Oxidation of 6,7-Dihydro-5H-Benzocycloheptene by Bacterial Strains Expressing Naphthalene Dioxygenase, Biphenyl Dioxygenase, and Toluene Dioxygenase Yields Homochiral Monol or *cis*-Diol Enantiomers as Major Products

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Bacterial strains expressing naphthalene, biphenyl, and toluene dioxygenase were examined for their abilities to oxidize 6,7-dihydro-5H-benzocycloheptene (benzocyclohept-1-ene). The major oxidation products were isolated, and their absolute configurations were determined by chiral ¹H nuclear magnetic resonance analysis of diastereomeric boronate esters, chiral stationary-phase high-pressure liquid chromatography, and stereochemical correlation. *Pseudomonas* sp. strain 9816/11 and *Sphingomonas yanoikuyae* (formerly identified as a *Beijerinckia* sp.) B8/36 expressing naphthalene and biphenyl dioxygenases, respectively, oxidized benzocyclohept-1-ene to a major product identified as (–)-(1*R*,2*S*)-*cis*-dihydroxybenzocycloheptane (>98% enantiomeric excess [ee], 50 and 90% yield, respectively). In contrast, *Pseudomonas putida* F39/D expressing toluene dioxygenase oxidized benzocyclohept-1-ene to (+)-(5*R*)-hydroxybenzocyclohept-1-ene (>98% ee, 90% yield) as the major metabolite and to the “opposite” diol, (+)-(1*S*,2*R*)-*cis*-dihydroxybenzocycloheptane (>98% ee, 10% yield). The results indicate that, for benzocyclohept-1-ene, the major reaction catalyzed by naphthalene and biphenyl dioxygenases is dioxygenation whereas toluene dioxygenase catalyzes mainly *R*-stereospecific benzylic monooxygenation. Although the type of reaction catalyzed by each organism was not predictable, the absolute configuration of the diol and monol products formed by naphthalene and toluene dioxygenases are consistent with the stereochemistry of the products formed by these enzymes from other benzocycloalkene substrates.

Bacterial aromatic ring dioxygenases are multicomponent enzyme systems capable of catalyzing the initial incorporation of molecular dioxygen and two hydrogen atoms into the aromatic substrate to produce a *cis*-diene diol (15). These enzymes play an important role in the activation and hydroxylation of resonance-stabilized aromatic compounds prior to further catabolism in nature. Three such enzymes are toluene dioxygenase (TDO) from *Pseudomonas putida* F1, naphthalene dioxygenase (NDO) from *Pseudomonas* sp. strain 9816-4, and biphenyl dioxygenase (BPO) from *Sphingomonas yanoikuyae* B1 (formerly identified as a *Beijerinckia* sp.), each of which catalyzes the enantiospecific dihydroxylation of their primary substrate to yield the homochiral *cis*-dihydrodiols shown in Fig. 1 (12, 14, 20). Strains expressing TDO, NDO, or BPO and containing mutations in the gene encoding the next enzyme, *cis*-dihydrodiol dehydrogenase, accumulate the *cis*-diols when induced cells are incubated with the substrate and an alternative energy source (12, 14, 20). The *cis*-diols from toluene, naphthalene, and biphenyl have the same relative stereochemistry for the hydroxyl-bearing carbon adjacent to the methyl and phenyl substituent of toluene and biphenyl dihydrodiols or to the bridgehead carbon of naphthalene dihydrodiol (Fig. 1) (20, 23, 36).

Interest in the reactions catalyzed by TDO, NDO, BPO, and other dioxygenases stems from their ability to produce enantiomerically pure *cis*-diols from an extremely wide range of substrates (15, 16, 33). More than 130 *cis*-diols have been produced by a relatively small number of dioxygenases, and several of these diols have been used as chiral synthons in the

preparation of a wide range of acyclic sugars and complex bioactive natural products, often in fewer steps than conventional synthetic methods (10, 11, 18, 33). However, the reactions catalyzed by TDO, NDO, and BPO are not limited to *cis* dihydroxylation of aromatic rings. Additional reactions catalyzed by aromatic ring dioxygenases include benzylic monooxygenation (4, 5, 8, 32, 35), desaturation (13, 28, 34), O dealkylation (28), N demethylation (25), and sulfoxidation (3, 24).

