Synthetic Communications: An International Journal for Rapid Communication of Synthetic Organic Chemistry

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lsyc20

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Version of record first published: 23 Sep 2006.

To cite this article: Deevi Basavaiah & Sarikonda Bhaskar Raju (1995): Enantioselective Hydrolysis of 2, 2-Disubstituted Oxiranes Mediated by Microsomal Epoxide Hydrolase, Synthetic Communications: An International Journal for Rapid Communication of Synthetic Organic Chemistry, 25:21, 3293-3306

To link to this article: http://dx.doi.org/10.1080/00397919508013848

SYNTHETIC COMMUNICATIONS, 25(21), 3293-3306 (1995)

ENANTIOSELECTIVE HYDROLYSIS OF 2,2-DISUBSTITUTED OXIRANES MEDIATED BY MICROSOMAL EPOXIDE HYDROLASE

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Abstract: 2-Aryl-2-methyloxiranes are enantioselectively hydrolyzed with microsomal epoxide hydrolase from pig liver to provide 1,2-diols containing a tertiary benzylic alcohol stereogenic centre upto 34% enantiomeric purities.

The microsomal epoxide hydrolase is an important enzyme involved in the metabolism of xenobiotic compounds playing a fundamental role in the detoxification of highly carcinogenic and mutagenic epoxides arising by the oxidation of alkenes and aromatic substrates by the cytochrome P-450 dependent monooxygenases.[1-3] In the metabolism epoxide hydrolase converts the toxic, mutagenic and carcinogenic epoxides into more easily excreted 1,2-diols by the *trans* addition of water molecule.

Liver microsomal epoxide hydrolases have been successfully employed for the opening of meso and racemic epoxides to provide the corresponding enantio-

merically enriched 1,2-diols.[4-9] The mechanism of opening of oxirane ring to provide 1,2-diols by the microsomal epoxide hydrolase is believed to involve either a general base catalyzed nucleophilic *anti*-addition of water molecule to the oxirane ring or the nucleophilic attack of the active site carboxylate on the oxirane ring followed by the base catalyzed hydrolysis of the resulting acyl enzyme.[10,11]

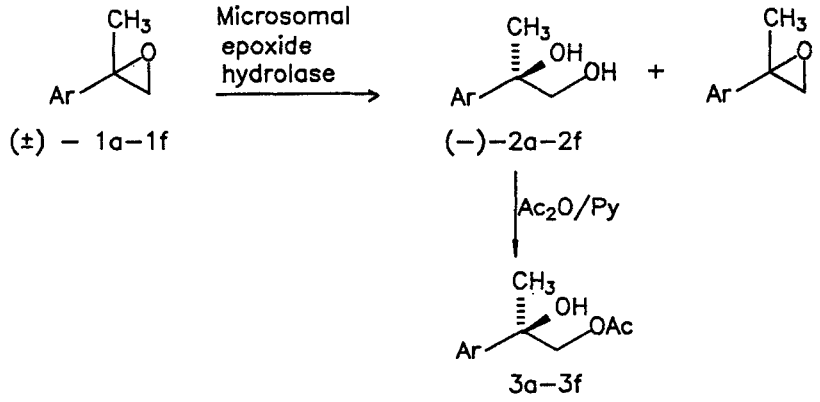
Enantiomerically pure 1,2-diols have been utilized as chiral directors in a number of stereoselective processes.[12-14] Despite recent developments in asymmetric synthesis a very few methods are available for the preparation of enantiomerically pure tertiary alcohols.[15-17] It occurred to us that microsomal epoxide hydrolase catalyzed hydrolysis of 2-aryl-2-methyloxiranes would provide the desired optically active 1,2-diols bearing a tertiary hydroxyl stereogenic centre. To the best of our knowledge these molecules have not been used as substrates for microsomal epoxide hydrolase catalyzed reactions. We have, therefore, studied the enantioselective hydrolysis of 2-aryl-2-methyloxiranes using microsomal epoxide hydrolase from pig liver and we, herein, report the results of our investigations.

