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Diverse reactions catalyzed by naphthalene dioxygenase from *Pseudomonas* sp strain NCIB 9816

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Naphthalene dioxygenase (NDO) from *Pseudomonas* sp strain NCIB 9816 is a multicomponent enzyme system which initiates naphthalene catabolism by catalyzing the addition of both atoms of molecular oxygen and two hydrogen atoms to the substrate to yield enantiomerically pure (+)-*cis*-(1*R*,2*S*)-dihydroxy-1,2-dihydronaphthalene. NDO has a relaxed substrate specificity and catalyzes the dioxygenation of many related 2- and 3-ring aromatic and hydroaromatic (benzocyclic) compounds to their respective *cis*-diols. Biotransformations with a diol-accumulating mutant, recombinant strains and purified enzyme components have established that in addition to *cis*-dihydroxylation, NDO also catalyzes a variety of other oxidations which include monohydroxylation, desaturation (dehydrogenation), *O*- and *N*-dealkylation and sulfoxidation reactions. In several cases, the absolute stereochemistry of the oxidation products formed by NDO are opposite to those formed by toluene dioxygenase (TDO). The reactions catalyzed by NDO and other microbial dioxygenases can yield specific hydroxylated compounds which can serve as chiral synthons in the preparation of a variety of compounds of interest to pharmaceutical and specialty chemical industries. We present here recent work documenting the diverse array of oxidation reactions catalyzed by NDO. The trends observed in the oxidation of a series of benzocyclic aromatic compounds are compared to those observed with TDO and provide the basis for prediction of regio- and stereospecificity in the oxidation of related substrates. Based on the types of reactions catalyzed and the biochemical characteristics of NDO, a mechanism for oxygen activation by NDO is proposed.

Keywords: asymmetric dihydroxylation; biocatalysis; biotransformation; dealkylation; desaturation; dioxygenation; monooxygenation; sulfoxidation; stereospecific oxidation

Introduction

Recent technical advances in molecular biology and analytical chemistry indicate that a gram of soil may contain more than 4000 bacterial species [97] and more than 100000 aromatic compounds of unknown structure and physiological activity [10]. These are daunting extremes of microbial and chemical diversity that pose the ultimate challenge to scientists in their quest to understand the interactions between man and his environment. This review focuses on a simple approach to this challenge. That is, the activities of a single enzyme from a single bacterial strain. We show that diversity at the level of enzyme activity may result in the identification of new useful compounds which may be produced by procedures that minimize the generation of chemical waste products.

Naphthalene is an aromatic hydrocarbon found in creosote and crude petroleum. It has been identified as a priority pollutant by the Environmental Protection Agency and has been used as a model compound for studies on the metabolism of polycyclic hydrocarbons by mammals and microorganisms. Consequently, a considerable amount of information is available on the metabolism of naphthalene by bacteria, fungi, and mammals. These results have been summarized in review articles [25,39,52,89,94,103]. The metabolic pathway for the conversion of 1,2-dihydroxynaphthalene to salicylate and the order of genes encoding the enzymes responsible for each reaction were first described in detail by Davies and Evans [27] and Yen and Gunsalus [102], and more recently by Eaton and Chapman [29]. The initial reaction in the bacterial oxidation of naphthalene by pseudomonads involves the stereospecific addition of both atoms of molecular oxygen at the 1,2-position to form (+)-*cis*-(1*R*,2*S*)-dihydroxy-1,2-dihydronaphthalene (*cis*-naphthalene dihydrodiol) [23,51,53]. This compound is then oxidized by a pyridine nucleotide-dependent dehydrogenase to 1,2-dihydroxynaphthalene [75].

We have studied the formation of *cis*-naphthalene dihydrodiol by *Pseudomonas* sp NCIB 9816-4. This strain has had a checkered history since its isolation as *Pseudomonas* strain P_G in 1965 [33]. The organism was subsequently deposited in the National Collection of Industrial Bacteria as *Pseudomonas* sp strain NCIB 9816 [24]. Four strains of 9816 are known which differ in the regulation of the pathways used for fission of catechol and also in the number and size of plasmids they contain [103]. In addition, at various times strain NCIB 9816 has been referred to as *Pseudomonas putida* by ourselves and others. However, speciation was based on preliminary unpublished experiments and the more accurate designation for *Pseudomonas* sp strain NCIB 9816-4 (strain 9816-4) described in this review refers to a pseudomonad which harbors the genes for naphthalene catabolism on a conjugative 83-kb plasmid named pDTG1 [86].

Cells of *Pseudomonas* sp 9816-4 contain an inducible multicomponent enzyme system designated naphthalene

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dioxygenase (NDO) [31] which catalyzes the formation of *cis*-naphthalene dihydrodiol (Figure 1). The system consists of an iron-sulfur flavoprotein (reductase$_{NAP}$) [46], a Rieske [2Fe-2S] protein (ferredoxin$_{NAP}$) [45], and an iron-sulfur protein (ISP$_{NAP}$) [30], which serves as the terminal oxygenase component. ISP$_{NAP}$ has an $\alpha_2\beta_2$ subunit composition and each α subunit contains a Rieske [2Fe-2S] cluster and mononuclear iron [55,92]. The Rieske cluster is believed to be an electron storage center that transfers electrons to mononuclear iron which is responsible for dioxygen activation and ultimately the catalytic reaction.

The genes encoding the NDO components in *Pseudomonas* sp 9816-4 have been cloned and expressed in *Escherichia coli* [91,93]. The nucleotide sequences of the genes encoding reductase$_{NAP}$ (*nahAa*), ferredoxin$_{NAP}$ (*nahAb*), and ISP$_{NAP}$ (*nahAcAd*) have been determined and show 93.3%, 93.3%, 96.9%, and 94.8% identity, respectively, at the predicted amino acid level with the isofunctional genes carried by the well-studied NAH7 plasmid in *P. putida* G7 ([62,88]; Parales and Gibson, unpublished results).

