Pergamon

Bioorganic & Medicinal Chemistry, Vol. 2, No. 7, pp. 727-734, 1994

0968-0896/94 $7.00 + .00

0968-0896(94)00084-0

Oxidation of 2-Methoxynaphthalene by Toluene, Naphthalene and Biphenyl Dioxygenases:[1] Structure and Absolute Stereochemistry of Metabolites†

Gregory M. Whited,[a] J. Corey Downie,[a] Tomas Hudlicky,*[b2] Stephen P. Fearnley,[b] Travis C. Dudding,[b] Horatio F. Olivo[b] and Denise Parker[b]

[a]*Genencor International, Inc., 180 Kimball Way, South San Francisco, CA 94080, U.S.A.*
[b]*Department of Chemistry, Virginia Polytechnic Institute & State University, Blacksburg, VA 24061, U.S.A.*

Abstract—2-Methoxynaphthalene was subjected to biooxidation by whole cells of six organisms: *Pseudomonas putida* F39/D containing toluene dioxygenase, *Escherichia coli* JM109(pDTG601), containing recombinant toluene dioxygenase from *Pp* F39/D, *Pseudomonas* sp. NCIB 9816/11, containing naphthalene dioxygenase, *E. coli* JM109(pDTG141), containing recombinant naphthalene dioxygenase from NCIB 9816/11, *E. coli* C534(ProR/Sac) containing recombinant naphthalene dioxygenase from *Pp* G7, and *Beijerinckia* sp. B8/36, containing biphenyl dioxygenase. The major product of oxidation by the naphthalene and biphenyl dioxygenases has been isolated and identified as (1*R*,2*S*)-dihydroxy-7-methoxy-1,2-dihydronaphthalene, **2c**. A minor product, (1*R*,2*S*)-dihydroxy-6-methoxy-1,2-dihydronaphthalene, **3c**, has also been detected. Oxidation by the toluene dioxygenase-containing organisms led to the isolation of **3c** as the major product. Minor products detected in these reactions were **2c**, and a third compound, (1*S*,2*S*)-dihydroxy-3-methoxy-1,2-dihydronaphthalene, **4c**. Structural studies and dehydration of the diols to a mixture of naphthols are described. The absolute stereochemistry of these new diols has been established by correlation with known compounds. The organisms' potential in the production of new metabolites as useful chiral synthons by biooxidation of 2-substituted naphthalenes is indicated.

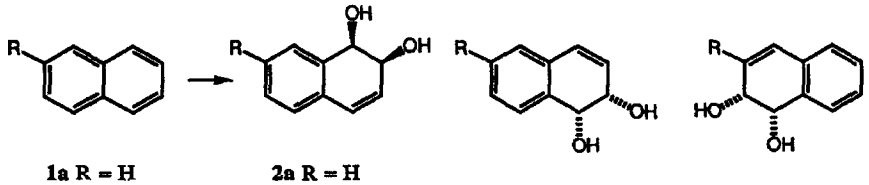
Introduction

The oxidation of polynuclear aromatic hydrocarbons by dioxygenase and monooxygenase enzyme systems is well documented and is known to lead to *cis*-diols or to arene oxides respectively.[3] The work of Jerina and Gibson led to the isolation of diols derived from naphthalene,[4] and other aromatic systems.[5] New metabolites are being isolated at an increasing rate.[6,7] Recently, 2-methylnaphthalene (**1b**) was oxidized with *Pseudomonas putida* 9816-11 to yield the corresponding diol **2b** as a major product.[8] The biotransformation of naphthol ethers of type **1c** would permit the use of diols such as **2c** in the preparation of chiral synthons, natural products or pharmaceuticals containing a functionalized perhydronaphthalene skeleton. The use of such synthons in the preparation of enantiomerically pure compounds has recently been reviewed.[9] In this manuscript we report the isolation and the complete structural and stereochemical assignment of diols **2c**, **3c** and **4c**.

Results and Discussion

Biotransformation

Six organisms were compared in their tendencies to accumulate arene *cis*-diols derived from 2-methoxynaphthalene; these are summarized in Table 1. The *Pp* 9816-11 strain that has been used in the past to generate diols **2a** and **2b**,[10] as well as diols of higher polynuclear aromatics,[5,6] was grown in a mineral salts broth (MSB) medium[11] and the cells harvested by centrifugation. Biooxidation of 2-methoxynaphthalene was then performed at 30 °C in 0.1 M phosphate buffer overnight. Similar fermentation procedures were followed for the remaining organisms, as outlined in the Experimental Section.



1a R = H
1b R = Me
1c R = OMe

2a R = H
2b R = Me
2c R = OMe

3b R = Me
3c R = OMe

4c R = OMe

†We dedicate this manuscript to Professor Bryan Jones, in recognition of his outstanding contributions to the field of biocatalytic asymmetric synthesis.

Following the extractive isolation of the crude diol mixtures, the major products, diols **2c**, **3c** and **4c**, were purified by column chromatography over deactivated silica (10 %, H_2O). Minor constituents of the crude mixture were phenols generated upon workup (5–10 %, distributed accordingly between compounds **5**–**8**). The gross structure of **4c** was readily apparent from NMR, while the regiochemistry of both **2c** and **3c** was initially inferred and subsequently proven by both spectroscopic means and a series of dehydration studies (*vide infra*). A comparison of the optical activities of metabolites **2c** and **3c**, produced by organisms containing either the naphthalene or toluene dioxygenase systems, showed no apparent difference in enzyme enantioselectivity. Table 2 summarizes the comparative performance of each organism in substrate biooxidation.

Structure determination

The gross structural identities of **2c** and **3c** were assigned by both spectroscopic means and dehydration studies. The structure assignment was supported by nÖe experiments which showed a 7 % enhancement of H-5 upon irradiation of H-4 in **2c**. Naphthols **5** and **6** were detected in dehydration mixtures from **2c**, and naphthols **7** and **8** were detected in dehydration experiments performed on the crude diol mix containing **3c**.