First we have selected the racemic 2-methyl-2-phenyloxirane as a substrate. The microsomal epoxide hydrolase from pig liver was prepared according to the

literature procedure.[18] The racemic epoxide **1a** was subjected to hydrolysis in 0.1M, pH 7.4 phosphate buffer at room temperature with pig liver microsomes. The hydrolysis was stopped at conversion ratio 37:63. The resulting optically active 2-phenyl-1,2-propanediol (**2a**) was obtained in 28% enantiomeric purity with R configuration as determined by comparing its optical rotation with the literature value[19,20] (Scheme 1).

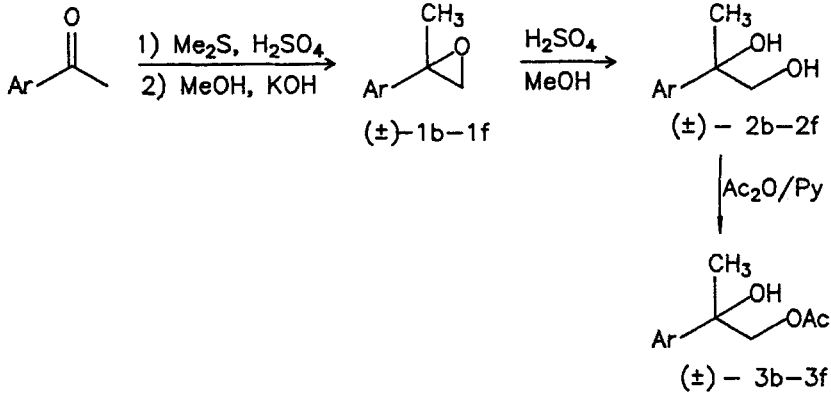
Encouraged by this result we have prepared a representative class of 2-aryl-2-methyloxiranes according to a recent literature procedure [21] (Scheme 2). These epoxides were subjected to hydrolysis by microsomal epoxide hydrolase to produce the resulting 1,2-diols in 13-34% enantiomeric purities (Table 1 and Scheme 1). We observed that there is no hydrolysis of the oxiranes in the absence of microsomal epoxide hydrolase. The stereoselectivity factor (E) values for these enzymatic reactions range from 1.4 to 2.4 as calculated using Sih equation[22] (Table 1).

The enantiomeric purities of these diols were determined by HPLC analysis (chiral column, chiralcel OD) of their corresponding monoacetates (Scheme 1) with reference to the corresponding racemic monoacetates (Scheme 2). The enantiomeric purities of the recovered epoxides from the enzymatic reactions were not determined, because their enantiomeric purities will not be appreciable.



1a: Ar = phenyl; **1b**: Ar = 4-methylphenyl; **1c**: Ar = 4-ethylphenyl; **1d**: Ar = 4-isobutylphenyl; **1e**: Ar = 4-chlorophenyl; **1f**: Ar = 3-bromophenyl

Scheme 1



Scheme 2

Table 1. Enantioselective hydrolysis of racemic epoxides (1a-1f) using microsomal epoxide hydrolase from pig liver.[a]

Substrate	Time	Conversion	(-)-diols (2a-2f)			Conf.	E[g]
	h	ratio[b]	Yield[c]	ee%	optical rotation, $[\alpha]_D^{20}$		
1a	3	37:63	84	28[d]	-2.5 (c5.25, Et_2O)	R	2.0
1b	2	36.64	80	22[e]	-2.6 (c1.90, $CHCl_3$)	R[f]	1.7
1c	3	34:66	83	20[e]	-3.5 (c2.01, $CHCl_3$)	R[f]	1.6
1d	4	30:70	77	27[e]	-4.5 (c1.55, $CHCl_3$)	R[f]	1.9
1e	2	31:69	88	13[e]	-2.0 (c4.55, $CHCl_3$)	R[f]	1.3
1f	3.5	35:65	87	34[e]	-4.2 (c1.85, $CHCl_3$)	R[f]	2.4

a) All reactions were carried out in 2 mM scale with 5 mL of microsomal solution. b) Determined by HPLC analysis. c) Yields of pure, isolated products after column purification and are based on conversion ratios. d) Based on $[\alpha]_D^{22}$ +8.99 (c5.8, ether), 100% ee (ref. 19) e) By HPLC analysis of the corresponding monoacetate using chiral column Chiralcel OD (Diacel, Japan). f) Tentatively assigned on the analogy with enzymatic hydrolysis of **2a**. g) Calculated according to Sih equation $E = \ln [1-c\ (1+ee_p)] / \ln [1-c\ (1-ee_p)]$ (ref. 22).