Interest in the substrate specificity of bacterial dioxygenases stems from initial studies on the degradation of benzene and toluene more than 25 years ago. A mutant strain of *P. putida* (strain F39/D) was shown to oxidize benzene and toluene to *cis*-1,2-dihydroxycyclohexa-3,5-diene (*cis*-benzene dihydrodiol) [36] and *cis*-(1*S*,2*R*)-dihydroxy-3-methylcyclohexa-3,5-diene (*cis*-toluene dihydrodiol) [37,59], respectively. The enzyme catalyzing these reactions, toluene dioxygenase (TDO), is remarkable in terms of its ability to produce enantiomerically-pure cyclohexadiene *cis*-diols from a wide range of aromatic substrates [39,40,87,90]. Hudlicky has pointed out that the advantages of arene *cis*-diols lie in the fact that they constitute an 'unusual and potentially useful high functional system for stereospecific organic synthesis' [20]. They can undergo a variety of reactions including asymmetric Diels–Alder reactions, epoxidation, photochemical oxygenation, metallation, diol cleavage, diene cleavage, carbene additions and ozonolysis. These in turn have led to a variety of synthetic products that are not readily obtainable by conventional chemical synthesis. Examples include conduritols, inositol phosphates, pinitol enantiomers, prostanoid and terpene synthons, and complex natural products such as (−)-zeylena and (+)-lycoricidine. Much of this work has been carried out in the laboratories of Hudlicky, Ley, and Carless and has been the subject of several reviews [20,21,49,70,87,101]. The number of *cis*-diols that have been isolated and identified exceeds 170 [50]. Many of these compounds were isolated by Hudlicky and his associates using TDO expressed in *P. putida* F39/D [20,90]. Boyd and his associates conducted extensive studies on a wide range of products formed by a constitutive TDO in *P. putida* UV4 [1,3,12–15,18,72] and some of these have been presented in review form [16]. Other arene *cis*-diols that have potential use in asymmetric synthesis are those formed from aromatic acids [84,101].

In contrast to the arene *cis*-diols and other oxygenated products formed by TDO, relatively little attention has been paid to the substrate specificity of NDO. The potential of

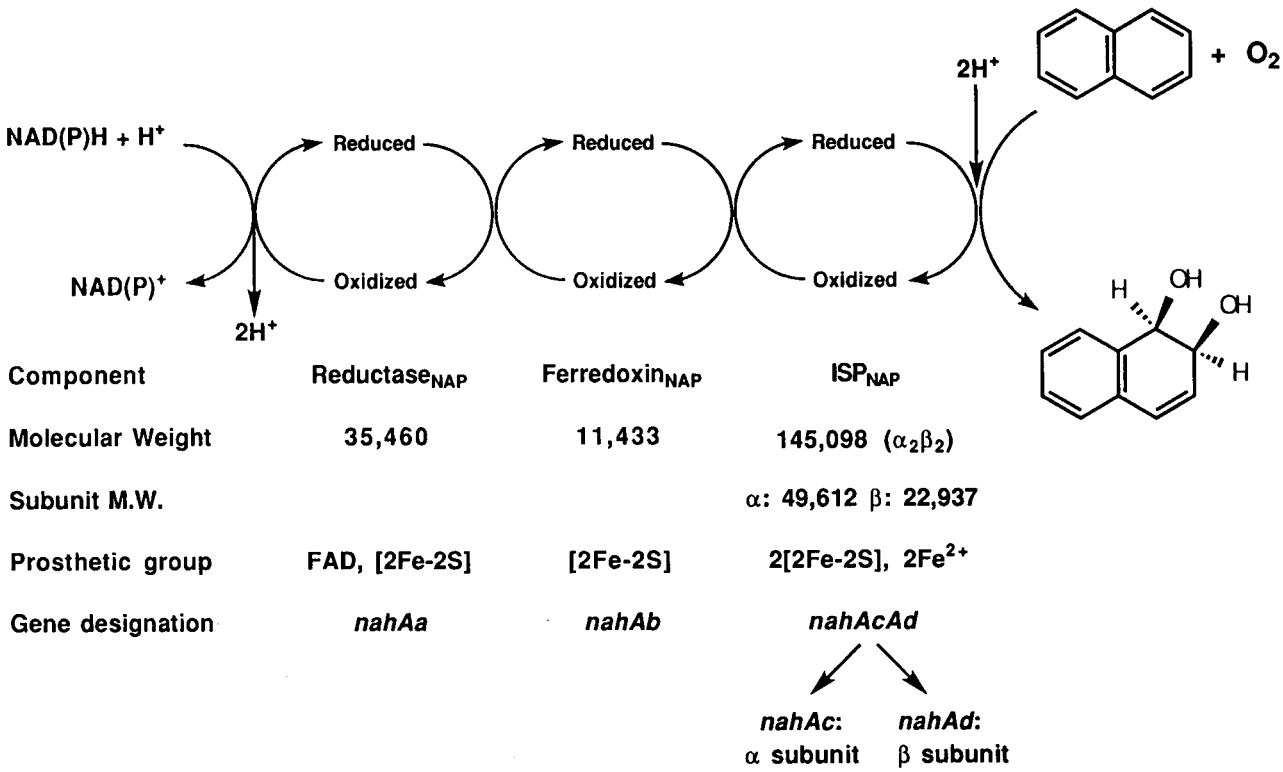


Figure 1 Sequence of electron transfer from NAD(P)H to the oxygenase component (ISP$_{NAP}$) of NDO, resulting in the formation of (+)-*cis*-(1*R*,2*S*)-dihydroxy-1,2-dihydronaphthalene. The individual components reductase$_{NAP}$, ferredoxin$_{NAP}$ and ISP$_{NAP}$ have been purified [30,45,46], and their structural genes have been cloned and sequenced [74,88]. The molecular weights of the protein components are deduced from nucleotide sequence data [74,88].

this enzyme to form products of opposite chirality to those formed by TDO was first noted in 1988 during studies on the oxidation of indan. The major product formed by TDO was (−)-(1*R*)-indanol (84% enantiomeric excess [ee]) whereas NDO produced (+)-(1*S*)-indanol (>92% ee) [98]. Subsequent studies with NDO have revealed further differences in substrate specificity and suggest that this enzyme may be a valuable additional source of new chiral synthons for the enantiospecific synthesis of biologically active products. Although this review focusses primarily on reactions catalyzed by NDO from *Pseudomonas* sp strain 9816-4, recent results on the reactions catalyzed by NDOs from *P. putida* NCIMB 8859 [3,4], *P. putida* G7 [85,100] and *P. fluorescens* TTC1 [9] have also been included in the text and appropriate tables.

Studies on NDO substrate specificity

Our knowledge of the types of reactions catalyzed and the range of substrates oxidized by NDO is based largely on biotransformation studies with *cis*-naphthalene dihydrodiol dehydrogenase (DDH) mutants, recombinant strains expressing NDO and purified NDO components. *Pseudomonas* sp 9816/11 is a DDH mutant of strain 9816-4 [57] which accumulates *cis*-naphthalene-1,2-dihydrodiol when induced cells are incubated with naphthalene and a suitable carbon source [96]. Studies with purified dioxygenase components have been crucial in the identification of reactions catalyzed by NDO in the absence of other host-associated enzyme activities which, through subsequent catalysis, have the potential to affect product distribution and/or stereochemistry [38,44,65,67,83].

