C=O); UV λ_{max} (CH_3CN) 257 nm (32 200). CD (CH_3CN): $\Delta\epsilon_{267}$ = +13.2, $\Delta\epsilon_{249}$ = −13.4.

Acknowledgment. This work was supported by grants from the Ministero della Pubblica Istruzione and from the Consiglio Nazionale delle Ricerche. We thank Dr. C. Rosini for the CD measurements.

Registry No. 1, 118319-30-5; 2, 118354-09-9; 4, 118319-31-6; 5, 1689-71-0; 6, 52340-78-0; MEH, 9048-63-9; 5H-dibenzo[a,d]-cycloheptene, 256-81-5.

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Hypochlorite-Induced Oxidative Decarboxylation of Trisubstituted Acetic Acids

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Received August 30, 1988

Hypohalite-induced oxidative decarboxylation of α-hydroxycarboxylic acids (1) is well documented,[1] and it is significant that reaction rates are dependent on pH, which may undergo substantial changes induced by consecutive reactions during oxidations.[2]

$$\underset{1}{RR'C(OH)CO_2H} \rightarrow RR'C{=}O + CO_2$$

R, R' = Ar, alkyl, or H

Initial attack upon compound 1 by the oxidant is plausible at either the alcohol or carboxyl function. Primary and secondary alcohols readily are oxidized by hypochlorite,[3] and certain tertiary alkyl hypohalites are decomposed by heat and/or light to produce ketones.[4] Alternately, hypohalites are known to effect oxidative decarboxylation of both benzoylformic acid (2, to benzoic acid)[1c,5] and phenylacetic acid (3, to benzaldehyde),[5] which lack the alcohol function.

$$\underset{2}{PhC(O)CO_2H} \qquad \underset{3}{PhCH_2CO_2H}$$

It has been suggested that α-keto acid 2 might react as an α-hydroxy compound through intermediate formation of the hydrate.[1c] Furthermore, compound 3 conceivably might be oxidized to the corresponding α-hydroxy acid under the reaction conditions used (100 °C for 8 h).[5]

In order to explore reactivity of hypochlorite at the carboxyl function in systems that could not develop α-hydroxy functionality, we have studied the interactions of trisubstituted acetic acids 4 with aqueous hypochlorite. Results obtained not only demonstrated that the α-hydroxy function is not requisite for oxidative decarboxylation but also revealed a hitherto unrecognized, hypo-

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