Stereochemical analysis of bacterial oxidation products can provide rational approaches to desired chiral intermediates and information regarding specific substrate-enzyme interactions. The benzylic monooxygenation of indan (35) and the dioxygenation of indene (13, 35) and 1,2-dihydronaphthalene (34) by TDO and NDO often lead to the formation of chiral products of opposite absolute configuration in high enantiomeric excess (ee). For example, benzylic monooxygenation of indan by TDO and NDO yields (1*R*)- and (1*S*)-indanol, respectively. In addition, both indene and 1,2-dihydronaphthalene undergo dioxygenation by TDO and NDO to yield *cis*-diols of 1*S*,2*R* and 1*R*,2*S* configurations, respectively (5, 13, 34, 35). The distinct differences in the regiospecificity and stereospecificity of oxidations catalyzed by TDO and NDO, with respect to benzocyclic aromatic compounds, have led us to examine the oxidation of other related benzocyclic compounds.

Different aromatic ring dioxygenases have also been used to determine the effect of cycloalkane/cycloalkene ring size on the types of reactions catalyzed by TDO, NDO, and BPO and to establish patterns which relate trends regarding the regiospecific and stereospecific oxidations observed (2, 13, 34, 35). In an elegant series of studies, Boyd and coworkers have used *P. putida* UV4, a mutant constitutive for the expression of a TDO (with properties slightly different from those of the TDO from

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FIG. 1. Reactions catalyzed by the mutant strains PpF39/D, 9816/11, and B8/36 expressing TDO, NDO, and BPO. The reactions catalyzed by TDO, NDO, and BPO require NAD(P)H and molecular oxygen (not shown) and result in the formation of (+)-*cis*-(1*S*,2*R*)-dihydroxy-3-methylcyclohexa-3,5-diene from toluene, (+)-*cis*-(1*R*,2*S*)-dihydroxy-1,2-dihydronaphthalene from naphthalene, and (+)-*cis*-(1*S*,2*R*)-dihydroxy-3-phenylcyclohexa-3,5-diene from biphenyl, respectively. X, a mutation which renders the *cis*-dihydrodiol dehydrogenases inactive.

strain F1), to examine the oxidations catalyzed for a series of benzocyclic aromatic compounds (1, 5–8), including 6,7-dihydro-5H-benzocycloheptene (benzocyclohept-1-ene or BCH-1-ene) (4). On the basis of the dioxygenase-catalyzed desaturation, monooxygenation, and dioxygenation reactions with the benzocycloalkene substrates indene and 1,2-dihydronaphthalene, we predicted that NDO would catalyze *cis* dihydroxylation of BCH-1-ene to yield a *cis*-diol of 1*R*,2*S* configuration. We have tested this hypothesis and, in addition, determined which reactions are catalyzed by different dioxygenases when the cycloalkene ring is increased in size by one carbon. Here we report the regio- and stereospecific oxidation of BCH-1-ene by bacterial strains expressing TDO, NDO, and BPO.

MATERIALS AND METHODS

Bacterial strains and growth. *Pseudomonas* sp. strain 9816/11, *S. yanoikuyae* (formerly identified as a *Beijerinckia* sp. [22]) B8/36, and *P. putida* F39/D are *cis*-dihydrodiol dehydrogenase mutants which express NDO, BPO, and TDO, respectively, and accumulate *cis*-dihydrodiols from their primary substrates when grown on an alternative substrate under inducing conditions (12–14, 34). Cultures of strains 9816/11, B8/36, and PpF39/D were grown under conditions which induce their respective dioxygenases and harvested as described previously (28).