Biotransformation products are most easily detected by thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), and/or gas chromatography-mass spectrometry (GC/MS). Preparative TLC, HPLC, column or radial dispersion chromatography are commonly used for the isolation of oxidation products prior to nuclear magnetic resonance (NMR) structural determination and/or stereochemical analysis. Methods for the determination of absolute stereochemistry of mono- and dihydroxylated compounds have included chemical transformation to known compounds [100], X-ray crystallography [59], conversion to diasteromers (ie Mosher's-esters/diesters [11,15]), $[\alpha]_D$ and stereochemical correlation. We recently described the use of chiral boronic acids in the ^{1}H NMR determination of absolute configuration and ee of bacterial and synthetic *cis*-diols [82]. The method is applicable to small sample amounts (<2 mg) and requires no purification of the product prior to NMR analysis of diastereomeric alkyl boronate esters. The above study and many other configurational assignments have been aided by the existing literature reports on the chiroptic properties and absolute stereochemistry of chiral metabolites formed by other dioxygenase systems. These data have facilitated correlation of stereochemistry through rotational properties, chiral HPLC or analysis of diastereomeric esters. The regio- and stereospecificity of oxidations catalyzed by NDO are discussed below; differences in the reactions catalyzed by NDO and TDO are also noted.

Dioxygenation

In addition to the enantiospecific *cis*-dihydroxylation of naphthalene, NDO catalyzes dioxygenation of a variety of multicyclic and heterocyclic aromatic compounds to produce, in many cases, chiral *cis*-dihydrodiols. The use of mutant and recombinant strains expressing NDO can lead to the formation of *cis*-diols as sole products in high yields and high enantiomeric purity. However, depending on the nature of the substrate, the *cis*-diols may be formed in variable yields and are sometimes produced with other diols or monols resulting from oxidation of the substrate at more than one position (relaxed regiospecificity). Substrates which are oxidized to *cis*-dihydrodiols by NDO include indene [3,38], 1,2-dihydronaphthalene [3,96], benzocycloclohept-1-ene [3,80], anthracene [2,54], phenanthrene [54,61], dibenzo[1,4]dioxan [57], acenaphthylene [85], 1- and 2-substituted naphthalenes [9,19,28,58,100], biphenyl (Resnick and Gibson, unpublished), fluorene [78], dibenzofuran [78], dibenzothiophene [78], 9,10-dihydroanthracene and 9,10-dihydrophenanthrene [77]. The structures and yields of these *cis*-diols are shown in Table 1.

It is of interest to note that in the benzocycloalkene series, NDO oxidizes indene, 1,2-dihydronaphthalene (1,2-DHN) and benzocyclohept-1-ene (BCH-1-ene) to (+)-*cis*-(1*R*,2*S*)-indandiol [38], (−)-*cis*-(1*R*,2*S*)-dihydroxy-1,2,3,4-tetrahydronaphthalene [96] and (−)-*cis*-(1*R*,2*S*)-dihydroxybenzocycloheptane [80], respectively as the major enantiomers. In addition to *cis*-dihydroxylation, NDO catalyzed the benzylic monooxygenation of indene (Table 2) and the desaturation of DHN (Table 3). Oxidation of the aromatic nucleus was not observed. However, NDO catalyzes the *cis*-dihydroxylation of (1*R*)-indenol to yield *cis*-1,2,3-indantriol [68]. In the present series, major differences have been noted in the reactions catalyzed by NDO and TDO. For example, TDO catalyzes the *cis*-dihydroxylation of indene, 1,2-DHN and BCH-1-ene to the opposite enantiomers of the *cis*-diols (noted above) and in lower yields [80,96,98]. Other differences include the ability of TDO (but not NDO) to catalyze benzylic monooxygenation of 1,2-DHN and BCH-1-ene [12,80,96].

The ability of a wild-type, naphthalene-utilizing *P. putida* (strain NCIMB 8859) to oxidize the benzocycloalkenes indene, DHN and BCH-1-ene to *cis*-diols of 1*R*,2*S*-configuration has been recently reported [3]. The authors have used the same strain to resolve racemic mixtures of *cis*-diols to yield the 1*S*,2*R*-enantiomers. This procedure is based on the enantiomeric specificity of *cis*-(1*R*,2*S*)-dihydroxy-1,2-dihydronaphthalene dehydrogenase [75] which was used to resolve racemic *cis*-dihydroxy-1,2-dihydronaphthalene into its *cis*-1*S*,2*R*-enantiomer [51].

The oxidation of fluorene by induced cells of strain 9816/11, expressing NDO, yielded a single dihydrodiol as major product which was identified as *cis*-(3*S*,4*R*)-dihydroxy-3,4-dihydrofluorene (Table 1) [78]. Although small amounts of a minor dihydrodiol were detected by NMR and by GC/MS (as phenolic dehydration products), we did not observe the complex mixture of 3,4- and 1,2-dihydrodiols of 9-fluorenol which were also reported as products of fluorene oxidation by NDO encoded by the NAH7 plasmid [85]. *cis*-Dihydroxylation was also the major reaction cata-

Table 1 *cis*-Dihydroxylation reactions catalyzed by NDO

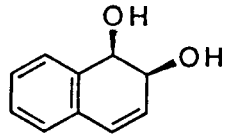
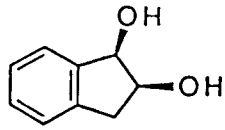
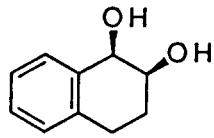
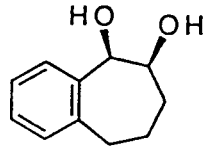
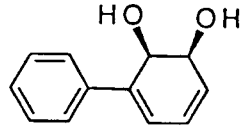
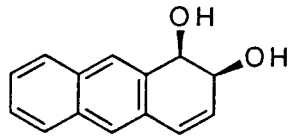
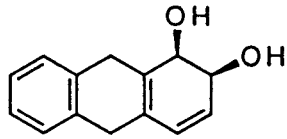
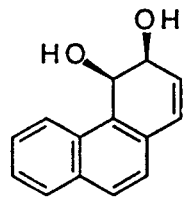
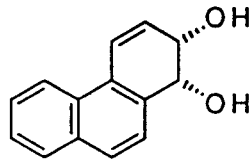
Substrate	Product[a]				Ref
	cis-Dihydrodiol	Stereochemistry	% ee	Relative yield (%)	
Aromatic hydrocarbons:					
Naphthalene		(+)-1*R*,2*S*	>98%	100%	[51,53]
Indene		(+)-1*R*,2*S* (+)-1*R*,2*S*	72–80% 85–90%	53–56% n.d.[b,c]	[38] [3]
1,2-Dihydronaphthalene		(−)-1*R*,2*S* (−)-1*R*,2*S*	>98% >98%	65% n.d.	[96] [3]
Benzocyclohept-1-ene		(−)-1*R*,2*S* (−)-1*R*,2*S*	>98% >98%	50% n.d.	[80] [3]
Biphenyl		(+)-2*R*,3*S*	>98%	100%	a.r.[d]
Anthracene		(+)-1*R*,2*S*	>98%	100%	[2,54]
9,10-Dihydroanthracene		(+)-1*R*,2*S*	>98%[e]	>95%	[77]
Phenanthrene		(+)-3*S*,4*R*	high	>90%	[54,61]
		1*R*,2*S*	high	<10%	[54,61]

