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ARTICLE *in* CHIRALITY · JANUARY 1995

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Oxidation of 2-(2-Bromoethyl)bromobenzene With Toluene Dioxygenase: Isolation and Identification of New Chiral Synthons

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ABSTRACT 2(2-Bromoethyl)bromobenzene was subjected to microbial oxidation by the whole cells of *Pseudomonas putida* 39/D and JM109(pDTG601) yielding (3R,4S)-2-(2-bromoethyl)-bromocyclohexa-1,5-diene-3,4-diol.

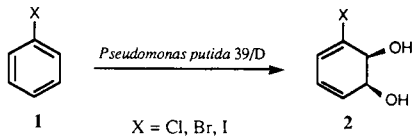
KEY WORDS: biooxidation, *Pseudomonas putida* 39/D, absolute stereochemistry

INTRODUCTION

Cyclohexadiene *cis*-diols derived from aromatic compounds through biooxidation have become popular in enantioselective synthesis in recent years as evidenced by recent reviews.[1] Several diols, for instance those derived from styrene, chlorobenzene, and bromobenzene, have been widely used in the synthesis of oxygenated natural products.[1] Few of such metabolites contain multiple or well-differentiated functionalities. As part of an on-going program aimed at widening the pool of chiral diols, we examined the biooxidation of 2-(2-bromoethyl) bromobenzene.[2] The new metabolite obtained from the title compound contains two electronically differentiated bromine atoms as well as the diene and the diol functionalities and presents a wide array of reactive sites necessary for a synthon attractive for use in asymmetric synthesis. Herein we report the regiochemistry and absolute stereochemistry of the fermentation product and several of its derivatives.

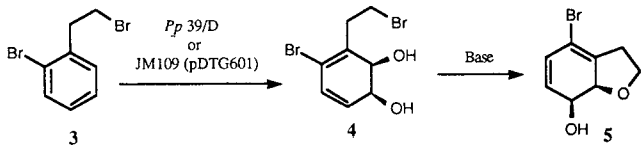
RESULTS AND DISCUSSION

In general, mono-substituted aromatics are oxidized by *Pseudomonas putida (Pp)* 39/D in the 2,3-position relative to the ring substituent with the stereochemistry as depicted in diene diol **2.** While a variety of monosubstituted benzenes are



1 X = Cl, Br, I 2

oxidized in this predictable manner, the regiochemistry of oxidation of disubstituted benzenes is more difficult to forsee. In this study, 2-(2-bromoethyl)bromobenzene **3** was subjected to standard fermentation protocol with *Pp* 39/D.[3] From the extract of the fermentation broth, 2.3 mg/liter of diol **4** and 3.1 mg/liter of tetrahydrofuran **5** were isolated. The typical fermentation procedure for the *Pp* 39/D results in an increase of pH and tetrahydrofuran **5** emerges as a by-product from the base-induced closure of the furan ring and is not a product of the biooxidation.



3 4 5

When the biooxidation was performed using the *E. coli* clone JM109 (pDTG601),[4] the yield of diol **4** was in the range 95–500 mg/liter. Also isolated was 42–250 mg/liter of the tetrahydrofuran **5**. In this case, a higher diol/furan ratio was obtained since the pH of the fermentation broth is typically kept at 7.0 and the product immediately extracted with ice-cold ethyl acetate. Existing as a white crystalline solid, the diene diol had limited stability at room temperature in solution containing a trace amount of acid ($t_{1/2}$ = 7 days in $CDCl_3$) but could be stored at −78°C for an indefinite period of time without decomposition.

The presence of the tetrahydrofuran by-product allowed for facile determination of the regioselectivity in the dihydroxylation reaction. The only metabolite with an orientation to allow for an S_N2 type ring closure was the diene diol resulting from a biooxidation directed by the 2-bromoethyl chain. The metabolite which could have been produced by an oxidation directed by the bromide was not detected in these experiments.

