Pergamon

*Tetrahedron: Asymmetry* Vol. 6, No. 2, pp. 537-542, 1995

0957-4166/95 $9.50+0.00

0957-4166(95)00037-2

# New Chiral Synthon from Bromoethylbenzene: Absolute Stereochemistry of A Biooxidation Metabolite

**Michele R. Stabile, Tomas Hudlicky,*‡ and Meredith L. Meisels§**

*Department of Chemistry, Virginia Polytechnic Institute and State University*
*Blacksburg, VA 24061-0212, U. S. A.*

**Abstract:** (2-Bromoethyl)benzene was subjected to biooxidation with whole cells of *Pseudomonas putida* 39/D to yield (1*S*, 2*R*) 3-(2-bromoethyl)-cyclohexa-3,5-diene-1,2-diol **1** with an optical purity of 96%.

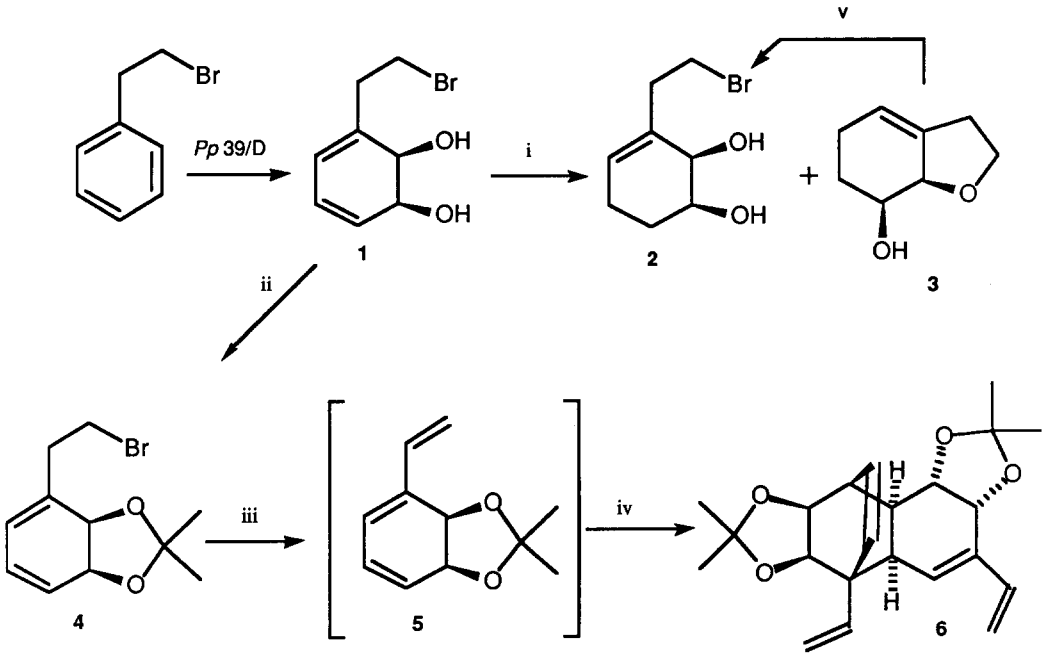
## INTRODUCTION

The importance of diol metabolites derived from aromatic compounds has increased considerably in recent years as evidenced by the growing number of papers and reviews in this area.[1] Since their initial discovery by Gibson,[2] these compounds have been widely used in asymmetric synthesis, and over 150 diverse cyclohexadiene *cis*-diols are known.[3] Surprisingly, only a few biooxidation products have been derived from aromatic compounds containing aliphatic side chains.[4] Only the metabolites of the following alkyl, alkenyl, or alkynyl arenes are known: toluene,[4a-g] ethylbenzene,[4h] styrene,[4b,i] 2-propenylbenzene,[4j,k] 2-butylbenzene,[4m] n-propylbenzene,[4n] n-butylbenzene,[4n] phenyl-acetylene,[4o] isopropylbenzene,[4p] and isobutylbenzene.[4p] While the diols containing aliphatic chains do not offer enough functionality to be useful in synthesis, those derived from styrene and acetylene have been used.[4o,5] We were interested in the metabolite derived from (2-bromoethyl)benzene, which would allow, through nucleophilic substitution, the preparation of functionalized derivatives that may not be available from the corresponding arenes if such were not readily selected as substrates by the microbial dioxygenase.