Further confirmation of regiochemistry and the proof of the absolute and relative stereochemistry of diol **2c** was obtained by adaption of Boyd's and Dalton's protocol.[7a] In this way, a series of simple chemical manipulations allowed stereochemical correlation with the known adipic

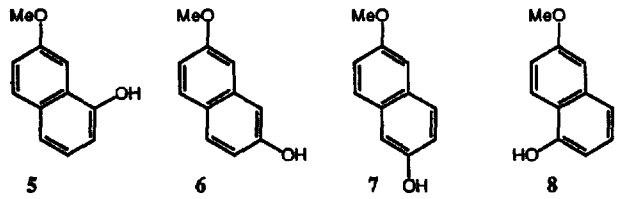
Table 1. Bacterial strains used in this study

Strain	Phenotype	Reference
Pseudomonas putida F39/D	Mutant of wild type strain PpF1 which contains toluene dioxygenase and oxidizes toluene to (*1S,2R*)-dihydroxy-3-methylcyclohexa-3,5-diene; dioxygenase is inducible by toluene.	18
Pseudomonas sp. NCIB 9816/11	Mutant of wild type strain NCIB 9816-4 which contains naphthalene dioxygenase and oxidizes naphthalene to (*1R,2S*)-dihydroxy-1,2-dihydronaphthalene; dioxygenase is inducible by naphthalene.	4
Beijerinckia sp. B8/36	Mutant of wild type strain B1 which contains biphenyl dioxygenase and oxidizes biphenyl to (*1S,2R*)-dihydroxy-3-phenylcyclohexa-3,5-diene; dioxygenase is inducible by biphenyl.	16, 19
E. coli JM109(pDTG601)	JM109 containing the structural genes for toluene dioxygenase (*todC12BA*) in pKK223-3; dioxygenase is inducible by isopropyl-β-*D*-thiogalactaside (IPTG); ampicillin and carbenicillen resistant (Amp).	17
E. coli JM109(pDTG141)	JM109 containing the structural genes for naphthalene dioxygenase from NCIB 9816-4 (*nahAaAbAcAd*) in pKK223-3; dioxygenase is inducible by IPTG; Amp.	20
E. coli C534(ProR/Sac)	C534 containing the structural genes for naphthalene dioxygenase from PpG7 (*nahAaAbAcAd*) in pAC1; dioxygenase is expressed constitutively (Lambda P_L promoter); Amp.	15

Table 2. Comparison of products from different organisms

Strain	Dioxygenase	Yield[a]	Ratio 2c:3c:4c[b]
PpF39/D	Toluene	202	12: 73: 15
JM109(pDTG601)	Toluene	226	17: 69: 14
9816/11	Naphthalene	242	93: 7: 0
JM109(pDTG141)	Naphthalene	272	93: 7: 0
C354(ProR/Sac)	Naphthalene	224	92: 8: 0
B8/36	Biphenyl	272	74: 26: 0

[a]Crude yield expressed as mg product ♦ g dry weight cells^{-1} ♦ L^{-1}. Details of biotransformation conditions are reported in the Experimental Section.
[b]The ratios of the diol regioisomers in the crude extracts were determined by ^{1}H NMR analysis, after integration of the methoxy signals.

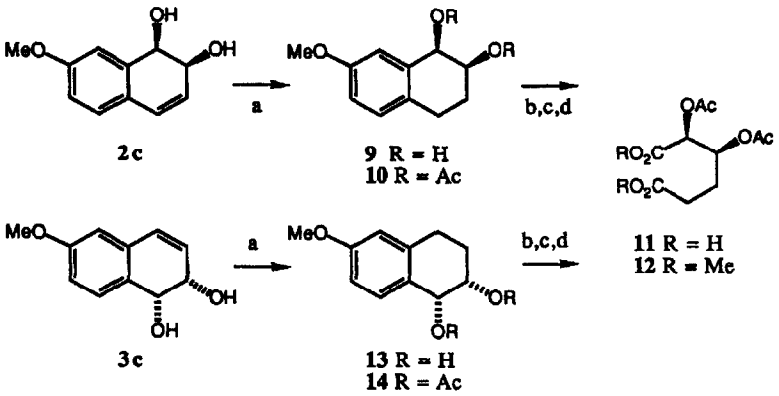


acid derivative **12**, similarly derived from a homochiral source, Scheme I. In an identical fashion, metabolite **3c** was also correlated to **12**, indicating that it has the stereochemistry shown. Thus the reported biooxidations of 2-methoxynapthalene appear to display exclusive enantiomeric selectivity, since compound **12** has been previously correlated with material derived from naphthalene *cis*-dihydrodiol (**2a**) the enantiomeric purity of which has been established.[4]

In a similar fashion, we have proven the absolute stereochemistry of diol **4c** by a series of reactions shown in Scheme 2. The ozonolysis of the acetonide-protected diol **15** furnished not the expected methyl ester but rather the mixture of anomeric hemiacetals **16**, which were reduced to diol **17** ($[\alpha]_D^{25}$: +76.8° (*c* = 0.25; $CHCl_3$). Identical series of reactions performed with **2a**[4] gave the *ent*-**17**, ($[\alpha]_D^{25}$ −76.8 ° (*c* = 0.25; $CHCl_3$) and this confirmed the absolute stereochemistry for **4c**.

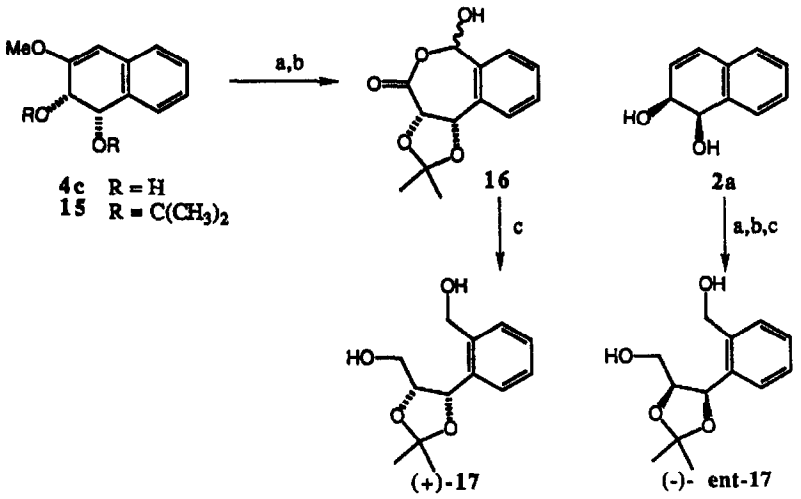
Dehydration studies

Both diols **2c** and **3c** dehydrated quantitatively to a mixture of naphthols under a variety of conditions ($CDCl_3$, 1 h, rt; 0.1 N H_2SO_4, 4 h, rt; acetone/silica gel, 8 h, rt). The resulting naphthol mixtures were subsequently compared with standard solutions of the known naphthols **5–8**,[12–14] (prepared or obtained from the Kodak depository) by both GC–MS and HPLC coinjection. Diols **2c** and **3c** dehydrated quantitatively to mixtures of **5** and **6**, and **7** and **8** respectively, thus confirming the regiochemistry of these materials. Although **2c** is extremely short-lived at room



Reagents: (a) H_2, Pd/C, EtOAc (b) Ac_2O, NEt_3, DMAP, CH_2Cl_2 (c) RuO_2, $NaIO_4$, MeCN/CCl_4/H_2O 2:2:3 (d) CH_2N_2

Scheme 1.