Though the optical purities of these 1,2-diols are not high, our study demonstrates the applicability of microsomal epoxide hydrolase for the synthesis of optically active 1,2-diols containing a tertiary hydroxyl stereogenic centre.

Experimental Section:

^{1}H and ^{13}C NMR spectra were recorded on Brucker-200 (200 MHz) spectrometer in chloroform-d solution with TMS as internal standard. IR spectra were recorded on Perkin Elmer 1310 or Jasco 5300 FT spectrometers. Elemental analyses were performed on a Perkin Elmer 240C-CHN analyzer. HPLC analysis was performed on Shimadzu LC-10AD equipped with SPD-10A detector. Enantiomeric purities were determined using chiralcel OD (Diacel, Japan) column. Column chromatography was performed on Acme's silica gel (100-200 mesh). Optical rotations were measured on Autopol II automatic polarimeter.

The racemic epoxide **1a** was prepared by treatment of α-methylstyrene with NBS and NaOH according to known procedure.[23] The racemic epoxides **1b-1f** were prepared from the corresponding aryl methyl ketones following the literature procedure.[21] The racemic diols were prepared by the acid (H_2SO_4) catalyzed opening of the racemic epoxides.[24] Monoacetates of the racemic and optically active diols were prepared by treatment with

acetic anhydride in presence of pyridine. Racemic diols and monoacetates have identical IR, ^{1}H and ^{13}C NMR spectral data to that of corresponding optically active molecules. (IPA = isopropyl alcohol).

Racemic epoxides (1a-1f):

α-Methylstyrene oxide (1a): Yield 85%, B.P. 78°C / 7mm, ^{1}H NMR : δ 1.71 (s, 3H), 2.80 (d, 1H, J=5.4 Hz), 2.96 (d, 1H, J=5.4 Hz), 7.34 (m, 5H). ^{13}C NMR : δ 21.82, 56.70, 56.92, 125.32, 127.43, 128.31, 141.26.

2-Methyl-2-(4-methylphenyl)oxirane (1b): Yield 65%, B.P. 83-85°C / 6 mm, ^{1}H NMR : δ 1.75 (s, 3H), 2.38 (s, 3H), 2.82 (d, 1H, J = 5.4 Hz), 2.98 (d, 1H, J = 5.4 Hz), 7.18 (d, 2H, J = 7.4 Hz), 7.30 (d, 2H, J = 7.4 Hz). ^{13}C NMR : δ 21.09, 21.93, 56.71, 57.04, 125.30, 129.06, 137.15, 138.27.

2-Methyl-2-(4-ethylphenyl)oxirane (1c): Yield 49%, B.P. 87°C / 5 mm, ^{1}H NMR : δ 1.26 (t, 3H, J = 6Hz), 1.76 (s, 3H), 2.68 (q, 2H, J = 6Hz), 2.84 (d, 1H, J = 5.5 Hz), 2.98 (d, 1H, J = 5.5 Hz), 7.19 (d, 2H, J = 7 Hz), 7.31 (d, 2H, J = 7 Hz). ^{13}C NMR : δ 15.50, 21.84, 28.47, 56.60, 56.91, 125.32, 127.79, 138.48, 143.46.

2-Methyl-2-(4-isobutylphenyl)oxirane (1d): Yield 67%, B.P. 95-97°C / 6 mm, ^{1}H NMR : δ 0.95 (2 doublets, 6H, J = 6.4 Hz), 1.74 (s, 3H), 1.93 (m, 1H), 2.52 (d, 2H, J = 6 Hz), 2.83 (d, 1H, J = 5 Hz), 2.97 (d, 1H, J = 5 Hz), 7.16 (d, 2H, J = 7 Hz), 7.31 (d, 2H, J = 7 Hz).