## RESULTS AND DISCUSSION

Toluene-dioxygenase-mediated dihydroxylation of (2-bromoethyl)benzene, accomplished with *Pseudomonas putida* 39/D according to a previously established protocol,[6a,b] afforded the bromoethyl benzene *cis*-dihydrodiol **1** as the sole product in a yield of 200 mg/L of culture. The diene diol was recrystallized from methylene chloride/hexanes to yield a white solid, which had fleeting stability at ambient temperature. A half-life study of the diene diol in deuterated chloroform at room temperature revealed the $t_{1/2}$ as 5 days. To ensure its stability, the diol in pure form can be stored for long periods of time at –78 °C

The diene diol was reduced with diimide, which afforded in a 10:1 ratio the desired cyclohexenediol **2** and the benzofuran derivative **3**, resulting from intramolecular $S_N2$ alkylation during the diimide reduction. The bromide was regenerated from **3** by reaction with $BF_3{\cdot}Et_2O$ and tetraethylammonium bromide.[7] Protection of **1** as an acetonide gave **4**, which was dehydrohalogenated to afford the known styrene diol derivative **5**. Although **5** could be isolated, correlation of absolute stereochemistry and establishment of optical purity proved difficult because of the commencement of dimerization and contamination of the sample with the known dimer **6**. As the absolute stereochemical configuration of **6** is known,[8] we decided to fully convert **5** (prepared from **1**) to **6** for evaluation of optical purity as well as correlation of absolute stereochemistry.




i. Potassium azodicarboxylate, AcOH, MeOH ii. 2,2-dimethoxypropane, acetone, pTsOH
iii. DBU, benzene, reflux 5 hr. iv. neat, RT, 2-3 weeks v. $BF_3{\cdot}Et_2O$, $NEt_4Br$

Transformation of **1** into the styrene dimer **6** began with protection of the diol as the acetonide followed by elimination of the bromide moiety with DBU in benzene at reflux. Upon isolation, the crude **5** was allowed to stand neat at room temperature for 5 days to fully dimerize. Although traces of the dimer were present as soon as **5** was isolated, additional reaction time was necessary to afford a 40% conversion of the starting material. The dimer **6**, isolated according to the published method, had an $[\alpha]_D^{25}$ of +71.0 (c = 0.41, $CHCl_3$), (Lit. +73.8 (c = 0.81, $CHCl_3$)),[8] which corresponds to an optical purity of 96% for diol **1**.

## CONCLUSION

Metabolite **1** will be exploited in further synthetic endeavors. We are in the process of studying nucleophilic additions to **1** and **4** to produce compounds otherwise unattainable by the microbial oxidation of corresponding arenes. The results will be reported in due course.

## EXPERIMENTAL SECTION

*General*

NMR spectra were determined in $CDCl_3$ on a Bruker WP-270 or a Varian Unity 400, $^1H$ at 270 MHz or 400 MHz. Coupling constants are given in Hertz. $^{13}C$ multiplicities were determined by APT experiments. IR spectra were obtained on a Perkin Elmer 283B instrument. All solvents used in reactions were dried according to standard procedure. Flash column chromatography was performed on Merck silica gel (grade 60, 230-400 mesh). Melting points were observed on a Thomas Hoover Uni-melt apparatus and are uncorrected. Optical rotations were measured on a Perkin Elmer model 241 polarimeter. Mass spectra were measured on a VG 7070 E-HF instrument. Elemental analyses were performed by Atlantic Microlabs, Norcross, GA.

**Microbial oxidation of (2-bromoethyl)benzene by *Pseudomonas putida* 39/D.** Fermentations were carried out as described earlier[6a,b] in a 2L fermentor without toluene induction since (2-bromoethy)benzene induces the production of toluene dioxygenase. From 5 g of (2-bromoethyl)benzene delivered into the fermentation broth, 500 mg of crude extract was isolated after centrifugation of the fermentor contents (6000 rpm, 15 min.), extraction of the 1.5 L of supernatant (4 x 200 ml ethyl acetate) and evaporation of the combined organics. The extract was purified on deactivated silica (1:1 hexane/ethyl acetate) to afford 300 mg of pure **1**.