Table 1 Continued

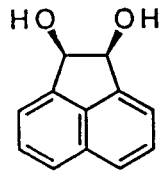
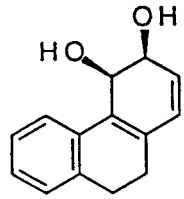
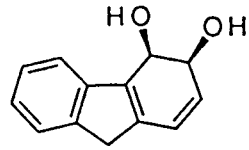
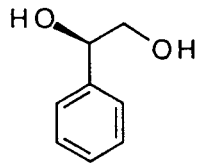
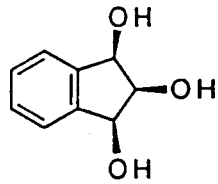
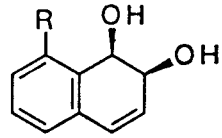
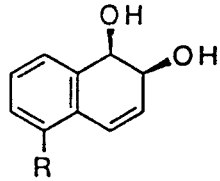
Substrate	Product[a]				Ref
	cis-Dihydrodiol	Stereochemistry	% ee	Relative yield (%)	
Acenaphthylene		*cis*-1,2	n.a.	>70%	[85]
9,10-Dihydrophenanthrene		3*S*,4*R*	>95%[e]	~70%	[77]
Fluorene		3*S*,4*R*	>95%[e]	>85%	[78]
Substituted aromatic hydrocarbons:					
Styrene (Vinylbenzene)		(−)-1*R*	>79%	100%	[66,69]
(1*R*)-Indenol		*cis*-1,2,3	n.a.	100%	[68]
1-substituted naphthalenes	 R = CH_3, CH_2CH_3, OCH_3, F, Cl, Br, $COOCH_3$	(+)-1*R*,2*S*	>95%	variable	[9]
	 R = OCH_3, $COOCH_3$, F, Cl, Br	1*R*,2*S*	>95%	variable	[9]

Table 1 Continued

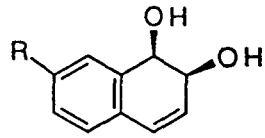
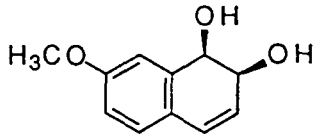
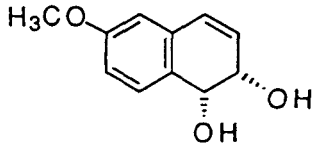
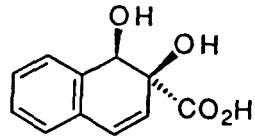
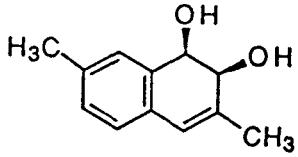
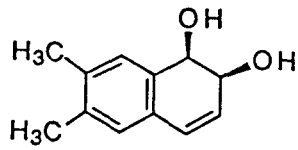
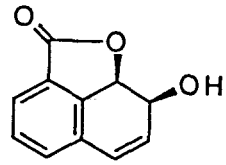
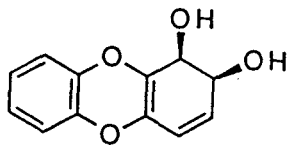
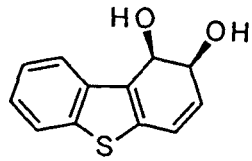
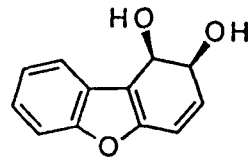
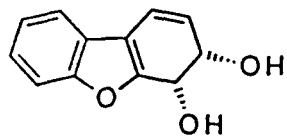
Substrate	Product[a]				Ref
	cis-Dihydrodiol	Stereochemistry	% ee	Relative yield (%)	
2-substituted naphthalenes	 R= CH_3, CH_2CH_3, Cl, Br, $COOCH_3$, NO_2	(+)-1*R*,2*S*	>95%	100%	[9,28]
2-Methoxynaphthalene		(+)-1*R*,2*S*-7-methoxy	>98%	93%	[100]
		(+)-1*R*,2*S*-6-methoxy	>98%	7%	[100]
2-Naphthoic acid		(+)-*cis*-1,2	n.d.	100%	[19,58]
2,6-Dimethylnaphthalene		(+)-*cis*-1*R*,2*S*	>95%	n.d.	[9]
2,3-Dimethylnaphthalene		(+)-*cis*-1*R*,2*S*	>95%	100%	[9]
1-Carbomethoxynaphthalene		(+)-*cis*-1*R*,2*S*	>95%	100%	[9]
Heterocyclic aromatic compounds:					
Dibenzo-1,4-dioxin		*cis*-1,2	n.d.	100%	[57]

Table 1 Continued

Substrate	Product[a]				Ref
	cis-Dihydrodiol	Stereochemistry	% ee	Relative yield (%)	
Dibenzothiophene		(+)-1*R*,2*S*	>95%[e]	86%	[78]
Dibenzofuran		1*R*,2*S*	>95%[e]	60–70%	[78]
		3*S*,4*R*	>95%[e]	30–40%	[78]

[a]Relative yields indicate the amount of the product(s) shown as a relative percentage of all products detected. ee, enantiomeric excess.
[b]n.d., not determined or not reported. n.a., not applicable.
[c]Results of Allen *et al* [3] were obtained with a wild-type naphthalene-utilizing strain (NCIMB 8859). In the indene biotransformation, the higher ee of indandiol was observed after shorter incubation time and 1-indenol was also observed.
[d]a.r., authors' results; Resnick and Gibson, unpublished.
[e]Absolute stereochemistry and ee determined by ^{1}H NMR analysis of diastereomeric esters prepared with (–)-*S*- and (+)-*R*-2-(1-methoxyethyl)-phenyl boronic acids (MPBA) [82].

lyzed by strain 9816/11 with the heterocyclic aromatic compounds dibenzothiophene and dibenzofuran [78]. However, differences were observed in the regiospecificity of NDO with these two related substrates. Dibenzothiophene was oxidized to the 1*R*,2*S*-dihydrodiol as the major diol while dibenzofuran was oxidized to two *cis*-dihydrodiols of 1*R*,2*S*- and 3*S*,4*R*-configuration in approximately a 7 : 3 ratio, respectively (Table 1). Sulfoxidation of dibenzothiophene led to the formation of dibenzothiophene sulfoxide as a minor product (see sulfoxidation below). Both the (+)-*cis*-1,2-dibenzothiophene dihydrodiol and the dibenzothiophene sulfoxide have been identified as products of dibenzothiophene oxidation by the biphenyl-oxidizing *Sphingomonas yanoikuyae* strain B8/36 [56,63].