Thus, for proof of the regiochemistry of the diol **4** and the formation of the bromofuranol **5** during work-up, the mixture of **4** and **5** was reduced with diimide to afford compounds **6** and **7,** which were stable indefinitely at room temperature. Exposure of the enediol **6** to Cs_2CO_3 in acetone resulted in a slow cyclization to furan **7** in 99% yield. Conversely, the tetrahydrofuran ring **7** was cleaved with BF_3/NEt_4Br to furnish the bromodiol **6** in 51% yield. Thus, the mixture of compounds could be converted to either diol **6** or hexahydrofuran **7** as desired. Dehydrohalogenation of the bromo

Received for publication April 3, 1995; accepted May 22, 1995.
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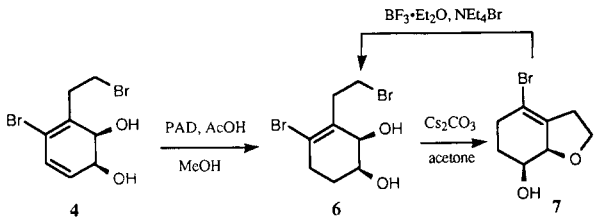
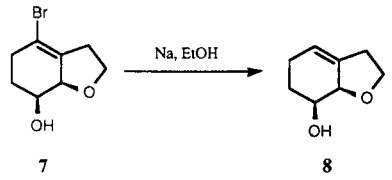
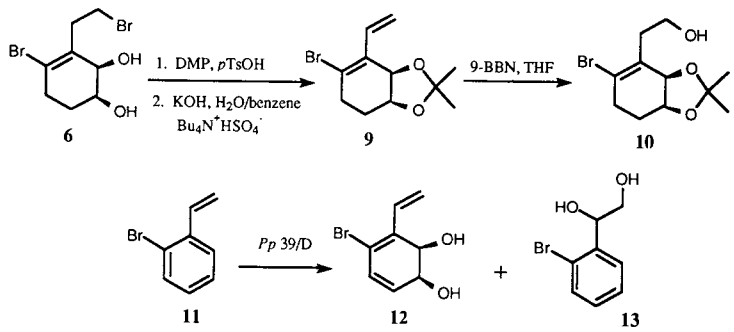
hexahydrofuranol **7** (sodium in refluxing ethanol) afforded known hexahydrofuranol **8**, providing the absolute stereo-



chemical proof for metabolite **4**. ($[\alpha]_D^{26}$ of **8** = +50.6° [c = 0.88 $CHCl_3$); lit[5] = +46.4° (c = 0.45 $CHCl_3$)]



Diene diol **6**, after protection as an acetonide, was dehydrohalogenated to afford the conjugated diene **9**, a compound similar to the minor metabolite **(12)** obtained from the toluene dioxygenase mediated oxidation of *o*-bromostyrene.[6b] The major metabolite of this oxidation was diol **13**, the product of



biooxidation of **11** on the side chain, which proved difficult to separate from the desired ring oxidized product **12**. Thus, isolation of **9** in this study provided a useful complement to the pool of metabolites available from *o*-disubstituted aryl bromides. In addition, the conjugated bromodiene **9** was further functionalized via hydroboration to the alcohol **10** in 56% yield.

CONCLUSION

This study substantiates the finding of earlier work regarding biooxidation of *o*, *m*, and *p*-substituted chlorostyrenes[3] in which the active site region of the ISP_{TOL} enzyme was predicted. The study further demonstrated that the "steric disposition of the substrate is probably the dominant factor determining regiochemistry of the oxidation."[3a] The *ortho* substitution does not affect the enantiomeric purity of isolated diol **4** since the optical purity (>95%) was shown to be comparable to that of the diol from the monosubstituted (2-bromoethyl)benzene.[5] This study also affirmed that the toluene dioxygenase system tolerates a variety of substitution patterns without interfering with the high selectivity of the enzyme catalyst, albeit at the expense of yields in the case of disubstituted substrates. The new metabolite **4** is currently being used as a chiral starting material in the synthesis of isoquinoline-containing natural products. Progress in the use of this and other metabolites will be reported in due course.