Reagents: (a) DMP/H^+; (b) O_3/DMS; (c) $LiAlH_4$.

Scheme 2.

temperature in solutions containing trace amounts of acid ($t_{1/2}$ = 45 min in chloroform), it can be purified and handled quite easily provided base-washed solvents and deactivated silica (10 %) are used. It is stable in crystalline form indefinitely when stored below –10 °C and is easily functionalized.

Conclusion

We have shown that the regiospecificity of naphthalene and biphenyl dioxygenases are opposite from that observed with toluene dioxygenase with respect to oxidation of **1c**, as shown in Scheme 3. In addition, a third product, **4c**, was produced by the reaction of **1c** with toluene dioxygenase and was not detected in the reactions of 2-methoxynaphthalene with naphthalene and biphenyl dioxygenases. Although the regiospecificity is different among the dioxygenases, the absolute stereochemistry of the products is the same for the toluene and naphthalene dioxygenase series of experiments. This was proven by comparison of optical rotations of samples of diols isolated from the two experiments and a similar comparison of the values of their hydrogenated derivatives **9** and **13**. In the biphenyl dioxygenase experiment insufficient amounts were isolated for comparison of the absolute configurations of the products, therefore the absolute stereochemistry is not implied in Scheme 3.

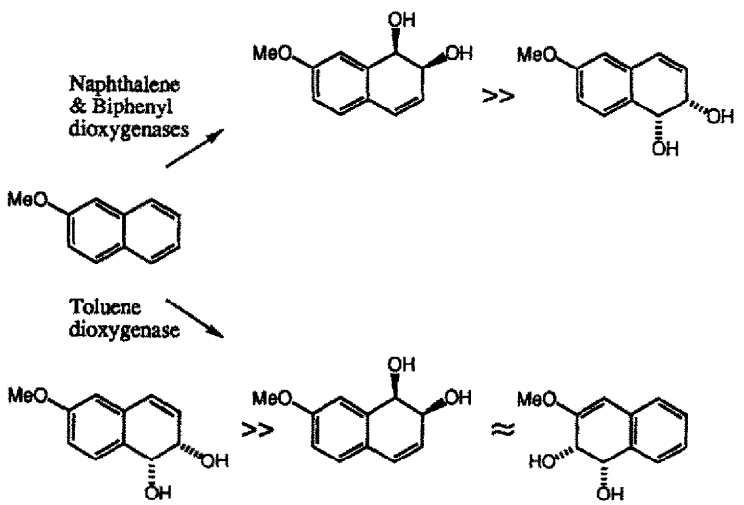
The isolation and further synthetic manipulations of both diols bode well for their use in the increasing pool of chiral synthons made available by biotransformation of aromatic hydrocarbons. Approaches to naphthalene-derived natural products should be made possible from diols manufactured from oxygenated naphthalenes. The results of such endeavors will be reported in due course.

Experimental Section

All non-aqueous reactions were carried out under argon using standard techniques for the exclusion of air and moisture. All solvents used were obtained anhydrous, either by appropriate distillation or by direct purchase. Where necessary, reagents were dried and purified according to the recommended methods. Thin layer chromatography was carried out on Merck Kieselgel 60 F_{254} glass plates. Flash chromatography was performed over Kieselgel 60 silica (EM Reagents, 230-400 mesh), previously deactivated with water (10 % v/v) by overnight agitation. Melting points were determined on an electrothermal apparatus and are uncorrected. Infra-red absorption spectra were recorded on a Perkin-Elmer FT-1600 instrument, as thin films, chloroform solutions or KBr discs. $^{1}H/^{13}C$ NMR Spectra were recorded on Bruker 270 MHz and Varian 400 MHz instruments at 270/60 MHz and 400/100 MHz, respectively, referenced to the appropriate deuterium lock. *J* Values are given in Hz. ^{13}C Multiplicities were determined by APT experiments. Mass spectra were measured on a VG 7070 EHF instrument. Percentile figures refer to relative intensity as a proportion of the base peak.

Microbial oxidation of 2-methoxynaphthalene (1c)

Cells of *Escherichia coli* C534(ProR/Sac)[15] were grown in 1 L cultures, in 2800 mL Fernbach flasks, with a rich growth medium containing 1 % glucose, 1 % tryptone, 1 % yeast extract, 0.1 M potassium phosphate buffer (pH 7.0) and 100 mg L^{-1} ampicillin. The culture was incubated, with shaking, at 37 °C until the maximum growth of cells was obtained (optical density monitored at 660 nm). The cells were then harvested by centrifugation at 9000 rpm and resuspended in 0.1 M phosphate buffer containing 0.2 % glucose. 2-Methoxynaphthalene (0.2 % w/v) was added followed by the addition of isopropanol (9 % v/v) and the mixture shaken at 37 °C overnight. At the end of the incubation period any unreacted/undissolved 2-methoxynaphthalene was filtered, the cells centrifuged and the supernatant extracted with ice-cold ethyl acetate (3 ×) and dichloromethane (2 ×). (The amount of naphthols in the crude reaction product was greatly reduced by performing the extraction with cold solvents.) Drying (Na_2SO_4), filtration and removal of solvents *in vacuo* afforded the crude diols; the yield immediately after



Scheme 3. Relative oxidation patterns of different dioxygenases.