A number of aromatic and heterocyclic aromatic compounds may undergo dihydroxylation reactions by NDO but do not yield isolable *cis*-diols. In these cases, the initial *cis*-dihydrodiol metabolites are thought to be unstable and undergo spontaneous loss of water to yield the corresponding phenol(s). The formation of phenols as dehydration products has also been observed for some of the *cis*-dihydrodiols formed from dibenzofuran, fluorene, 2-methoxynaphthalene and other related bicyclic aromatics [6,57,95,100]. Substrates for NDO where *cis*-diols have not been detected include indole and 9*H*-carbazole which are oxidized to indigo and 3-hydroxycarbazole, respectively [32,81]. The formation of indigo probably occurs through the spontaneous condensation (air oxidation) of indoxyl, the major dehydration product that would be expected from '*cis*-indole-2,3-dihydrodiol' [32]. The bioconversion of indole to indigo has far-reaching applications in that: i) formation of the insoluble blue dye has provided a screen for dioxygenase expression in molecular biology, facilitating the cloning of many other oxygenases which also catalyze the reaction; and ii) biological synthesis from renewable resources (ie glucose) offers an alternative route for the commercial production of an important dye that is presently derived from petrochemical resources [73].

The oxidation of styrene to (–)-(1*R*)-phenyl-1,2-ethanediol by NDO [66] differs from that of TDO in which the major reaction observed is *cis*-dihydroxylation of the aromatic ring [47]. This oxidative attack is consistent with several other reactions, discussed below, which result from the oxidation of substituents on substrates that contain a single aromatic ring (alkyl- and *O*-, *S*- and *N*-alkyl substituted benzenes).

In cases where their absolute stereochemistry has been determined, the *cis*-diols formed by NDO (Table 1) have the same absolute configuration as (+)-*cis*-(1*R*,2*S*)-dihydroxy-1,2-dihydronaphthalene. To date, all chiral *cis*-1,2-diols formed by NDO are of *R*-configuration at the hydroxyl-bearing (benzylic) carbon adjacent the bridgehead carbon.

Monooxygenation

In addition to *cis*-dihydroxylation, NDO catalyzes the benzylic monooxygenation of a number of benzocyclic and

Table 2 Monohydroxylation reactions catalyzed by NDO

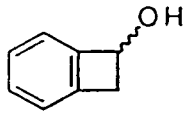
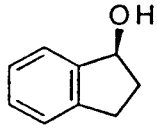
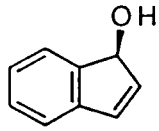
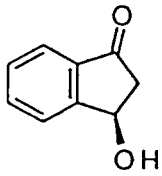
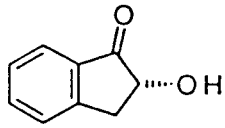
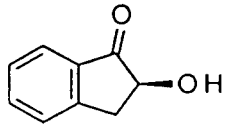
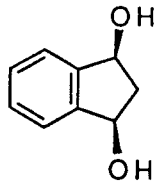
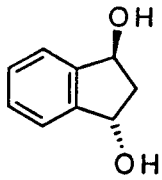
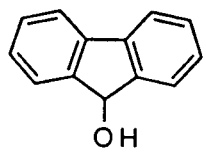
Substrate	Product[a]				Ref
	Alcohol	Stereochemistry	% ee	Relative yield (%)	
Benzocyclobutene		Racemic	0%	100%	[95], a.r.[b]
Indan		(+)-1*S*	58–92%	54–67%	[38,98]
Indene		(+)-1*S*	62–88%	42–58%	[38]
1-Indanone		(−)-3*R*	56–62%	90%	[83]
		(−)-2*R*	2–22%[c]	10%	[83]
2-Indanone		(+)-2*S*	6–76%[c]	100%	[83]
(1*R*)-Indanol		*cis*-1*R*,3*S*	>95%	>70%	[68]
(1*S*)-Indanol		*trans*-1*S*,3*S*	>95%	>85%	[68]
Fluorene		n.a.[d]	n.a.	n.d.[c] ~15%	[85] [78]

Table 2 Continued

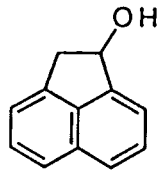
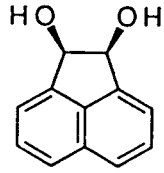
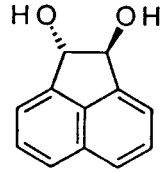
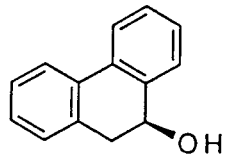
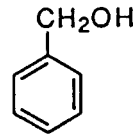
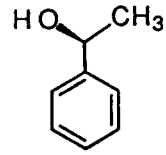
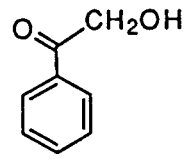
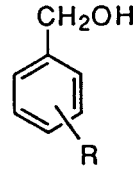
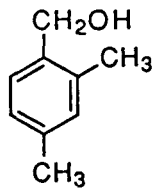
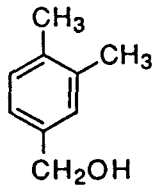
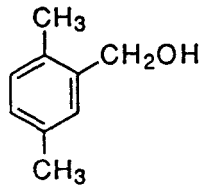
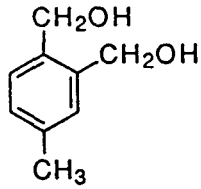
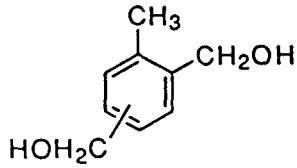
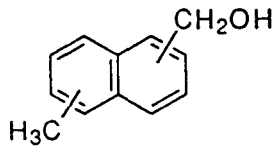
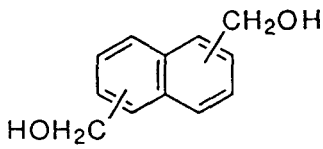
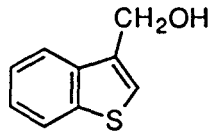
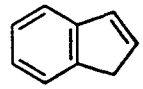
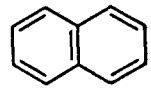
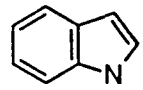
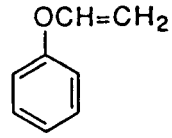
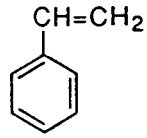
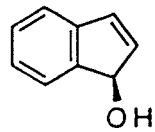
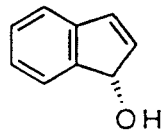
Substrate	Product[a]				Ref
	Alcohol	Stereochemistry	% ee	Relative yield (%)	
Acenaphthene		n.d.	n.d.	n.d.	[85]
Acenaphthen-1-ol		*cis*-1,2	n.a.	~15%	[85]
		*trans*-1,2	n.d.	~15%	[85]
9,10-Dihydrophenanthrene		(+)-9*S*	>98%	~30%	[77]
Toluene		n.a.	n.a.	>90%	[67,69]
Ethylbenzene		(−)-1*S*	77%	>74%	[67]
Acetophenone		n.a.	n.a.	100%	[67]
o-, *m*-, *p*-Xylene (R=CH_3) *o*-, *m*- *p*-Nitrotoluene (R=NO_2)		n.a.	n.a.	>90%	[67]