Chemicals. Chemicals were commercially available and used as received or prepared as described below. BCH-1-ene was prepared by dehydration (80% H_2SO_4 , 16 h) of 1-benzosuberol and then purified by radial dispersion chromatography (RDC; see below; 4-mm-thick silica, CHCl_3 elution at 10 ml/min). 1-Benzosuberol was obtained by treatment of 1-benzosuberone with sodium borohydride (1.5 eq, 2 h) in ethanol. Optically pure (–)-(*S*)- and (+)-(*R*)-2-[1-methoxyethyl]-phenylboronic acids (MPBA) were prepared by methods previously described (31).

Biotransformation of BCH-1-ene. Induced cells of strains 9816/11, B8/36, and PpF39/D (800-ml cultures) were washed in 0.05 M sodium-potassium phosphate buffer (pH 7.3), suspended in 250 ml of the same buffer ($A_{600} = 3.6$ to 6.5), and transferred to 2.8-liter Fernbach flasks containing 0.1% (vol/vol) BCH-1-ene and 0.5% pyruvate. The suspensions were incubated (30°C, 220 rpm) for 20 h, the cells were removed by centrifugation, and the supernatants were analyzed as described below.

Product isolation, identification, and analysis. Supernatants from biotransformation reactions were extracted with ethyl acetate and analyzed by thin-layer chromatography (TLC) on Silica Gel 60 F_{254} sheets using chloroform-acetone (8:2) as developing solvent (30). Metabolites were isolated by preparative TLC or RDC using a Chromatotron (Harrison Research, Palo Alto, Calif.), and specific optical rotations ($[\alpha]_D$), chiral stationary-phase high-pressure liquid chromatography (CSP-HPLC), gas chromatography-mass spectrometry (GC-MS), and nuclear magnetic resonance spectrometry (NMR) (^1H and ^{13}C) were performed under conditions reported previously (32). A Chiralcel OJ column eluted with a mobile phase of hexane-2-propanol (9:1; flow rate, 0.5 ml/min) was used for CSP-HPLC analyses.

BCH-1-ene oxidation products were isolated by RDC on silica (2mm thick-

ness) Chromatotron plates eluted with a chloroform-acetone step gradient (0 to 60% acetone, 20% steps over 1 h) at a flow rate of 7 ml/min. TLC of fractions (10 to 30 ml) revealed compound I and/or compound II as the major products in biotransformations by strains 9816/11, B8/36, and PpF39/D.

Diastereomeric boronate esters for the stereochemical analysis of *cis*-diols were prepared by adding optically pure (–)-(*S*)- and (+)-(*R*)-MPBA (1 eq) to isolated *cis*-diols in 4 ml of CHCl_3 and stirring at 25°C for 1 h. The MPBA esters were passed through small plugs of silica-sodium sulfate with CHCl_3 , concentrated to dryness, and redissolved in 0.6 ml of CDCl_3 (containing 0.05% [vol/vol] tetramethylsilane) for ^1H NMR analysis of the chemical shifts of the diastereomeric methoxyl and methyl group signals (31).

The absolute configurations of isolated metabolites were determined initially by comparing their specific rotations with those of known compounds (4) and thereafter were determined by chiral ^1H nuclear magnetic resonance analysis of diastereomeric boronate esters or by CSP-HPLC.

RESULTS

BCH-1-ene oxidation by strain 9816/11. Induced cells of strain 9816/11 ($A_{600} = 6.3$) incubated with 0.1% BCH-1-ene yielded 80 mg of a crude ethyl acetate-soluble, off-white solid. TLC showed a major polar product with an R_f of 0.2 and several nonpolar metabolites with R_f values of 0.4, 0.6, and 0.7. GC-MS analysis of the extracted material showed a major product with M^+ at m/z 178 (compound I, 50% total ion current area) and minor products with M^+ at m/z of 160 (15%, 12.1 min), 162 (18%, 12.4 min), and 158 (12%, 12.9 min). The major product, compound I (R_f of 0.2; 40 mg), was isolated by RDC and characterized by GC-MS, and ^1H and ^{13}C NMR (Table 1). The mass and ^1H NMR spectra of compound I were consistent with those previously reported for (±)-1,2-*cis*-dihydroxybenzocycloheptane [(±)-1,2-*cis*-dihydroxybenzosuberane or BCH-diol] (27). In addition, the product had a specific rotation of $[\alpha]_D = -20.8^\circ$ (c 0.40, MeOH). The sign and magnitude of the (–)-*cis*-1,2-BCH-diol formed by 9816/11 suggested that the product was of high ee but of 1*R*,2*S* absolute stereochemistry “opposite” to that of the (+)-*cis*-(1*S*,2*R*)-BCH-diol ($[\alpha]_D = +25.2^\circ$ (CHCl_3)) formed by strain UV4 (4). CSP-HPLC and chiral MPBA esters were used to confirm the absolute stereochemistry and enantiomeric composition of compound I formed by strain 9816/11.