isolation was 1.48 g (75 %) per 2 g of 2-methoxynaphthalene used (i.e. 1 L of culture). Further purification by flash chromatography (hexane/ethyl acetate 4:1; R_f = 0.3; 10 % deactivated flash silica), followed by recrystallization from hexane/ethyl acetate (needles) or acetone/hexane (plates) yielded an analytically pure sample of (1*R*,2*S*)-1,2-dihydroxy-7-methoxy-1,2-dihydronaphthalene, **2c**; mp: 108–111°C; $[\alpha]_D^{25}$ +247 ° (*c* = 1.0; MeOH); IR: ($CHCl_3$) υ 3564, 1608, 1570, 1498, 1220, 1036, 938, 831 cm^{-1}; UV: (MeOH) 272 nm (ε_0 = 14,400); ^1H NMR: (DMSO, 400 MHz) δ 7.04 (d, *J* = 8.2 Hz, 1H), 7.02 (d, *J* = 2.6 Hz, 1H), 6.75 (dd, *J* = 8.1, 2.6 Hz, 1H), 6.41 (d, *J* = 9.6 Hz, 1H), 5.84 (dd, *J* = 9.6, 4.7 Hz, 1H), 4.99 (d, *J* = 6.6 Hz, exchanges with D_2O, 1H), 4.61 (d, *J* = 5.6 Hz, exchanges with D_2O, 1H), 4.41 (t, *J* = 5.4 Hz, 1H), 4.06 (~q, *J* = ~5 Hz, 1H), 3.75 (s, 3H); ^{13}C NMR: (DMSO, 100 MHz) δ 158.8 (C), 139.6 (C), 127.25 (CH), 127.23 (CH), 125.2 (C), 112.4 (2 × CH), 111.8 (CH), 70.2 (CH), 66.1 (CH), 55.0 (CH_3); MS: (EI, 70 eV) m/z = 192 $[M]^+$ 40 %, 174 $[M–H_2O]^+$ 100 %; HRMS: calcd for $C_{11}H_{12}O_3$: 192.078664; found: 192.07832; anal.: calcd for $C_{11}H_{12}O_3$: C, 68.74; H, 6.29; found: C, 68.68; H, 6.39.

Secondly, the more polar minor metabolite, (1*R*,2*S*)-1,2-dihydroxy-6-methoxy-1,2-dihydronaphthalene, **3c**; R_f: 0.20 (hexane/ethyl acetate 1:1); mp: 109–111 °C; $[\alpha]_D^{25}$ + 165.5° (*c* = 1.0; MeOH); IR: (KBr disc) ν 3260.0, 2919.2, 1609.7, 1571.1, 1262.4, 1064.8, 1033.9, 800.1 cm^{-1}; UV: (MeOH) 266 nm (ε_0 = 5750); ^1H NMR: (DMSO, 400 MHz) δ 7.28 (d, *J* = 8.4 Hz, 1H), 6.76 (dd, *J* = 8.1, 2.6 Hz, 1H), 6.72 (d, *J* = 2.6 Hz, 1H), 6.46 (d, *J* = 9.3 Hz, 1H), 5.93 (dd, *J* = 9.7, 3.7 Hz, 1H), 4.76 (m, 2 × OH, exchanges with D_2O), 4.36 (t, *J* = 5.2 Hz before exchange, collapses with D_2O, 1H), 4.15 (m before exchange, collapses with D_2O, 1H), 3.73 (s, 3H); ^{13}C NMR: (DMSO, 100 MHz) d 159.5 (C), 134.1 (C), 131.9 (CH), 129.6 (C), 129.2 (CH), 112.9 (CH), 112.5 (CH), 69.7 (CH), 68.1 (CH), 55.7 (CH_3); MS: (CI, 70 eV) m/z = 193 $[M+H]^+$ 8 %, 192 $[M]^+$ 10 %, 175 $[M+H-H_2O]^+$ 100 %; HRMS: calcd for $C_{11}H_{12}O_3$: 192.078664; found: 192.078827.

With organisms containing the toluene dioxygenase system, an additional metabolite, (1*S*,2*S*)-1,2-dihydroxy-3-methoxy-1,2-dihydronaphthalene, **4c**, was also identified; R_f: 0.45 (hexane/ethyl acetate 1:2); mp: 154–156 °C; $[\alpha]_D^{25}$: – 225° (*c* = 0.5, MeOH); IR: (KBr disc) υ 3242.2, 1638.9, 1030.6 cm^{-1}; ^1H NMR: (DMSO-d_6, 400 MHz) δ 7.38 (d, *J* = 7.2 Hz, 1H), 7.08 (m, 2H), 7.00 (d, *J* = 7.2 Hz, 1H), 5.62 (s, 1H), 5.18 (d, *J* = 7.3 Hz, D_2O exchanged, 1H), 4.99 (d, *J* = 5.0 Hz, D_2O exchanged, 1H), 4.54 (dd, *J* = 6.6, 5.5 Hz before exchange, collapsed with D_2O, 1H), 3.86 (t, *J* = 4.9 Hz before exchange, collapsed with D_2O, 1H); ^{13}C NMR: (DMSO-d_6, 100 MHz) δ 159.8 (C), 135.0 (C), 132.9 (C), 126.8 (CH), 124.8 (CH), 124.7 (CH), 124.6 (CH), 97.3 (CH), 70.9 (CH), 69.5 (CH), 54.8 (CH_3); MS: (EI, 70 eV) m/z = 192 $[M]^+$ 12 %, 175 [M = H – $H_2O]^+$ 100 %; HRMS: (CI) calcd for $C_{11}H_{12}O_3$: 192.0786444; found: 192.078629.

Large scale oxidation of **1c** for structure determination with JM109(pDTG601) was conducted similarly to the small scale except that the final culture was grown in a 20 L fermentor with additional glucose feeding to increase the cell yield. After growth, 25 g of **1c** was added to approximately 13.5 L of culture and the oxidation allowed to proceed for 5 h. The broth was processed as described earlier and a crude yield of 19.4 g was obtained.