EXPERIMENTAL SECTION

General

NMR spectra were determined on a Bruker WP-270 or a Varian Unity 400, at 270 or 400 MHz. Coupling constants are given in Hertz. IR spectra were obtained on a Perkin Elmer 283B instrument. Flash column chromatography was performed on Merck silica gel (Grade 60, 230-400 mesh).

(3R,2S)-1-Bromo-2-(2-bromoethyl)-cyclohexa-1,5-diene-3,4-diol (4)

Fermentations using *Pp* 39/D were carried out as described earlier using toluene as the inducer.[3] From 5 g of 1-bromo-2-(2-bromoethyl) benzene introduced into the 15 liter fermentation, 174 mg of crude extract was obtained following standard work-up. [Centrifugation of the cells at 8000 rpm, extraction of the base-adjusted supernatant with ethyl acetate (4 × 400 ml), drying the combined organic portions over Na_2SO_4 and removal of the solvent in vacuo.] After HPLC purification of the mixture (Microsorb C18 pre column, 70/30 methanol/water, 10 ml/min) the pure compounds eluted as follows: 87 mg of toluene diol as a result of the induction, 25 mg (3.1 mg/liter) of the furan **5** and 18 mg (2.3 mg/liter) of the desired diene diol **4**. When JM109 cells were used, from the same volume of broth (7 liters) a total of 7.7 g of crude diol **4** and furan **5** was isolated. For preparative scale this crude mixture was reduced (see below) to a mixture of diol **6** and tetrahydrofuran **7**, which was purified by column chromatography (9:1 CH_2Cl_2/acetone) to afford 4 g (51%) of **6** and 2 g (38%) of **7**.

R_f: 0.37 (1:1 hex/EtOAc); mp: 59–63°C; $[\alpha]_D^{26}$: +72.1° (c = 0.64, MeOH); ^1H NMR: ($CDCl_3$) (400 MHz) δ 6.08 (1H, dd, J = 9.9, 1.5), 5.86 (1H, dd, J = 9.9, 3.5), 4.37 (1H, m),

4.21 (1H, m), 3.57 (2H, m), 3.05 (1H, m), 2.95 (1H, p), 2.43 (1H, d, J = 8.9), 2.27 (1H, d, J = 8.4).

^{13}C NMR: ($CDCl_3$) (400 MHz) δ 135.7 (C), 130.0 (CH), 129.9 (CH), 119.0 (C), 71.1 (CH), 67.9 (CH), 36.5 (CH_2), 29.9 (CH_2).

MS *m/z* (rel. int.) EI+ 300 (M+2, 1), 298 (M, 2), 280 (28), 199 (37), 185 (65), 91 (100).

(7S,7aR)-4-Bromo-2,3,7,7a-tetrahydrobenzofuran-7-ol (5)

^{1}H NMR: ($CDCl_3$)(400 MHz) δ 6.16 (1H, d, J = 9.61), 5.97 (1H, ddd, J = 1, 5.73, 9.74), 4.30 (2H, m), 4.22 (1H, t, J = 5.70) 4.00 (1H, m), 2.67 (2H, m), 2.19 (1H, s).

^{13}C NMR: ($CDCl_3$) (400 MHz) δ 140.1 (C), 131.9 (CH), 125.4 (CH), 106.6 (C), 81.7 (CH), 68.2 (CH_2), 61.8 (CH), 32.1 (CH_2).

(3R,4S)-1-Bromo-2-(2-bromoethyl)-cyclohexa-1-ene-3,4-diol (6)

Procedure A: To an ice cooled solution of diol **4** (~1.19 g, 3.69 mmol) in methanol (10 ml) was added potassium azodicarboxylate (1.43 g, 7.38 mmol). A solution of acetic acid (4.2 ml, 73.8 mmol) in methanol (10 ml) was added dropwise over 1 hour. The solution was allowed to gradually warm to room temperature and stirred overnight. After slow addition of sat. $NaHCO_3$ solution (10 ml) the solvent was concentrated under reduced pressure. The remaining residue was dissolved in water (10 ml) and brine (15 ml) and extracted with ethyl acetate (3 × 15 ml). The organic layers were combined, dried over Na_2SO_4, filtered, and evaporated. Purification by flash column chromatography yielded 553 mg of a white solid, **6,** 50% and 258 mg, 32%, of a yellow oil, **7.**