^{13}C NMR : δ 21.87, 22.41, 30.23, 45.12, 56.62, 57.01, 125.15, 129.10, 138.52, 140.94.

2-Methyl-2-(4-chlorophenyl)oxirane (1e): Yield 51%, B.P. 83-84°C / 4 mm, (lit[25] B.P. 106-108°C / 11 mm), ^{1}H NMR : δ 1.72 (s, 3H), 2.76 (d, 1H, J = 5.5 Hz), 2.98 (d, 1H, J = 5.5 Hz), 7.31 (m, 4H). ^{13}C NMR : δ 21.64, 56.25, 56.88, 126.78, 128.47, 133.33, 139.88.

2-Methyl-2-(3-bromophenyl)oxirane (1f): Yield 65%, B.P. 95-97°C / 5 mm, ^{1}H NMR : δ 1.72 (s, 3H), 2.76 (d, 1H, J = 5.5 Hz), 2.94 (d, 1H, J = 5.5 Hz), 7.15-7.52 (m, 4H). ^{13}C NMR : δ 21.58, 56.18, 56.91, 122.60, 124.05, 128.52, 129.93, 130.57, 143.71.

Preparation of pig liver microsomes:

Pig liver (100g) was homogenized in 0.32M sucrose solution. The homogenate was centrifuged at 5000 rpm to remove debris and unbroken cells. The supernatant was then centrifuged at 10,000 X g for 15 min and the resultant supernatant was further centrifuged at 1,00,000 X g for 1 hour. The microsomal pellet thus, obtained, was resuspended in the sucrose solution to a final concentration of 9 mg/mL and stored at-20°C.

Microsomal epoxide hydrolase catalyzed hydrolysis of racemic 1a-1f:

General procedure: To a solution of racemic epoxide (2mM) in 4 mL of ethanol and 0.1M pH 7.4 phosphate buffer (20 mL), microsmal solution (5 mL) was added and

the contents were stirred at room temperature. Hydrolysis was monitored by HPLC. After appropriate hydrolysis (Table 1) the reaction mixture was filtered and the filtrate was extracted with ethyl acetate to afford a mixture of optically active diol and epoxide, which were separated by column chromatography on silica gel (hexane:ethyl acetate / 80:20).

The enzymatic hydrolysis results are summarized in Table 1. IR, ^{1}H and ^{13}C NMR spectral data, optical rotations, methods of ee determination are given below.

(R)-(-)-2-Phenyl-1,2-propanediol (2a):

Obtained by the enzymatic hydrolysis of the racemic epoxide **1a**. Yield 84%, $[\alpha]_D^{20}$ -2.5 (c5.25, ether), 28% ee {lit[19] $[\alpha]_D^{22}$ +8.99 (c5.8, ether) 100% ee, conf.S}. IR (neat): 3385 cm^{-1}. ^{1}H NMR : δ 1.50 (s, 3H), 2.28 (br, 1H, D_2O washable), 2.86 (br, 1H, D_2O washable), 3.62 (d, 1H, J = 10Hz), 3.78 (d, 1H, J = 10 Hz), 7.24-7.42 (m, 5H). ^{13}C NMR: δ 25.80, 70.61, 74.81, 125.05, 126.90, 128.16, 145.08.

(R)-(-)-2-(4-Methylphenyl)-1,2-propanediol (2b):

Obtained by the enzymatic hydrolysis of the racemic epoxide **1b**. Yield 80%, $[\alpha]_D^{20}$ -2.6 (c1.9, $CHCl_3$), 22% ee. IR (neat): 3360 cm^{-1}. ^{1}H NMR : δ 1.52 (s, 3H), 2.35 (s, 3H), 2.12-2.45 (br, 2H, D_2O washable), 3.55 (d, 1H, J = 11 Hz), 3.76 (d, 1H, J = 11 Hz), 7.15 (d, 2H, J = 7 Hz), 7.31 (d, 2H, J = 7 Hz). ^{13}C NMR: δ 20.94, 26.03, 71.11, 74.78, 125.04, 129.11, 136.81, 142.09. Anal.