Small scale oxidations of **1c**, to determine product yields and isomeric ratios were conducted as follows:

*Pp*F39/D was grown in a 2.8 L Fernbach flask containing 500 mL of MSB[11] medium supplemented with 0.2 % fructose. The culture medium was inoculated with an overnight preculture grown in Lauria Broth (10 g L^{-1} tryptone, 5 g L^{-1} yeast extract, and 5 g L^{-1} NaCl; 25 mL in a 250 mL flask, 30 °C on a rotary shaker) and the culture was incubated at 30 °C on a rotary shaker in the presence of toluene vapors. Cells were harvested during active growth by centrifugation and the supernatant (which contained *cis*-toluene diol as determined by an absorbance maximum at 266 nm in the UV spectrum) was discarded. A portion of the pellet was resuspended to a final optical density at 660 nm of 4.7 in 50 mL of 100 mM KPO_4 buffer, pH 7.2 containing 0.2 % fructose and 0.1 % **1c** in a 250 mL flask. The cell suspension was incubated at 30 °C on a rotary shaker for 9 h after which time there was no further increase in the absorbance of the supernatant in the UV region (final absorbance at 268 nm was 20). The cells and unoxidized **1c** were removed by filtering the suspension through cheesecloth followed by centrifugation. The clarified supernatant, 38 mL, was extracted 2 times with equal volumes of ethyl acetate. The extract was dried over anhydrous Na_2SO_4 and evaporated to yield 18 mg of a light yellow oil.

NCIB 9816/11 was grown in a 2.8 L Fernbach flask containing 500 mL of MSB medium supplemented with 0.2 % succinate and 0.1 % naphthalene. The culture medium was inoculated with an overnight preculture grown in Lauria Broth (25 mL in a 250 mL flask, 30 °C on a rotary shaker) and the culture was incubated at 30 °C on a rotary shaker. Cells were harvested during active growth by centrifugation and the supernatant (which contained *cis*-naphthalene diol as determined by an absorbance maximum at 264 nm in the UV spectrum) was discarded. A portion of the pellet was resuspended to a final optical density at 660 nm of 1.5 in 25 mL of 100 mM KPO_4 buffer, pH 7.2 containing 0.2 % succinate and 0.1 % **1c** in a 250 mL flask. The cell suspension was incubated at 30 °C on a rotary shaker for 20 h after which time there was no further increase in the absorbance of the supernatant in the UV region (final absorbance at 272 nm was 16). The cells and unoxidized **1c** were removed by filtering the suspension through cheesecloth followed by centrifugation. The clarified supernatant, 22 mL, was extracted 2 times with equal volumes of ethyl acetate. The extract was dried over anhydrous Na_2SO_4 and evaporated to yield 4 mg of a dry white material.

B8/36 was grown in a 2.8 L Fernbach flask containing 500 mL of MSB medium supplemented with 0.2 % fructose,

0.05 % yeast extract and 0.1 % biphenyl. The culture medium was inoculated with an overnight preculture grown in Lauria Broth (25 mL in a 250 mL flask, 30 °C on a rotary shaker) and the culture was incubated at 30 °C on a rotary shaker. Cells were harvested during active growth by centrifugation and the supernatant (which contained *cis*-biphenyl diol as determined by an absorbance maximum at 304 nm in the UV spectrum) was discarded. A portion of the pellet was resuspended to a final optical density at 660 nm of 4.9 in 50 mL of 100 mM KPO_4 buffer, pH 7.2 containing 0.2 % succinate and 0.1 % **1c** in a 250 mL flask. The cell suspension was incubated at 30 °C on a rotary shaker for 20 h after which time there was no further increase in the absorbance of the supernatant in the UV region (final absorbance at 270 nm was 40). The cells and unoxidized **1c** were removed by filtering the suspension through cheesecloth followed by centrifugation. The clarified supernatant, 33 mL, was extracted 2 times with equal volumes of ethyl acetate. The extract was dried over anhydrous Na_2SO_4 and evaporated to yield 22 mg of a dry white material.

JM109(pDTG601) was grown in a 2.8 L Fernbach flask containing 500 mL of MSB medium supplemented with 0.2 % glucose, 1 mM thiamine, 10 mg L^{-1} isopropyl-β-D-thiogalactaside (IPTG) and 50 mg L^{-1} carbenicillin. The culture medium was inoculated with an overnight preculture grown in Lauria Broth containing 50 mg L^{-1} carbenicillin (25 mL in a 250 mL flask, 30 °C on a rotary shaker) and the culture was incubated at 30 °C on a rotary shaker. Cells were harvested during active growth by centrifugation and the supernatant was discarded. A portion of the pellet was resuspended to a final optical density at 660 nm of 5.0 in 50 mL of 100 mM KPO_4 buffer, pH 7.0 containing 0.2 % glucose and 0.1 % **1c** in a 250 mL flask. The cell suspension was incubated at 35 °C on a rotary shaker for 20 h after which time there was no further increase in the absorbance of the supernatant in the UV region (final absorbance at 266 nm was 35). The cells and unoxidized **1c** were removed by filtering the suspension through cheesecloth followed by centrifugation. The clarified supernatant, 39 mL, was extracted 2 times with equal volumes of ethyl acetate. The extract was dried over anhydrous Na_2SO_4 and evaporated to yield 22 mg of a light yellow oil.

JM109(pDTG141) was grown in a 2.8 L Fernbach flask containing 500 mL of MSB medium supplemented with 0.2 % glucose, 1 mM thiamine, 10 mg L^{-1} IPTG and 50 mg L^{-1} carbenicillin. The culture medium was inoculated with an overnight preculture grown in Lauria Broth containing 50 mg L^{-1} carbenicillin (25 mL in a 250 mL flask, 35 °C on a rotary shaker) and the culture was incubated at 35 °C on a rotary shaker. Cells were harvested during active growth by centrifugation and the supernatant was discarded. A portion of the pellet was resuspended to a final optical density at 660 nm of 5.7 in 50 mL of 100 mM KPO_4 buffer, pH 7.0 containing 0.2 % glucose and 0.1 % **1c** in a 250 mL flask. The cell suspension was incubated at 35 °C on a rotary shaker for 7 h after which time there was no further increase in the absorbance of the supernatant in the UV region (final absorbance at 272 nm was 57). The cells and unoxidized **1c** were removed by filtering the suspension through cheesecloth followed by centrifugation. The clarified supernatant, 45 mL, was extracted 2 times with equal volumes of ethyl acetate. The extract was dried over anhydrous Na_2SO_4 and evaporated to yield 35 mg of a white material.