CSP-HPLC analysis of the (–)-*cis*-(1*R*,2*S*)-BCH-diol formed by strain 9816/11 showed a single peak at 26.8 min. The enantiomeric purity was confirmed by analyzing diastereomeric boronate esters of the (–)-*cis*-diol prepared with (–)-(*S*)- and (+)-(*R*)-MPBA. ^1H NMR analysis showed that the OMe and Me signals of the ester formed via (–)-(*S*)-MPBA at 3.288 and 1.434 ppm, respectively, while the corresponding signals of the (+)-(*R*)-MPBA ester were at 3.247 and 1.496 ppm (Fig. 2). The single diastereomeric OMe and Me signals in the ^1H NMR spectra of the esters formed with (–)-(*S*)-MPBA and (+)-(*R*)-MPBA, together with CSP-HPLC analysis, indicated that the (–)-*cis*-(1*R*,2*S*)-BCH-diol formed by strain 9816/11 was enantiomerically pure (>98% ee) (Fig. 2). The structures of the minor products present were not established in this study.

BCH-1-ene oxidation by strain B8/36. Induced cells of strain B8/36 ($A_{600} = 6.4$) incubated with BCH-1-ene gave 50 mg of a crude ethyl acetate-soluble, off-white solid. The major product (R_f of 0.2; TLC) was isolated by RDC to yield 40 mg of a product with mass and ^1H and ^{13}C NMR spectra identical to those of compound I formed by strain 9816/11 (Table 1). The sign of the rotation of compound I formed by B8/36 ($[\alpha]_D = -16.3^\circ$ (c 0.24, MeOH)) indicated that the absolute configuration was the same as that of the diol formed by strain 9816/11. CSP-HPLC analysis of the diol and ^1H NMR analysis of its (–)-(*S*)-MPBA and (+)-(*R*)-MPBA esters indicated that compound I formed by B8/36 was enantiomerically pure (–)-*cis*-

TABLE 1. Physical properties of the major oxidation products formed from benzocyclohept-1-ene by bacterial strains expressing TDO, NDO, and BPO

Compound	TLC R_f^a	R_t (min) ^b	m/z of fragment ions (% relative intensity)	¹ H and ¹³ C NMR chemical shifts ^c	Product ^d
I	0.2	14.5	M^+ 178 (5), 160 (31), 142 (70), 131 (50), 119 (22), 107 (100)	¹ H (acetone- d_6) δ : 7.31–7.34 (m, 1H), 7.02–7.13 (m, 3H), 4.85 (s, 1H), 4.20 (d, J = 3.6 Hz, 1H; D ₂ O exchangeable), 3.82 (m, 1H), 3.44 (s, 1H; D ₂ O exchangeable), 3.06 (t, J = 12.0 Hz, 1H), 2.60 (dd, J = 13.8 Hz, 8.7 Hz, 1H), 2.12–2.20 (m, 1H), 1.75–1.90 (m, 2H), 1.45–1.54 (m, 1H); ¹³ C δ : 142.7, 140.8, 129.8, 129.1, 127.7, 126.2, 79.4, 73.7, 35.2, 34.9, 25.2	1,2- <i>cis</i> -Dihydroxybenzocycloheptane
II	0.5	12.8	M^+ 160 (5), 142 (100), 131 (16), 115 (32)	¹ H (CDCl ₃) δ : 7.40 (d, J = 7.4 Hz, 1H), 7.15–7.24 (m, 3H), 6.39 (d, J = 12.0 Hz, 1H), 5.92 (dt, J = 12.0 Hz, 5.0 Hz, 1H), 4.85 (dd, J = 6.2 Hz, 1.5 Hz, 1H), 2.40–2.54 (m, 2H), 2.02–2.22 (m, 2H); ¹³ C δ : 142.5, 134.0, 132.4, 131.3, 129.3, 127.2, 126.7, 126.5, 73.2, 33.9, 26.8	5-Hydroxybenzocyclohept-1-ene