Calcd for $C_{10}H_{14}O_2$: C, 72.25; H, 8.48. Found: C, 72.42; H, 8.50.

1-Acetoxy-2-(4-methylphenyl)-2-propanol (**3b**): IR (neat): 3385, 1720 cm^{-1}. ^1H NMR: δ 1.55 (s, 3H), 2.06 (s, 3H), 2.35 (s, 3H), 2.45 (br, 1H, D_2O washable), 4.14 and 4.25 (AB q, 2H, J = 11 Hz), 7.12 (d, 2H, J = 8 Hz), 7.28 (d, 2H, J = 8 Hz). ^{13}C NMR: δ 20.99, 21.15, 26.75, 72.01, 73.66, 125.14, 129.21, 137.14, 141.64, 171.27. HPLC analysis (solvent system, hexane: IPA / 98:2) shows 22% optical purity.

(R)-(-)-2-(4-Ethylphenyl)-1,2-propanediol (2c):

Obtained by the enzymatic hydrolysis of the racemic epoxide **1c**. Yield 83%, $[\alpha]_D^{20}$ -3.5 (c2.01, $CHCl_3$), 20% ee. M.P. 61-63°C, IR (KBr): 3360 cm^{-1}. ^1H NMR : δ 1.24 (t, 3H, J = 7.6 Hz), 1.52 (s, 3H), 2.57-2.72 (m, 4H, 2H D_2O washable), 3.60 (d, 1H, J = 11 Hz), 3.78 (d, 1H, J = 11Hz), 7.19 (d, 2H, J = 8.2 Hz), 7.37 (d, 2H, J = 8.2 Hz). ^{13}C NMR: δ 15.38, 25.96, 28.32, 71.04, 74.72, 125.04, 127.83, 142.22, 143.07. Anal. Calcd for $C_{11}H_{16}O_2$: C, 73.30; H 8.94. Found: C, 73.42; H, 8.96.

1-Acetoxy-2-(4-ethylphenyl)-2-propanol (**3c**): IR (neat): 3470, 1740 cm^{-1}. ^1H NMR: δ 1.26 (t, 3H, J = 6Hz), 1.54 (s, 3H), 2.05 (s, 3H), 2.40 (br, 1H, D_2O washable), 2.68 (q, 2H, J = 6Hz), 4.09 and 4.12 (AB q, 2H, J = 11 Hz), 7.24 (d, 2H, J = 7 Hz), 7.32 (d, 2H, J = 7 Hz). ^{13}C NMR: δ 15.41, 20.79, 26.52, 28.39, 71.84, 73.45, 125.02, 127.79, 141.69, 143.28, 171.06. HPLC analysis

(solvent system, Hexane : IPA / 98 : 2) shows 20% enantiomeric purity.

(R)-(-)-2-(4-Isobutylphenyl)-1,2-propanediol (2d):

Obtained by the enzymatic hydrolysis of the racemic epoxide **1d**. Yield 77%, $[\alpha]_D^{20}$ -4.5 (c1.55, $CHCl_3$), 27% ee. M.P. 82-84°C, IR (KBr) : 3350 cm^{-1}. ^1H NMR : δ 0.92 (d, 6H, J = 6.5 Hz), 1.54 (s, 3H), 1.62-2.02 (m, 3H, 2H D_2O washable), 2.47 (d, 2H, J = 7.2 Hz), 3.62 (d, 1H, J = 10 Hz), 3.74 (d, 1H, J = 10 Hz), 7.17 (d, 2H, J = 7 Hz), 7.35 (d, 2H, J = 7 Hz). ^{13}C NMR: δ 22.38 26.01, 30.14, 44.98, 71.18, 74.74, 124.83, 129.17, 140.64, 142.22. Anal. Calcd for $C_{13}H_{20}O_2$: C, 74.96; H, 9.67. Found: C, 74.85; H, 9.66.