C534(ProR/Sac) was grown in a 2.8 L Fernbach flask containing 500 mL of Lauria Broth supplemented with 0.2 % glucose and 50 mg L^{-1} carbenicillin. The culture medium was inoculated with an overnight preculture grown in Lauria Broth containing 50 mg L^{-1} carbenicillin (25 mL in a 250 mL flask, 35 °C on a rotary shaker) and the culture was incubated at 35 °C on a rotary shaker. Cells were harvested during active growth by centrifugation and the supernatant was discarded. A portion of the pellet was resuspended to a final optical density at 660 nm of 5.7 in 50 mL of 100 mM KPO_4 buffer, pH 7.0 containing 0.2 % glucose and 0.1 % **1c** in a 250 mL flask. The cell suspension was incubated at 35 °C on a rotary shaker for 8 h after which time there was no further increase in the absorbance of the supernatant in the UV region (final absorbance at 272 nm was 57). The cells and unoxidized **1c** were removed by filtering the suspension through cheesecloth followed by centrifugation. The clarified supernatant, 25 mL, was extracted 2 times with equal volumes of ethyl acetate. The extract was dried over anhydrous Na_2SO_4 and evaporated to yield 16 mg of a white material.

Dehydration of diols

A small sample of pure diol was left stirring in $CDCl_3$ at room temperature and monitored by NMR until the formation of naphthols was complete (< 4 h). The naphthols **5–8** were identified by GC–MS (Hewlett-Packard) co-injection.

(1R,2S)-1,2,3,4-Tetrahydro-7-methoxynaphthalene-1,2-diol (9). To a solution of methoxynaphthalenediol (**2c**) (300 mg, 1.56 mmol) in ethyl acetate (7 mL), was added palladium on carbon (30 mg, 10 % Pd/C) and the resulting suspension subjected to hydrogenation at 50 psi. After 2 h, the mixture was filtered through Celite and evaporated *in vacuo* to yield **9** as white crystals (257.6 mg, 1.326 mmol, 85 %); R_f: 0.32 (hexane/ethyl acetate 7:3); mp: 96–97 °C; $[\alpha]_D^{25}$ – 16.5° (*c* = 0.5; MeOH); IR: (KBr disc) υ 3284, 1617, 1502, 1216, 1038 cm^{-1}; ^1H NMR: ($CDCl_3$, 270 MHz) δ 7.01 (d, *J* = 8.4 Hz, 1H), 6.96 (d, *J* = 2.7 Hz, 1H), 6.80 (dd, *J* = 8.4, 2.7 Hz, 1H), 4.65 (d, *J* = 3.6 Hz, 1H), 4.00 (dt, *J* = 9.3, 2 × 3.7 Hz, 1H), 3.79 (s, H), 2.88 (dt, *J* = 16.8, 2 × 5.8 Hz, 1H), 2.70 (m, 1H), 2.25 (bs, 2H), 2.1–1.8 (m, 2H); ^{13}C NMR: ($CDCl_3$, 60 MHz) δ 158.2 (C), 137.5 (C), 129.5 (CH), 128.1 (C), 114.9 (CH), 113.9 (CH), 70.1 (CH), 69.5 (CH), 55.3 (CH_3), 26.5 (CH_2), 25.8 (CH_2); MS: (EI, 70 eV) m/z = 194 $[M]^+$ 10 %, 134 100 %; anal.: calcd for $C_{11}H_{14}O_3$: C, 68.02; H, 7.26; found: C, 68.02; H, 7.30.

Using an identical procedure, the minor metabolite **3c** (150 mg, 0.78 mmol) yielded, after further purification by flash chromatography (silica ratio 50:1, hexane/ethyl acetate

1:2), diol **13** as white crystals (128 mg, 0.66 mmol, 85 %); R_f: 0.19 (hexane/ethyl acetate 1:2); mp: 117–118 °C; $[\alpha]_D^{25}$ – 35.4° (*c* = 0.5; $CHCl_3$); IR: (KBr disc) υ 3228.3, 2945.3, 1611.4, 1498.2, 1268.9, 1074.8, 797.6 cm^{-1}; ^1H NMR: ($CDCl_3$, 400 MHz) δ 7.33 (d, *J* = 8.5 Hz, 1H), 6.78 (dd, *J* = 8.4, 2.6 Hz, 1H), 6.63 (d, *J* = 2.7 Hz, 1H), 4.66 (m, 1H), 3.96 (m, 1H), 3.77 (s, 3H), 2.92 (dt, *J* = 17.3, 2 × 5.1 Hz, 1H), 2.76 (m, 1H), 2.27 (d, *J* = 7.3 Hz, 1 × OH), 2.06 (d, *J* = 5.0 Hz, 1 × OH), 1.99 (m, 1H), 1.91 (m, 1H); ^{13}C NMR: ($CDCl_3$, 100 MHz) δ 159.4 (C), 137.7 (C), 131.3 (CH), 128.7 (C), 112.9 (CH), 112.8 (CH), 69.7 (CH), 69.6 (CH), 55.2 (CH_3), 27.5 (CH_2), 26.1 (CH_2); MS: (EI, 70 eV) m/z = 195 $[M+H]^+$ 15 %, 194 $[M]^+$ 19 %, 177 $[M+H - H_2O]^+$ 100 %; HRMS: (CI) calcd for $C_{11}H_{15}O_3$: 195.1021195; found: 195.102142.