**(5*S*,6*R*)-1-(2-bromoethyl)cyclohexa-1,3-diene-5,6-diol (1).** $R_f$ = 0.33 (1:1 hexane/ethyl acetate) mp = 49-50 °C (recryst. hexane/methylene chloride) $[\alpha]_D^{25}$ = +89.8 (c = 1.1, $CHCl_3$) $^1H$ NMR (270 MHz) δ 5.94 (1H, m), 5.84 (1H, d, *J* = 5), 5.79 (1H, d, *J* = 3), 4.27 (1H, br s), 4.06 (1H, d, *J* = 6), 3.53 (2H, t, *J* = 6.5), 3.15 (2H, br s), 2.77 (2H, m). $^{13}C$ NMR (400 MHz) δ 137.7 (C), 128.1 (CH), 124.3 (CH), 121.9 (CH), 69.3 (CH), 68.6 (CH), 37.1 ($CH_2$), 31.3 ($CH_2$) MS *m/z* (rel. int.) (EI+) 219 (M, 2), 202 (12), 107 (60), 79 (100).

**(1*S*,2*R*)-3-(2-bromoethyl)cyclohex-5-ene-1,2-diol (2).** To an ice cooled solution of diol **1** (440 mg, 2.01 mmol), in 2 mL methanol was added potassium azodicarboxylate (975 mg, 5.02 mmol). A solution of acetic acid (0.69 mL, 12.06 mmol) in 2 mL methanol was added dropwise over 1 hour. The solution was gradually warmed to room temperature and stirred overnight. After slow addition of saturated aqueous $NaHCO_3$ (3 ml), the solvent was concentrated under reduced pressure. The remaining residue was dissolved in water (3 mL) and brine (8 mL) and extracted with ethyl acetate (3 x 10 mL). The organic layers were combined, dried over $Na_2SO_4$, and evaporated to yield 186 mg of **2** (42%) and 18 mg of **3** (6%) after purification by flash column chromatography (1:1 hexane/ethyl acetate) $R_f$ = 0.35 (1:1 hexane/ethyl acetate) mp = 95-96 °C (from hexane/$CH_2Cl_2$). $[\alpha]_D^{25}$ = -63.6 (c = 1.02, $CHCl_3$) IR (KBr) 3260, 2810, 980 $cm^{-1}$. $^1H$ NMR (400 MHz) δ 5.66 (1H, s), 4.01 (1H, d, *J* = 4), 3.77 (1H, ddd, *J* = 14, 6.8, 4), 3.52 (2H, t, *J* = 7.6), 2.94 (2H, brs), 2.74 (1H, m), 2.62 (1H, M), 2.20 (1H, m), 2.09 (1H, m), 1.71 (2H, m) $^{13}C$ NMR (400 MHz) δ 134.5 (C), 128.4 (CH), 69.5 (CH), 68.5 (CH), 37.9 ($CH_2$), 32.0 ($CH_2$), 25.2 ($CH_2$), 23.8 ($CH_2$). MS *m/z* (rel. int.) (EI+) 220 (M, 2), 176 (100), 97 (80), 83 (95) Anal. Calcd. for $C_8H_{13}O_2Br$: C, 43.46; H, 5.89. Found: C, 43.23; H, 5.89.

**(7*S*,7a*R*)-2,3,5,6,7,7a-Hexahydrobenzofuran-7-ol (3).** $R_f$ = 0.32 (1:1 hexane/ethyl acetate) $[\alpha]_D^{25}$ = + 46.4 (c=0.45 1 ml $CHCl_3$) IR (neat) 3490 (br), 2920 $^1H$ NMR (400 MHz) δ 5.61 (1H, s), 4.22 (1H, s), 4.02 (1H, s), 3.92 (2H, m), 2.55 (2H, m), 2.21 (2H, m), 2.02 (2H, m), 1.61 (2H, m) $^{13}C$ NMR (400 MHz) δ 133.6 (C), 119.4 (CH), 76.9 (CH), 66.9 ($CH_2$), 64.4 (CH), 30.7 ($CH_2$), 25.1 ($CH_2$), 20.7 ($CH_2$). MS *m/z* (rel. int.) (EI+) 140 (M, 15), 96 (98), 83 (70), 76 (100) HRMS calcd for $C_8H_{12}O_2Br$ 140.0837 found 140.0835 error -1.3 ppm.