1-Acetoxy-2-(4-isobutylphenyl)-2-propanol (**3d**): IR (neat): 3476, 1743 cm^{-1}. ^1H NMR: δ 0.89 (d, 6H, J = 6.5Hz), 1.56 (s, 3H), 1.64 (br, 1H, D_2O washable), 1.82 (m, 1H), 2.05 (s, 3H), 2.47 (d, 2H, J = 7.1 Hz), 4.18 and 4.32 (AB q, 2H, J = 11.3 Hz), 7.13 (d, 2H, J = 8.2 Hz), 7.36 (d, 2H, J = 8.2 Hz). ^{13}C NMR: δ 20.84, 22.38, 26.46, 30.18, 45.00, 71.88, 73.51, 124.79, 129.06, 140.80, 141.60, 171.12. HPLC analysis (solvent system, Hexane : IPA / 98 : 2) shows 27% enantiomeric purity.

(R)-(-)-2-(4-Chlorophenyl)-1,2-propanediol (2e):

Obtained by the enzymatic hydrolysis of the racemic epoxide **1e**. Yield 88%, $[\alpha]_D^{20}$ -2.0 (c4.55, $CHCl_3$), 13% ee. IR (neat): 3383 cm^{-1}. ^1H NMR : δ 1.32 (s, 3H), 3.10 (br, 2H, D_2O washable), 3.46 and 3.59 (AB q, 2H, J

= 10 Hz), 7.15 (m, 4H). ^{13}C NMR: δ 26.01, 70.79, 74.63, 126.72, 128.50, 133.08, 143.71. Anal. Calcd for $C_9H_{11}ClO_2$: C, 57.91; H, 5.94. Found: C, 57.89; H, 5.96.

1-Acetoxy-2-(4-chlorophenyl)-2-propanol (**3e**): IR (neat): 3466, 1728 cm^{-1}. ^1H NMR: δ 1.54 (s, 3H), 2.05 (s, 3H), 2.52 (br, 1H, D_2O washable), 4.20 and 4.32 (ABq, 2H, J = 10 Hz), 7.38 (m, 4H). ^{13}C NMR: δ 20.81, 26.71, 71.66, 73.49, 126.66, 128.53, 133.14, 142.95, 171.07. HPLC analysis (solvent system, Hexane : IPA / 95 : 5) shows 13% enantiomeric purity.

(R)-(-)-2-(3-Bromophenyl)-1,2-propanediol (2f):

Obtained by the enzymatic hydrolysis of the racemic epoxide **1f**. Yield 87%, $[\alpha]_D^{20}$ -4.2 (c1.85, $CHCl_3$), 34% ee. M.P. 78-79°C, IR (KBr): 3350 cm^{-1}. ^1H NMR : δ 1.51 (s, 3H), 2.14 (br, 2H, D_2O washable), 3.62 and 3.78 (AB q, 2H, J = 11 Hz), 7.15-7.72 (m, 4H). ^{13}C NMR: δ 26.03, 70.81, 74.57, 122.75, 123.79, 128.54, 129.97, 130.26, 147.56. Anal. Calcd for $C_9H_{11}BrO_2$: C, 46.77; H, 4.79. Found: C, 46.65; H, 4.78.

1-Acetoxy-2-(3-bromophenyl)-2-propanol (**3f**): IR (neat): 3468, 1740 cm^{-1}. ^1H NMR: δ 1.54 (s, 3H), 2.06 (s, 3H), 2.59 (br, 1H, D_2O washable), 4.18 and 4.29 (AB q, 2H, J = 11 Hz), 7.23-7.65 (m, 4H). ^{13}C NMR: δ 20.80, 26.65, 71.59, 73.42, 122.70, 123.76, 128.54, 129.95, 130.49, 146.81, 171.07. HPLC analysis (solvent system, Hexane : IPA / 95 : 5) shows 34% enantiomeric purity.

Acknowledgement: We thank DST (New Delhi) for funding this project. SBR thanks (UGC, New Delhi) for financial assistance. We thank UGC (New Delhi) for COSIST and Special Assistance Programs in School of Chemistry, University of Hyderabad.

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(Received in the UK 13 March 1995)