(2S,3S)-Dimethyl (2,3)-diacetoxyadipate (12). To a stirred solution of diol **9** (194 mg, 1.0 mmol, 1 eq) and dimethylaminopyridine (12 mg, 0.1 mmol, 0.1 eq) in dichloromethane (10 mL) at 0 °C under argon, was added dropwise acetic anhydride (377 μL, 4.0 mmol, excess), followed by triethylamine (558 μL, 4.0 mmol, excess), and the mixture allowed to warm to room temperature. After 48 h, saturated aqueous ammonium chloride (25 mL) was added and the resulting mixture extracted with dichloromethane (5 × 25 mL). The combined organic fractions were dried ($MgSO_4$), filtered and reduced *in vacuo* to yield the crude diacetate **10** as a white solid, (289 mg), which was used directly without further purification. To a stirred biphasic mixture of this diacetate **10** and sodium metaperiodate (6.42 g, 30 mmol, excess) in carbon tetrachloride (4 mL), acetonitrile (4 mL) and water (6 mL), was added a catalytic quantity of ruthenium (IV) dioxide hydrate (2 mg, 0.015 mmol), and vigorous stirring at room temperature continued for some time. After 5 days, aqueous 1 M hydrochloric acid (40 mL), previously saturated with sodium chloride, was added and the resulting mixture extracted with ethyl acetate (5 × 20 mL). The combined organic fractions were dried ($MgSO_4$), filtered and reduced *in vacuo* to yield the crude diacid **11** as a brown oil, (343 mg). ^1H NMR Analysis of the crude reaction product confirmed that the aromatic ring of diacetate **10** had been cleaved and thus was used directly without further purification. To a stirred solution of this crude diacid **11** in methanol (2 mL) at 0 °C, was added dropwise with caution an excess of freshly prepared diazomethane as a solution in diethyl ether, and the mixture allowed to warm to room temperature. After 2.5 h, excess diazomethane was removed under a stream of argon and the solvents removed *in vacuo* to yield a brown oil (277 mg). Further purification by flash chromatography (gradient elution, hexanes/ethyl acetate 6:1 to 2:1, silica ratio 100:1) yielded pure **12** as a colorless oil (110 mg, 0.379 mmol, 37.9 % from **9**), the ^1H NMR and optical rotation of which closely matched the given literature values;[7a] $[\alpha]_D^{25}$: –15.1° (*c* = 2.92; $CHCl_3$); lit.:[7a] –14° (*c* = 2.92; $CHCl_3$); ^1H NMR: ($CDCl_3$, 270 MHz) δ 5.25 (m, 2H), 3.75 (s, 3H), 3.64 (s, 3H), 2.34 (m, 2H), 2.14 (s, 3H), 2.03 (s, 3H), 1.87–2.15 (m, 2H).

Using an identical procedure, diol **13** (107 mg, 0.552 mmol), yielded **12** (109.3 mg, 0.376 mmol, 68.2 %) whose data closely matched that of the previously obtained sample; $[\alpha]_D^{25}$: –15.5° (*c* = 2.96; $CHCl_3$).

Degradation of (1S,2S)-1,2-dihydroxy-3-methoxy-1,2-dihydronaphthalene (4c) and proof of absolute stereochemistry. To a stirred solution of diol **4c** (125.9 mg, 0.655 mmol) in 2,2-dimethoxypropane (20 mL), was added a catalytic quantity of *p*-toluenesulphonic acid, and stirring continued for 1.5 h, whereupon saturated aqueous sodium bicarbonate (20 mL) was added and the reaction mixture extracted with dichloromethane (4 × 20 mL). The combined organic fractions were dried ($MgSO_4$), filtered, and reduced *in vacuo* to yield the crude acetonide **15** as a colorless oil, used immediately in the next step. A solution of this acetonide **15** in dichloromethane (3 mL) was cooled to –78 °C and a stream of ozone in oxygen was bubbled through until a faint blue coloration persisted (~20 min). The mixture was purged with a stream of argon, dimethyl sulfide (0.42 mL, excess) added, and allowed to warm to room temperature. Solvent was removed *in vacuo* to yield the crude lactols **16** as a colorless oil (254 mg), which was used directly without further purification. To a stirred solution of these lactols **16** in tetrahydrofuran, was added, in one portion, lithium aluminum hydride (50 mg, excess), and stirring continued. After 18 h, water (50 μL) was added, followed by 10 % aqueous sodium hydroxide (50 μL) and further water (150 μL). The resulting granular suspension was filtered through Celite and concentrated *in vacuo* to yield the crude diol **17** as a colorless oil (165 mg). Further purification by flash chromatography (hexanes/ethyl acetate 1:1, silica ratio 150:1) yielded pure (+)-**17** as a colorless oil (80.6 mg, 0.388 mmol, 52 % from **4c**); $[\alpha]_D^{25}$ + 76.8° (*c* = 0.25; $CHCl_3$); ^1H NMR: ($CDCl_3$, 200 MHz) δ 7.60 (d, *J* = 7.2 Hz, 1H), 7.30 (m, 3H), 5.64 (d, *J* = 6.9 Hz, 1H), 4.76 (d, *J* = 12.1 Hz, 1H), 4.56 (m, 2H), 3.34 (dd, *J* = 11.1, 6.3 Hz, 1H), 3.12 (dd, *J* = 11.0, 6.6 Hz, 1H), 1.63 (s, 3H), 1.50 (s, 3H).

Using an identical sequence of reactions, (1*R*,2*S*)-dihydroxy-(1,2)-dihydronaphthalene (**2a**) yielded (–)-*ent*-**17**, identical in all respects except optical rotation; $[\alpha]_D^{25}$: –76.8° (*c* = 0.25; $CHCl_3$).

Acknowledgements

The authors wish to thank Frank Pettrone, William Anthony and Jeff Gerstner for technical assistance, and to Eastman Kodak Co. for the Summer Visiting Scientist Fellowship to T. H.

References

1. A portion of this work was performed at Eastman Kodak Laboratories, Rochester, NY, Summer, 1990.

2. Recipient of the American Cyanamid Faculty Research Award, 1992.

3. a) For a recent report on arene oxides see: Lee, Y. T.; Fisher, J. F. *J. Org. Chem.* **1993**, *58*, 3712; for other leading references see: b) Lehr, R. E.; Wood, A. W.; Levin, W.;

Conney, A. H.; Jerina, A. M. In *Polycyclic Aromatic Hydrocarbon Carcinogenesis*, Vol. 1, pp. 31–58, Yang, S. K.; Silverman, B. D., Eds; CRC Press; Boca Raton, FL, 1988; c) Chada, A.; Sayer, J. M.; Yeh, H. J. C.; Yagi, H.; Cheh, A. M.; Pannell, L. K.; Jerina, D. M. *J. Am. Chem. Soc.* **1989**, *111*, 5456; d) Thakker, D. R.; Yagi, H.; Levin, W.; Wood, A. W.; Conney, A. H.; Jerina, D. M. In *Bioactivation of Foreign Compounds*, p. 177, Anders, M. W., Ed.; Academic Press; New York, 1985; e) Jerina, D. M.; Daly, J. W.; Witkop, B.; Zaltzman-Nireuberg, P.; Udenfriend, S. *Arch. Biochem. Biophys.* **1968**, *128*, 176.