Procedure B: Recycling from tetrahydrofuran **7:** To a dry, one-piece reaction vessel with condenser under argon was added Et_4NBr (599 mg, 2.85 mmol). A solution of **7** (417 mg, 1.9 mmol) in 25 ml CH_2Cl_2 was transferred to the reaction vessel followed by dropwise addition of 350 μl of $BF_3 \cdot Et_2O$. After heating the mixture at reflux for 18 h and quenching with 30 ml water, the solution was extracted with CH_2Cl_2. The combined organics were dried over $MgSO_4$, filtered, and evaporated. Purification of the crude mixture by flash column chromatography (9:1 CH_2Cl_2/acetone) yielded 218.2 mg of **6,** 51%.

R_f: 0.42 (1:1 hex/EtOAc); mp: 120°C; $[\alpha]_D^{26}$: −69.0° (c = 0.93, $CHCl_3$); IR: (KBr) 3250(br), 2980, 1490 cm^{-1}.

^{1}H NMR: ($CDCl_3$) (400 MHz) δ 4.18 (1H, d, J = 3.21), 3.86 (1H, m), 3.54 (2H, m), 2.90 (2H, m), 2.63 (3H, m), 1.93 (1H, m), 1.77 (2H, m).

^{13}C NMR: ($CDCl_3$) (400 MHz) δ 133.4 (C), 127.4 (C), 70.2 (CH), 68.2 (CH), 37.7 (CH_2), 34.7 (CH_2), 29.8 (CH_2), 27.1 (CH_2).

MS *m/s* (rel. int.) EI+ 302 (M+2, 5), 300 (M, 11), 283 (64), 175 (58), 67 (100).

HRMS calcd for $C_8H_{12}O_2Br_2$ (M-OH) 280.9177, found 280.9184, error 2.6 ppm.

(7S,7aR)-4-Bromo-2,3,5,6,7,7a-hexahydrobenzofuran-7-ol (7)

The diol **(6)** (18 mg, 0.061 mmol) was dissolved in acetone (1 ml). Cs_2CO_3 (59 mg, 0.183 mmol) was added and the mixture was stirred for 5 h. Additional Cs_2CO_3 (50 mg) was added and the mixture was stirred overnight. After filtration through celite and purification on a flash silica gel column (8:2 methylene chloride/acetone), 13 mg of a colorless oil was obtained in a 99% yield.

R_f: 0.32 (1:1 hex/EtOAc); $[\alpha]_D^{26}$: + 50.6° (c = 0.8, $CHCl_3$).

IR (neat) 3450, 2950, 1310 cm^{-1}.

^{1}H NMR: ($CDCl_3$) (400 MHz) δ 4.27 (1H, s), 4.06 (2H, m), 3.93 (1H, m), 2.63 (3H, m) 2.40 (1H, m), 2.25 (1H, s), 2.07 (1H, m), 1.73 (1H, m).

^{13}C NMR: ($CDCl_3$) (400 MHz) δ 133.7 (C), 115.9 (C), 79.2 (CH), 66.3 (CH_2), 63.7 (CH), 32.4 (CH_2), 30.6 (CH_2), 27.2 (CH_2).

MS *m/z* (rel. int.) EI+ 221 (M+2, 8), 219 (M, 9), 139 (76), 95 (100).

Anal calcd for $C_8H_{11}O_2Br$: C 43.85, H 5.07; found C 43.84, H 5.05.

(7S,7aR)-2,3,5,6,7,7a-Hexahydrobenzofuran-7-ol (8)

The bromofuranol **(7)** (100 mg, 0.4566 mmol) was reacted with freshly cut and cleaned sodium in refluxing ethanol (3 ml) for 2 h. The reaction was carefully quenched with ice water (5 ml). Extraction with ethyl acetate (3 × 8 ml) followed by drying over Na_2SO_4 and purification on a flash column (1:1 hexane/ethyl acetate) yielded 30 mg of furan **8.** The oil had identical spectral properties as those of the previously reported compound.[5]

$[\alpha]_D^{26}$: +50.6° (c = 0.88, $CHCl_3$).