^a Determined in chloroform-acetone (8:2).^b Determined by GC-MS as described in the text.^c ¹H and ¹³C NMR were recorded in the deuterated solvent listed at 360 and 90 MHz, respectively. Chemical shift multiplicities, coupling constants (J values in Hz), and proton integrations are listed in parentheses.^d Identified by R_t and spectral (mass and NMR) comparison. The characteristics of compounds I and II are consistent with those reported in previous studies (4, 27).

(1*R*,2*S*)-BCH-diol (>98% ee). Several minor products detected by GC-MS prior to isolation had retention times (R_t) of 12.4 min (M^+ 162), 14.8 min (M^+ 160), 15.0 min (M^+ 158), and 16.8 min (M^+ 174). While the compound with an R_t of 12.4 min was also present in reactions with strain 9816/11, the minor products from B8/36 represented less than 10% of the total ion current area, and their structures were not further investigated.

BCH-1-ene oxidation by strain PpF39/D. Toluene-induced cells of strain PpF39/D (A_{600} = 3.6) incubated with BCH-1-ene yielded 47 mg of an ethyl acetate-soluble clear oil. TLC analysis showed a major product (R_f of 0.5) and a minor product (R_f of 0.2), and both products were isolated by RDC. The major product formed by PpF39/D (90% yield; R_f 0.5; 44 mg), designated compound II, was characterized by GC-MS and NMR (Table 1). Compound II was optically active and had a specific rotation of $[\alpha]_D^{25} = +187^\circ$ (c 0.44, MeOH). The above data are in excellent agreement with data reported for 1,2-benzocyclohepta-1,3-dien-7-ol, referred to here as 5-hydroxy-BCH-1-ene (¹H NMR [19]). The $[\alpha]_D^{25}$ value of $+187^\circ$ indicated that the monol formed by strain PpF39/D was of 5*R* configuration and identical to the (+)-(*5R*)-hydroxybenzocyclohept-1-ene formed by strain UV4 (>98% ee; $[\alpha]_D^{25} = +162^\circ$ (CHCl₃)) (4).

The minor product formed by PpF39/D (10% yield; R_f of 0.2; 3.5 mg) had mass and ¹H NMR spectral characteristics identical to those of compound I (Table 1). The enantiomeric composition of the diol (compound I) formed by PpF39/D was determined by CSP-HPLC and ¹H NMR analysis of diastereomeric MPBA esters. The (+)-(*R*)-MPBA ester of the diol formed by PpF39/D showed OMe and Me resonances at 3.289 and 1.434 ppm, respectively. The same chemical shifts were observed for the diastereomeric ester obtained via (–)-(*S*)-MPBA and the diol formed by strain 9816/11, indicating that the *cis*-diol formed by PpF39/D was (+)-*cis*-(1*S*,2*R*)-BCH-diol. The enantiomeric composition was shown to be >98% ee by CSP-HPLC, which resolved the PpF39/D BCH-diol at 26.8 min; none of the opposite enantiomer (obtained via strain 9816/11; R_t of 19.6 min) was observed. A trace amount of a nonpolar metabolite (R_f of 0.7; <0.5 mg) was also obtained by RDC. This compound was identified as 5-keto-BCH-1-ene by GC-MS (R_t of 12.5 min; M^+ 158) and ¹H NMR (acetone- d_6). In addition to the signals representing the four aromatic and