**(1*S*,2*R*) -1,2-isopropylidene-dioxy-3-(2-bromoethyl)-cyclohexa-3,5-diene (4).** The diol **1** (1.0 g, 4.5 mmol) was dissolved in acetone (30 mL) with stirring. 2,2-Dimethoxypropane (1 mL, 5.5 mmol) was added followed by a catalytic amount of p-toluenesulfonic acid. Stirring continued at room temperature until no starting material was observed by TLC monitoring (approximately 15 min.). The reaction was quenched with 10% aqueous NaOH solution (8 ml) and extracted with ether (3 x 10 mL). The organic layers were combined, washed with brine, dried over $Na_2SO_4$, and evaporated to yield 0.92 g of a colorless oil, 78% yield. $R_f$ = 0.46 (7:3 hexane/ethyl acetate) $[\alpha]_D^{25}$ = +101.5 (c = 1.2, $CHCl_3$) IR (neat) 2980, 2880, 1370, 1040 $^1H$ NMR (400 MHz) δ 5.97 (1H, dd, *J* = 9.5, 5.5), 5.83 (1H, dd, *J* = 9.5, 4), 5.77 (1H, d, *J* = 5.5), 4.63 (1H, dd, *J* = 8.5, 4), 4.54 (1H, d, *J* = 8.5), 3.53 (2H, m), 2.82 (1H, m), 2.69 (1H, m), 1.37 (3H, s), 1.35 (3H, s) $^{13}C$ NMR (400 MHz) δ 134.9 (C), 124.4 (CH), 123.5 (CH), 120.8 (CH), 105.4 (C), 72.8 (CH), 70.9 (CH), 37.2 ($CH_2$), 30.6 ($CH_2$), 26.8 ($CH_3$), 25.0 ($CH_3$) MS *m/z* (rel. int.) (CI+) 259 (M, 3), 201 (50), 121 (100) HRMS cacld for $C_{11}H_{15}O_2Br$ 259.0334 found 259.0346 error 4.8 ppm.

**(5*S*,6*R*)-5,6-[(Isopropylidene)dioxy]-1-ethenylcyclohexa-1,3-diene (5)** The bromoethylacetonide **4** (57 mg, 0.22 mmol) was dissolved in benzene (10 mL). DBU (0.05 mL, 0.33 mmol) was added and the mixture was brought to reflux for 6 hours. After cooling the solution to room temperature, it was poured into 8 mL of water. The aqueous layer was separated and extracted with $CH_2Cl_2$ (2 x 5 mL). Combination of the organic fractions followed by drying over $Na_2SO_4$ and evaporation yielded 62 mg of a crude oil. Flash column chromatography of the residue (7:3 hexane/ethyl acetate) gave the styrene acetonide **6** (21 mg, 53%) as a colorless oil.

**(1*S*,2*S*,5*S*,6*S*,7*R*,8*S*,9*S*,10*S*)-1,4-Diethenyl-5,6,9,10-bis([isopropylidene)dioxy]tricyclo[6.2.2.0]dodeca-3,11-diene.(6)** The acetonide **5** was left at room temperature for 2 weeks. Purification by flash column chromatography, 8:2 hexane/ethyl acetate yielded 8 mg of the major dimer whose $^1H$ NMR spectrum and $\alpha_D$ corresponded to the literature values.[8]

## ACKNOWLEDGMENTS

The authors are grateful to Genencor International, Inc., TDC Research, Inc. and NSF CHE-9315684 for financial support of this work. We also thank Dr. Gregg Whited and Dr. Kurt Königsberger for helpful discussions regarding the procedures for microbial oxidations.