Table 2 Continued

Substrate	Product[a]				Ref
	Alcohol	Stereochemistry	% ee	Relative yield (%)	
1,2,4-Trimethylbenzene		n.a.	n.a	28%	[85]
		n.a.	n.a.	24%	[85]
		n.a.	n.a.	20%	[85]
		n.a.	n.a.	9%	[85]
		n.a.	n.a.	2%	[85]
1,5-, 2,6-, 2,7- and 1,8-Dimethylnaphthalene		n.a.	n.a.	17–75%	[85,9]
		n.a.	n.a.	<15%	[85,9]
3-Methylbenzothiophene		n.a.	n.a.	35%	[85]

[a]Relative yields indicate the amount of the product shown as a relative percentage of all products detected. ee, enantiomeric excess.
[b]a.r., authors' results; Resnick and Gibson, unpublished.
[c]The higher ee of (2*S*)-hydroxy-1-indanone was observed in reactions catalyzed by purified NDO.
[d]n.a., not applicable.
[e]n.d., not determined or not reported.

Table 3 Desaturation reactions catalyzed by NDO

Substrate	Initial desaturation product		Ref
	Structure	Relative yield (%)[a]	
Indan (1,2-dihydroindene)		<3%[b]	[38]
1,2-Dihydronaphthalene		<5%[b]	[96]
Indoline (2,3-Dihydroindole)		n.d.[b,c]	[44]
Phenetole (Ethoxybenzene)		42%	[79]
Ethylbenzene		<1%[b]	[67]
(1*R*)-Indanol		<2%[b]	[68]
(1*S*)-Indanol		<1%[b]	[68]

[a]Relative yields indicate the amount of the product shown as a relative percentage of all products detected. Several products of desaturation were further oxidized by NDO.
[b]Indene, naphthalene, styrene, (1*R*)-indenol and (1*S*)-indenol formed via desaturation were observed as transient products since they serve as substrates for monooxygenation and/or dioxygenation. Indole was subsequently oxidized to indigo.
[c]n.d., not determined.

alkyl-substituted aromatic compounds. In many cases, the benzylic hydrogen atoms are prochiral. The products formed from members of a series of benzocyclic aromatic compounds have contributed to the current understanding of the regio- and stereospecificity of reactions catalyzed by NDO. Each substrate in the series contains an aromatic ring fused with either a cycloalkane or cycloalkene ring and therefore offers a number of potential positions for oxygenase attack. The effects of increasing the size of the cycloalkane ring and the presence or absence of a double bond have also been examined. Some of the trends and differences relating to NDO-catalyzed monooxygenation of members of the series are shown in Table 2 and discussed below.

The oxidation of benzocyclobutene by a naphthalene-oxidizing pseudomonad led to the formation of (±)-1-hydroxybenzocyclobutene suggesting that NDO catalyzed the benzylic monooxygenation reaction [95]. No dioxygenation of the aromatic ring was observed and the involvement of NDO in the formation of the racemic alcohol has been confirmed using a recombinant *E. coli* strain expressing NDO (Resnick and Gibson, unpublished). The reaction catalyzed by NDO differs from those of TDO from strains UV4 and PpF1 which both catalyze dihydroxylation of the aromatic ring as well as monohydroxylation of the cycloalkane ring ([18]; Resnick and Gibson, unpublished).

The stereospecific oxidation of indan and indene has been examined with mutant and recombinant strains expressing NDO and with purified NDO [38,98]. These studies showed that NDO oxidizes indan to (+)-(1*S*)-indanol as the major product (Table 2). Other products formed by NDO from indan included indene, (+)-*cis*-(1*R*,2*S*)-indandiol (Table 1) and (+)-(1*S*)-indenol resulting from desaturation, dioxygenation and monooxygenation of indan or its products [38]. NDO catalyzed the benzylic monooxygenation of indene to (+)-(1*S*)-indenol (Table 2) and also

dioxygenation to yield (+)-*cis*-(1*R*,2*S*)-indandiol as mentioned in the previous section.

The results obtained when the cycloalkane ring size is increased by one methylene unit (ie benzocyclobutene to indan/indene) help to define the size requirement for the facial selectivity which results in the stereospecific oxidation of the prochiral benzocyclopentane and benzocyclopentene substrates to their corresponding alcohols of *S*-configuration. It is of interest in the present discussion to note that benzocyclic aromatic compounds with cycloalkene rings greater than five carbons are not substrates for benzylic monooxygenation by NDO. For example, 1,2-DHN and BCH-1-ene were clearly not substrates for benzylic monooxygenation but have instead yielded products via desaturation and/or dioxygenation (discussed in these sections). 1,2,3,4-Tetrahydronaphthalene was not oxidized efficiently by strains expressing NDO (Resnick and Gibson, unpublished). In contrast to the benzylic hydroxylation reactions catalyzed by NDO, similar studies with TDOs have yielded secondary alcohols of *R*-configuration from indan, 1,2-DHN, BCH-1-ene, and several heterocyclic analogues [13,16,18,80,96,98].