IR: (neat) 3460, 2950, 1075 cm^{-1}.

(3S,4R)-Bromo-2-ethenylcyclohexene-3,4-dimethylmethylenedioxy-3,4-diol (9)

A round-bottom flask was charged with potassium hydroxide (1.0 g, 117.6 mmol) and water (5 ml). After the KOH had dissolved, a solution of the dibromide **6** (100 mg, 0.294 mmol) in benzene (5 ml) was added with a catalytic amount of Bu_4NHSO4. The solution was stirred vigorously for 1 h and the layers were separated. Extraction of the aqueous layer with benzene (2 ml), drying of the combined organic fractions, and concentration of the solvent in vacuo yielded 62 mg of **9,** a colorless oil, 81%. As the compound had limited stability, it was used immediately in the next reaction.

^{1}H NMR: δ 6.82 (1H, dd, J = 17.7, 11.2), 5.60 (1H, d, J = 16.6), 5.29 (1H, d, J = 11.3), 4.81 (1H, d, J = 5.3), 4.39 (1H, m), 2.85 (1H, m), 2.54 (1H, m), 2.02 (1H, m), 1.86 (1H, m), 1.41 (3H, s), 1.39 (3H, s).

(3S,4R)-Bromo-2-ethyloxycyclohexene-3,4-dimethylmethylenedioxy-3,4-diol (10)

An argon-filled round-bottom flask was charged with the olefin **9** (550 mg, 2.122 mmol), and THF (5 ml). After addition of 9-BBN (12.8 ml of a 05 M soln in THF, 6.37 mmol), the reaction was stirred at room temperature overnight. Conversion of the olefin was ~50% at this point, and the mixture was heated to 50°C for 30 min. Additional 9-BBN (5 ml) was added and the reaction was stirred for 1 h. The mixture was cooled (0°C) and water (500 μl), NaOH (3M, 1.5 ml), EtOH (2.5 ml), and H_2O_2 (30%, 2.5 ml) were added. After evolution of gases,

the reaction was heated to 50°C for 20 min. Na_2SO_3 (0.5 g) was added and the solvents were concentrated in vacuo. Brine (30 ml) was added and the solution was extracted wth ethyl acetate (2 × 30 ml; 2 × 15 ml). The combined organic extracts were dried ($MgSO_4$), filtered and the volume reduced. Purification of the residue via flash column chromatography (7:3 ethyl acetate/hexane) afforded 331 mg (56%) of diol **10.**

R_f: 0.5 (7:3 hexane/ethyl acetate); $[\alpha]_D^{26}$: +87.4 (c = 1.0, $CHCl_3$).

IR 3405, 2948, 2932 cm^{-1}.

^1H NMR: δ 4.52 (1H, d, J = 5.6), 4.38 (1H, m), 3.77 (2H, m), 2.56 (2H, m), 2.41 (3H, m), 2.00 (1H, m), 1.90 (1H, m), 1.38 (3H. s), 1.28 (3H, s).

^{13}C NMR: 132.0, (C), 126.2 (C), 109.0 (C), 76.1 (CH), 72.6 (CH), 60.9 (CH_2), 38.1 (CH_2), 32.0 (CH_2), 27.6 (CH_3), 26.8 (CH_2), 26.2 (CH_3).

MS *m/z* (rel. int.) 277 (M, 80), 261 (38), 200 (82), 139 (100).

Anal calcd for $C_{11}H_{17}O_3Br$: C 47.67, H 6.18; found C 47.50, H 6.30.