FIG. 2. Expanded regions of the ¹H NMR spectra showing the diastereomeric OMe and Me signals resulting from the esters formed with the strain 9816/11 (–)-*cis*-(1*R*,2*S*)-BCH-diol and the (–)-(*S*)-MPBA (δ_{OMe} = 3.288 ppm, δ_{Me} = 1.434 ppm) (A), the (+)-(*R*)-MPBA (δ_{OMe} = 3.247 ppm, δ_{Me} = 1.496 ppm) (B), and a mixture of (+)- and (–)-MPBA (C). The signal at 1.55 ppm is due to water. Chemical shifts of diastereomeric esters are discussed in the text.

FIG. 3. Major oxidation products formed from BCH-1-ene by strains 9816/11 and B8/36 expressing NDO and BPO (A) and strain F39/D expressing TDO (B), respectively. Product yields are given in the text.

four methylenic protons, ^1H NMR chemical shifts at 6.24 and 6.55 ppm (1H each) corresponded to the two alkene protons present in the cycloheptene ring.

DISCUSSION

The results presented show differences in the regioselective and stereospecific oxidation of BCH-1-ene by mutant strains expressing NDO, BPO, and TDO. Strains 9816/11 and B8/36 expressing NDO and BPO, respectively, oxidized BCH-1-ene to a *cis*-diol (compound I) as the major product. The major reaction catalyzed by strains B8/36 and 9816/11 is shown in Fig. 3A. The (-)-*cis*-(1*R*,2*S*)-BCH-diol formed by these strains was shown to be enantiomerically pure by CSP-HPLC and chiral ^1H NMR methods and was of opposite absolute configuration to the (+)-*cis*-(1*S*,2*R*)-diol formed as a minor product by TDO-containing strains PpF39/D and UV4 (4). The formation of the (-)-*cis*-(1*R*,2*S*)-diol by strains 9816/11 and B8/36 represents a direct route to this single enantiomer in high yield. Recently, a naphthalene-utilizing wild-type *P. putida* strain (NCIMB 8859) was shown to oxidize BCH-1-ene to (-)-*cis*-(1*R*,2*S*)-BCH-diol (>98% ee, 24 h). The same strain resolved synthetic racemic *cis*-BCH-diol to yield (+)-*cis*-(1*S*,2*R*)-diol (35% ee) (2). These results demonstrated the presence of enantiospecific *cis*-diol dehydrogenase in strain 8859 that oxidizes the (-)-*cis*-(1*R*,2*S*)-diol. Our data indicate that higher yields of this diol may be obtained through the use of a mutant strain that lacks *cis*-dihydrodiol dehydrogenase activity.

Oxidation of BCH-1-ene by strain PpF39/D, expressing TDO, indicated that the major reaction catalyzed was benzylic monooxygenation (90% yield), with facial selectivity resulting in the replacement of the pro-*R* hydrogen to yield enantiomerically pure (+)-(5*R*)-hydroxybenzocyclohept-1-ene (Fig. 3B, compound II). The oxidation of other benzocyclic aromatic compounds to their corresponding (*R*)-alcohols by TDOs from strains F39/D and UV4 has previously been observed with benzocyclobutene (8, 29), indan (8, 9, 35), indene (5), 1,2-dihydronaphthalene (5, 34), and 1-indanone (32). The minor oxidation product formed by strain PpF39/D was (+)-*cis*-(1*S*,2*R*)-BCH-diol (>98% ee, 10% yield, compound I), the configuration of which was also consistent with the diols formed by the TDO-catalyzed *cis*-dihydroxylation of indene (5, 35) and 1,2-dihydronaphthalene (1, 5, 34). The trace amount of 5-keto-BCH-1-ene (>1%) identified was presumably formed by either enzymatic dehydrogenation or isomerization of the

major product, (+)-(5*R*)-hydroxybenzocyclohept-1-ene. The yields and stereochemistries of the monol and *cis*-diol products formed by strain F39/D in the present study were identical to those reported for strain UV4 (4).