Benzylic monooxygenation was observed in the NDO-catalyzed oxidation of 1- and 2-indanone and (1*S*)- and (1*R*)-indanol. Induced cells of strains 9816/11 and purified NDO catalyzed the oxidation of 1-indanone to (−)-(3*R*)-hydroxy-1-indanone as the major product and (2*R*)-hydroxy-1-indanone as the minor product [83]. Studies with purified NDO in the presence of an [^{18}O]-oxygen atmosphere confirmed the incorporation of one atom of molecular oxygen into these benzocyclic ketones. The (3*R*)-hydroxy-1-indanone was formed via benzylic monooxygenation in greater yield and enantiomeric purity than the (2*R*)-hydroxy-1-indanone (Table 2). The absolute stereochemistry of the hydroxylated products was also opposite to that observed in the oxidation of indan and indene by NDO; TDO did not oxidize 1-indanone. The oxidation of 2-indanone by purified NDO (and TDO) yielded (+)-(2*S*)-hydroxy-1-indanone as the major product [83]. The formation of this product in relatively high enantiomeric purity suggests that it may arise via benzylic monooxygenation through 1-hydroxy-2-indanone which rapidly tautomerizes in the active site to the observed chiral product. In contrast to the stereospecificity observed in the oxidation of 1- and 2-indanone, purified NDO and recombinant strains expressing the enzyme catalyze the stereospecific benzylic

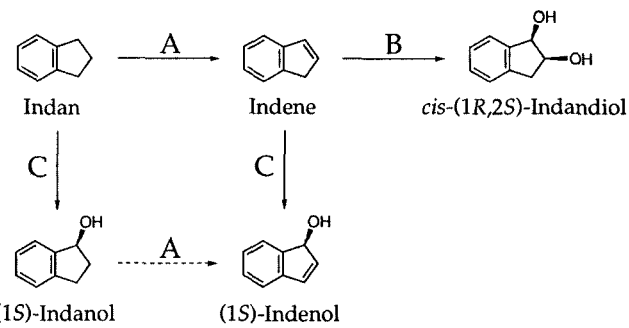


Figure 2 Major products formed from indan by NDO. Reactions: A, desaturation; B, *cis*-dihydroxylation; C, monooxygenation. The dashed line indicates a minor reaction. Relative yields are given in the text [38].

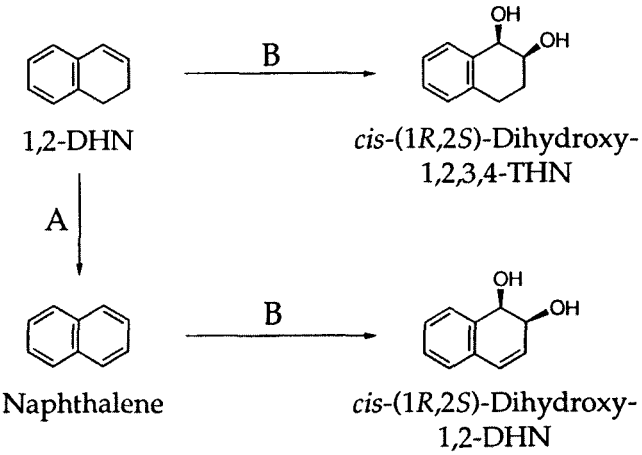


Figure 3 Products formed from 1,2-dihydronaphthalene (DHN) by NDO. Reactions: A, desaturation; B, *cis*-dihydroxylation. The reaction sequences shown are supported by NMR studies of products formed from deuterium-labeled substrate [96].

monooxygenation of (1*S*)-indanol and (1*R*)-indanol to *trans*-(1*S*,3*S*)-indandiol and *cis*-(1*R*,3*S*)-indandiol, respectively (Table 2) [68]. The latter reactions are catalyzed with a predominant facial selectivity, as observed for the oxidation of indan and indene by NDO, in which the benzylic pro-*S* hydrogen is oxidized.

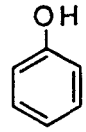
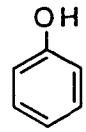
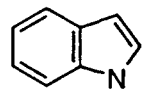
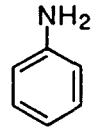
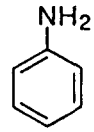
Recent studies showed that purified NDO catalyzes the benzylic monooxygenation of toluene to benzyl alcohol, and ethylbenzene to (−)-(*S*)-1-phenethyl alcohol, acetophenone and 2-hydroxyacetophenone (Table 2) [67,69]. In addition, NDO oxidizes the methyl groups of nitrotoluenes and xylenes to their corresponding benzyl alcohols (Table 2) [67]. NDO from the NAH7 plasmid expressed in *P. aeruginosa* PAO1 has recently been shown to catalyze esssentially non-(regio)specific methyl group oxidation of 1,2,4-trimethylbenzene and several dimethylnaphthalenes (Table 2) [85].

Desaturation

The term desaturation is used here to describe reactions involving an oxygen-dependent net abstraction of two hydrogen atoms resulting in the formation of a double bond. Chemically, desaturation is indistinguishable from dehydrogenation (both are two-electron oxidations), however we use the former term to describe the enzymatic reaction since the latter term is more often associated with NAD(P)$^+$-dependent processes. NDO-catalyzed oxygen-dependent desaturation reactions have been demonstrated for benzocyclic, heterocyclic and alkyl-substituted aromatic compounds (Table 3).

The ability of NDO to catalyze desaturation was first observed in the transformation of indan and indoline by purified NDO components [44]. Recent studies with mutant and recombinant strains expressing NDO, as well as purified NDO, have confirmed these initial observations [38]. Since indene is also a substrate for NDO (see above), it was further oxidized to products shown in Figure 2. Thus NDO catalyzes the enantiospecific monooxygenation of indan to (+)-(1*S*)-indanol and the desaturation of indan to

Table 4 *O*- and *N*-Dealkylation reactions catalyzed by NDO

Substrate	Dealkylation product		Ref
	Structure	Relative yield (%)[a]	
Anisole (Methoxybenzene)		100%	[79]
Phenetole (Ethoxybenzene)		58%	[79]
N-Methylindole		n.d.[b]	a.r.[c]
N-Methylaniline		100%	[64]
N,N-Dimethylaniline		100%	[64]

[a]Relative yields indicate the amount of the product shown as a relative percentage of all products detected.
[b]n.d., not determined. Indole was detectable by GC/MS but was subsequently oxidized to indigo.
[c]a.r., authors' result; Torok, Resnick and Gibson, unpublished.