ACKNOWLEDGMENTS

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

LITERATURE CITED

1. (a) Hudlicky, T., Reed, J.W. In: Advances in Asymmetric Synthesis, Vol. 1. Hassner, A., ed. Greenwich, CT: JAI Press, 1995: 271. (b) Brown, S.M., Hudlicky, T. The use of arene-*cis*-diols in synthesis. In: Organic Synthesis: Theory and Practice, Vol. 2. Hudlicky, T., ed. Greenwich, CT: JAI Press, 1993: 113. (c) Carless, H.A.H. The use of cyclohexa-3,5-diene-1,2-diols in enantiospecific synthesis. Tetrahedron: Asymm. 3:795–826, 1992. (d) Widdowson, D.A., Ribbons, D.W., Thomas, S.D. The use of substituted cyclohexadiene diols as versatile chiral synthons. Janssen Chim. Acta 8(3):3–9, 1990.

2. Petigara, R.B., Yale, H.L. Novel polycyclic heterocycles. XI. Synthesis of 11,12-dihydropyrido[2,1-b][1,3]benzodiazepines, 6H-pyrido[1,2-c][1,3,5] benzoxadiaze-pines, and 6H-pyrido[1,2-c][1,3,5]benzothiadiazepines. J. Heterocyclic Chem. 11(3):331–337, 1974. For this study, the 2-(2-bromoethyl)bromobenzene was prepared in a manner similar to the procedure of Petigara and Yale. The commercially available *ortho*-bromophenylacetic acid was reduced with borane in THF to yield the (*o*-bromophenyl)ethyl alcohol (80%). Formation of the mesylate followed by displacement with LiBr afforded the desired *ortho*-substituted arene (77%).

3. (a) Hudlicky, T., Boros, E.E., Boros, C.E. New diol metabolites derived by biooxidation of chlorostyrenes with *Pseudomonas putida:* Determination of absolute sterochemistry and enantiomeric excess by convergent syntheses. Tetrahedron: Asymm. 4:1365–1386, 1993. (b) Hudlicky, T,. Boros, C.E., Boros, E.E. A model study directed towards a practical enantioselective total synthesis of (−)-morphine. Synthesis 174–178, 1992. (c) Gibson, D., Hensley, M., Yoshioka, H., Mabry, T.J. Formation of (+)-cis-2,3-dihydroxy-1-methylcyclohexa-4,6-diene from toluene by *Pseudomonas putida.* Biochemistry 9:1626–1630, 1970.

4. Gibson, D.T., Zylstra, G.J. Toluene degredation by *Pseudomonas putida* F1 nucleotide sequence of the todC1C2BADE genes and their expression in *Escherichia coli.* J. Biol. Chem. 264:14940–14946, 1989. The recombinant organism, JM109 (pDTG601) overexpresses the toluene dioxygenase enzyme and does not require induction with the known inducers (toluene, chlorobenzene, bromobenzene, styrene). The induction of protein synthesis is instead dependent on the presence of isopropyl-β-D-thiogalactopyranoside (Sigma). This facilitates the testing of the recombinant strain for new substrates and eliminates the problem of separation for the diols derived from the aromatic inducers that would otherwise be present in the fermentation broth. The yields of metabolites are generally 10 times greater than when using *Pp* 39/D.

5. Stabile, M.R., Hudlicky, T., Meisels, M.L. New chiral synthon from bromoethylbenzene: Absolute stereochemistry of a biooxidation metabolite. Tetrahedron: Asymm. 6:537–542, 1995.

6. (a) Whited, G.M., McCombie, W.R., Kwart, L.D., Gibson, D.T. Identification of *cis*-diols as intermediates in the oxidation of aromatic acids by a strain of *Pseudomonas putida* that contains a TOL plasmid. J. Bacteriol. 166:1028–1039, 1986. (b) Königsberger, K., Hudlicky, T. Microbial oxidation of 2-bromostyrene by *Pseudomonas putida* 39D. Isolation and identification of metabolites. Tetrahedron: Asymm. 4:2469–2474, 1993. (c) Hudlicky, T., Boros, C.H. New Diels-Alder dimers of (1S,2R)-*cis*-1,2-isopropylidene-dioxy-3-ethenylcyclohexa-3,5-diene. Tetrahedron Lett. 34: 2557–2560, 1993.