4. Jerina, D. M.; Daly, J. W.; Jeffrey, A. M.; Gibson, D. T. *Arch. Biochem. Biophys.* **1971**, *142*, 394.

5. For a discussion of bacterial metabolism of aromatic compounds see: a) Bayly, R.C.; Barbour, M. G. In *Microbial Degradation of Organic Compounds*, Chapter 8, Gibson, D. T., Ed.; Marcel Dekker; New York, 1984; b) Trudgill, P. W. In *Microbial Degradation of Organic Compounds*, Chapter 9, Gibson, D. T., Ed.; Marcel Dekker; New York, 1984; c) Ensley, B. D., Jr In *Microbial Degradation of Organic Compounds*, Chapter 10, Gibson, D. T., Ed.; Marcel Dekker; New York, 1984; d) Reineke, W. In *Microbial Degradation of Organic Compounds*, Chapter 11, Gibson, D. T., Ed.; Marcel Dekker; New York, 1984; e) Safe, S. H. In *Microbial Degradation of Organic Compounds*, Chapter 12, Gibson, D. T., Ed.; Marcel Dekker; New York, 1984; f) Ribbons, D. W.; Keyser, P.; Eaton, R. W.; Anderson, B. N.; Kunz, D. A.; Taylor, B. F. In *Microbial Degradation of Organic Compounds*, Chapter 13, Gibson, D. T., Ed.; Marcel Dekker; New York, 1984.

6. For recent compilations of known metabolites derived from the microbial oxidation of aromatic compounds see: a) McMordie, R. A. S. Ph.D Thesis, The Queen's University of Belfast, 1989; b) Stabile, M. M.S. Thesis, Virginia Polytechnic Institute and State University, 1993.

7. a) Boyd, D. R.; Sharma, N. D.; Boyle, R.; Malone, J. F.; Chima, J.; Dalton, H. *Tetrahedron: Asymmetry* **1993**, *4*, 1307; b) Boyd, D. R.; Sharma, N. D.; Dority, M. R. J.; Hand, M. V.; McMordie, R. A. S.; Malone, J. R.; Porter, H. P.; Dalton, H.; Chima, J.; Sheldrake, G. N. *J. Chem Soc., Perkin Trans 1* **1993**, 1065; c) Engesser, K. H.; Auling, G.; Busse, J.; Knackmuss, H. J. *Arch. Microbiol.* **1990**, *153*, 193; d) Boyd, D. R.; Sharma, N. D.; Stevenson, P. J.; Chima, J.; Gray, D. J.; Dalton, H. *Tetrahedron Lett.* **1991**, 3887; e) Boyd, D. R.; Sharma, N. D.; Boyle, R.; McMurray, T. T.; Evans, T. A.; Malone, J. F.; Dalton, H.; Chima, J.; Sheldrake, G. N. *J. Chem. Soc. Chem. Commun.* **1993**, 49; f) Boyd, D. R.; Sharma, N. D.; Boyle, R.; McMordie, R. A. S.; Chima, J.; Dalton, H. *Tetrahedron Lett.* **1992**, 1241; g) Hudlicky, T.; Boros, E. E.; Boros, C. H. *Synlett* **1992**, 391; *Tetrahedron: Asymmetry* **1993**, *4*, 1365.

8. Deluca, M.; Hudlicky, T. *Tetrahedron Lett.* **1990**, *31*, 13.

9. Reviews: a) Carless, H. A. J. *Tetrahedron: Asymmetry* **1992**, *3*, 795; b) Brown, S. M.; Hudlicky, T. *Organic Synthesis: Theory and Applications*, Vol. 2, p. 113, Hudlicky, T., Ed.; JAI Press; Greenwich, CT, 1993; c) Widdowson, D. A.; Ribbons, D. A.; Thomas, S. D. *Janssen Chim. Acta* **1990**, *8*, 3; d) Ribbons, D. W.; Williams, J. O. *Advances in Nat. Prod. Chem.* (in press); e) Hudlicky, T.; Reed, J. W. In *Advances in Asymmetric Synthesis*, Vol. 2, pp. 271–311, Hassner, A., Ed.; JAI Press; Greenwich, CT, 1994.

10. a) Ensley, B. D.; Gibson, D. T. *J. Bacter.* **1983**, *155*, 505; b) Ensley, B. D.; Ratzkin, B. J.; Osslund, T. D.; Simon, M. J.; Wackett, L. P.; Gibson, D. T. *Science* **1983**, *222*, 167; c) Ensley, B. D.; Gibson, D. T.; Laborde, A. L. *J. Bacter.* **1982**, *149*, 948.

11. Cohen-Bazire, G.; Sistrom, W. R.; Stanier, R. Y. *J. Cellular Comp. Physiol.* **1957**, *49*, 25; see also Hudlicky, T.; Boros, C. H.; Boros, E. E. *Synthesis* **1992**, 174.

12. All compounds obtained yielded full spectroscopic and analytical data in accordance with their proposed structures.

13. Samples of several of these compounds were available from the Kodak Chemical Sample Library; **4**: KAN # 029880; **6**: KAN # 184489; **7**: KAN # 907932.

14. For leading references to the preparation and physical properties of naphthols **4**–**7**, see the following: **4**: Giles, R. B. F.; Hughes, A. B.; Sargent, M. V. *J. Chem. Soc., Perkin Trans 1*, **1991**, 1581; **5**: Prince, P.; Gandour, R. D. *Synlett* **1991**, 405; **6**: Kidwell, R. L.; Murphy, M.; Darling, S. D. *Org. Synth.* **1969**, *49*, 90; **7**: Khasnis, D. D.; Mane, R. *Indian J. Chem, Pt. B* **1991**, *30B*, 448.

15. Serdar, C. M.; Murdock, D. C.; Ensley, B. D. U.S. Patent 5,173,425.

16. Gibson, D. T.; Roberts, R. L.; Wells, M. C.; Kobal, V. M. *Biochem. Biophys. Res. Commun.* **1973**, *50*, 211; see also reference 5e.

17. Zylstra, G. J.; Gibson, D. T. *J. Biol. Chem.* **1989**, *264*, 14940.

18. Gibson, D. T.; Hensley, M.; Yoshioka, H.; Mabry, T. J. *Biochemistry* **1970**, *9*, 1626.

19. Ziffer, H.; Kabuto, K.; Gibson, D. T.; Kobal, V. M.; Jerina, D. M. *Tetrahedron* **1977**, *33*, 2491.

20. Suen, W.-C. Ph. D. Dissertation, 1991, University of Iowa, Iowa City, IA.

(*Received* 13 *June* 1994)