## REFERENCES

*‡Address correspondence to this author at:
Chemistry Department, University of Florida, Gainesville, FL 32611.
§ Undergraduate research participant 1993-1994

1. (a) Hudlicky, T.; Reed, J. W. In *Advances in Asymmetric Synthesis*; Hassner, A., Ed.; JAI Press: Greenwich, CT, **1994**, Vol. 2, p. 271. (b) Brown, S. M.; Hudlicky, T. In *Organic Synthesis: Theory and Practice*; Hudlicky, T., Ed.; JAI Press: Greenwich, CT, **1993**; Vol. 2, p. 113. (c) Carless, H. A. H. *Tetrahedron: Asymm.* **1992**, *3*, 795. (d) Widdowson, D. A.; Ribbons, D. W.; Thomas, S.D. *Janssen Chimica Acta* **1990**, *8* (3), 3.
2. Gibson, D. T.; Koch, J. R.; Schuld, C. L.; Kallio, R. E. *Biochemistry* **1968**, *7*, 3795.
3. (a) McMordie, R. A. S. Ph.D Thesis, School of Chemistry, The Queen's University of Belfast, Belfast BT9 5AG, U. K., 1991. (b) Stabile, M. M.S. Thesis, Virginia Polytechnic Institute and State University, Blacksburg, VA, 1993.

4. (a) Boyd, D.; Dorrity, M.; Hand, M.; Malone, J.; Sharma, N.; Dalton, H.; Gray, D.; Sheldrake, G. *J. Am. Chem. Soc.* **1991**, *113*, 666. (b) Hudlicky, T.; Luna, H.; Barbieri, G.; Kwart, L. D. *J. Am. Chem. Soc.*, **1988**, *110*, 4735. (c) Jenkins, R. O.; Stephens, G.M.; Dalton, H. *Biotechnol. and Bioeng.*, **1987**, XXIX, 873. (d) Ziffer, H.; Kabuto, K.; Gibson, D. T.; Kobal, V. M.; Jerina, D. M. *Tetrahedron*, **1977**, *33*, 2491. (e) Kobal, V. M.; Gibson, D. T.; Davis, R. E.; Garza, A. *J. Am. Chem. Soc.*, **1973**, *95*, 4420. (f) Ziffer, H.; Jerina, D. M.; Gibson, D. T.; Kobal, V. M. *J. Am. Chem. Soc.*, **1973**, *95*, 4048. (g) Gibson, D. T.; Hensley, M.; Yoshioka, H.; Mabry, T. J. *Biochemistry*, **1970**, *9*, 1626.. (h) Gibson, D. T.; Gschwendt, B.; Yeh, W. K; Kobal, V. M. *Biochemistry*, **1973**, *12*, 1520. (i) Hudlicky, T.; Seoane, G.; Pettus, T. *J. Org. Chem.*, **1989**, *54*, 4239. (j) Bestetti, G., Balli, E.; Benigni, C.; Orsini, F.; Pelizzoni, F. *Appl. Microbiol. Biotechnol.*, **1989**, *30*, 252. (k) Omori, T.; Jigami, Y.; Minoda, Y. *Agr. Biol. Chem.* **1974**, 3S, 409. (m) Baggi, G.; Catelani, D.; Galli, E.; Treccani, V. *Biochem. J.* **1972**, *126*, 1091. (n) Genencor International Inc., private communication. (o) Williams, M. G.; Olson, P. G. Tautvydas, K. J.; Bitner, R. M.; Mader, R. A.; Wackett, L. P. *Appl. Microbiol. Biotechnol.* **1990**, *34* , 316. (p) Jigami, Y.; Omori, T.; Minoda, Y. *Agr. Biol. Chem.* **1975**, *39*, 1781.

5. (a) Hudlicky, T.; Seoane, G.; Pettus, T. *J. Org. Chem.* **1989**, *54*, 4239.

6. (a) Hudlicky, T.; Boros, E. E.; Boros, C. E. *Tetrahedron: Asymm.* **1993**, *4*, 1365. (b) Hudlicky, T.; Boros, C. E.; Boros, E. E. *Synthesis* **1992**, 174.

7. Hudlicky, T. and Gum, A. unpublished results.

8. Hudlicky, T.; Boros, C. H. *Tetrahedron Lett.* **1993**, *34*, 2557.

(*Received in USA* 28 *November* 1994)