While both NDO and TDO catalyze the desaturation and dioxygenation of 1,2-dihydronaphthalene (34) (NDO also catalyzes the desaturation of indan [13]), the desaturation of BCH-1-ene was not observed at significant levels with strains expressing NDO, BPO, and TDO. Strain 9816/11 did yield a product with an M^+ at m/z 158 (12%; R_f of 12.95 min), but examination of mass and two-dimensional NMR spectra (not shown) indicated this product to be 3- or 4-keto-BCH-1-ene (presumably formed via hydroxylation followed by nonspecific dehydrogenation) rather than a hydroxy-BCH-1,3-diene resulting from desaturation and hydroxylation of BCH-1-ene.

It is of interest that the ^1H NMR directional shifts in Fig. 2 for the diastereomeric OMe and Me signals of the MPBA esters formed with the *cis*-BCH-diol enantiomers were reversed with regard to the trends previously reported for a series of homochiral *cis*-diols (31). This result suggests that the spatial orientations of the OMe and Me groups (with respect to the aromatic ring of the benzocyclic diol) of the diastereomeric esters formed with the "floppy" 7-membered ring diol are completely opposite to their orientation in esters derived from 5- and 6-membered ring benzocycloalkane and benzocycloalkene *cis*-diols. Similar anomalies have been reported for the "chromatographic behavior" on a Pirkle chiral HPLC column of the 7-membered ring alcohol, (*S*)-1-benzosuberol, which was eluted in the reverse order relative to the corresponding 5- and 6-membered ring alcohols (21).

In addition to their unique ability to catalyze the stereospecific *cis*-dihydroxylation of the aromatic nucleus, aromatic ring dioxygenases (TDO, NDO, and/or BPO) are capable of catalyzing a diverse array of reactions that include benzylic monooxygenation (5, 8, 13, 32, 35), desaturation (13, 28, 34), O dealkylation (28), N demethylation (25), and sulfoxidation (3, 24). Each of these reactions except *cis*-dihydroxylation is also catalyzed by cytochrome P-450 (17, 26). In general, the reactions catalyzed are determined by both the structure of the substrate and the intrinsic properties of the specific oxygenases. Studies with *P. putida* UV4, expressing TDO, have shown that benzocycloalkenes (benzocyclobutene, indene, 1,2-dihydronaphthalene, and BCH-1-ene) undergo preferential monooxygenation of the cycloalkene ring to yield secondary alcohols of *R* configuration (8). In the case of BCH-1-ene, *R*-specific monooxygenation was observed as the major reaction (90%), while dioxygenation to the (+)-(1*S*,2*R*)-*cis*-diol was observed as a minor reaction (10%) (4).

The present study provides additional insight regarding the oxidation of benzocyclic aromatic hydrocarbons by aromatic ring dioxygenases. For example, the results indicate that increasing the benzocycloalkene ring size by one methylene unit (i.e., indene to 1,2-dihydronaphthalene to BCH-1-ene) has a pronounced effect on the types of reactions catalyzed by NDO and TDO. While both NDO and TDO catalyze desaturation and dioxygenation of 1,2-dihydronaphthalene (34), no products resulting from desaturation (and subsequent oxygenation) were produced from BCH-1-ene. Instead, NDO and BPO preferentially catalyze the enantiospecific 1*R*,2*S* dihydroxylation of BCH-1-ene, whereas TDO catalyzes mainly the *R*-stereospecific benzylic monooxygenation of this substrate. The differences in the reactions catalyzed for BCH-1-ene by TDO (monooxygenation and 1*S*,2*R* dioxygenation, 9:1) and NDO/BPO (both catalyzing mainly 1*R*,2*S* dioxygenation) probably reflect differences in the orientation of the substrate in the active site of the specific oxygenases. The identification of the trends and

differences in the regiospecific and enantioselective reactions catalyzed by different dioxygenases can assist in the rational prediction of chiral products obtainable through the microbial oxidation of prochiral substrates.

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