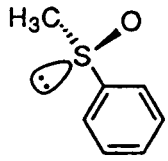
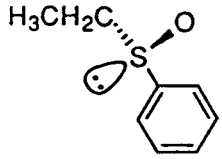
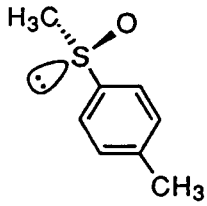
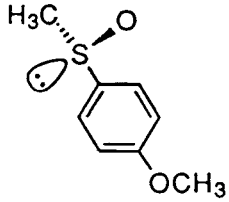
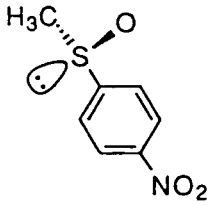
indene, which then serves as a substrate for the formation of (+)-*cis*-(1*R*,2*S*)-indandiol and (+)-(1*S*)-indenol. The yields of products formed directly or indirectly by desaturation of indan (ie indene, 1-indenol, *cis*-1,2-indandiol) ranged from 15 to 37% overall yield in reactions containing mutant and recombinant strains expressing NDO, and with purified NDO components. The stereochemistry and relative yields of *cis*-1,2-indandiol and 1-indenol formed from indan were essentially identical to the same products formed from indene indicating that their formation involved desaturation of indan to indene which then undergoes mono- and dioxygenation reactions [38].

Elucidation of the sequence of reactions involved in the oxidation of benzocyclic aromatic compounds by NDO was obtained through deuterium (^{2}H)-NMR studies with [4-^{2}H]-1,2-dihydronaphthalene (DHN). The results demonstrated that NDO catalyzed the desaturation of DHN to produce naphthalene, which serves as a substrate for dioxygenation to yield (+)-*cis*-(1*R*,2*S*)-dihydroxy-1,2-dihydronaphthalene (*cis*-naphthalene dihydrodiol) [96]. The finding that the *cis*-dihydrodiol contained equal amounts of deuterium label at positions 1, 4, 5 and 8 indicated that the reaction sequence involved desaturation of DHN followed by *cis*-dihydroxylation of naphthalene to the *cis*-dihydrodiol (Figure 3). These results are in accord with the suggestion made by Boyd *et al* [1,16] that TDO-catalyzed oxidation of DHN could involve formation of a benzyl radical followed by a second hydrogen abstraction to yield naphthalene which then undergoes *cis*-dihydroxylation. The equal distribution of deuterium label in *cis*-naphthalene dihydrodiol also eliminated its formation through reaction sequences involving: i) *cis*-dihydroxylation of DHN and desaturation of the resulting *cis*-1,2-dihydroxy-1,2,3,4-tetrahydronaphthalene; and/or ii) benzylic monooxygenation of DHN to 1-hydroxy-1,2-dihydronaphthalene followed by *cis*-dihydroxylation and subsequent dehydration of the putative triol [13]. The latter sequence involving *cis*-naphthalene dihydrodiol formation from DHN through a triol intermediate has been reported to occur with *P. putida* strain UV4 expressing TDO [1,13,17], whereas strains expressing TDO from PpF1 appear to oxidize DHN by a sequence similar to that described above for NDO [96].

The effect of increasing the benzocycloalkene ring size has been determined by examining the oxidation of benzocyclohept-1-ene (BCH-1-ene) by strains expressing NDO. In contrast to results obtained with DHN where desaturation followed by dioxygenation accounted for up to 30% of the yield of isolable products [96], NDO did not catalyze desaturation of BCH-1-ene at significant levels and the main reaction observed was *cis*-dihydroxylation [80].

NDO has also been shown to catalyze desaturation of phenetole (ethoxybenzene)[79] and ethylbenzene [67]. Cells of a mutant pseudomonad and recombinant *E. coli* expressing NDO catalyzed the desaturation of phenetole, in

Table 5 Sulfoxidation reactions catalyzed by NDO

Substrate	Product[a]				Ref
	Sulfoxide	Stereochemistry	% ee	Relative yield (%)	
Methyl phenyl sulfide		*S* *S*	>98% 91%	100% n.d.[b,c]	[65] [4]
Ethyl phenyl sulfide		*S* *S*	93% 84%	100% n.d.	[65] [4]
Methyl *p*-tolyl sulfide		*S*	>98%	100%	[65]
p-Methoxyphenyl methyl sulfide		*S*	>98%	100%	[65]
Methyl *p*-nitrophenyl sulfide		*S*	>98%	100%	[65]

continued on next page

low yields, to a compound identified as ethenyloxybenzene (phenol was also identified and is presumably formed via *O*-dealkylation [79]). In addition to the benzylic monooxygenation of ethylbenzene (mentioned above), purified NDO catalyzed the desaturation of ethylbenzene to yield styrene (Table 3) which was further oxidized to (1*R*)-phenyl-1,2-ethanediol (Table 1) [67].

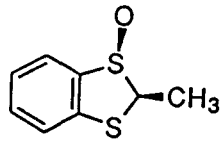
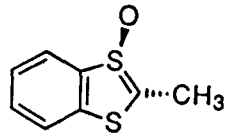
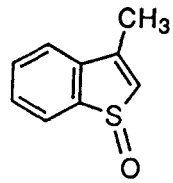
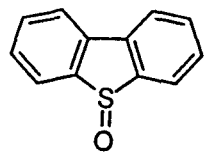
O- and *N*-Dealkylation

A study with mutant and recombinant strains expressing NDO showed that the enzyme was capable of catalyzing *O*-dealkylation reactions (Table 4). These strains catalyzed the *O*-demethylation of anisole and the *O*-deethylation of phenetole to yield phenol in both cases [79]. In addition to *O*-deethylation, NDO also catalyzed the desaturation of phenetole (Table 3). Strains expressing NDO did not oxidize the aromatic ring of anisole or phenetole, suggesting that the alkoxy-substituents were in close proximity to the reactive oxygen species at the enzyme active site. *O*-Dealkylation reactions involve hydroxylation of the alkyl moiety followed by non-enzymatic breakdown of the resulting unstable hemi-acetal intermediate to yield phenol and the corresponding aldehyde [8,26]. In addition to *O*-dealkylation of alkyl aryl ethers, NDO also catalyzes the *N*-dealkylation of both *N*-methylaniline and *N*,*N*-dimethylaniline to aniline [64] and *N*-methylindole to indole (Torok, Resnick and Gibson, unpublished) (Table 4).

Sulfoxidation

Recent studies have shown that purified NDO catalyzes the stereospecific sulfoxidation of aryl alkyl sulfides to yield sulfoxides of *S*-absolute configuration. Thus, NDO oxidized

Table 5 Continued

Substrate	Product[a]				Ref
	Sulfoxide	Stereochemistry	% ee	Relative yield (%)	
2-Methylbenzo-1,3-dithiole		*cis*-1*R*,2*S*	82%	n.d.	[4]
		*trans*-1*R*,2*R*	38%	n.d.	[4]
3-Methylbenzothiophene		n.d.	n.d.	45%	[85]
Dibenzothiophene		n.a.[d]	n.a.	14%[e]	[78]

[a]Relative yields indicate the amount of the product shown as a relative percentage of all products detected. ee, enantiomeric excess.
[b]n.d., not determined or not reported.
[c]Results of Allen *et al* [4] were obtained with a wild-type naphthalene-utilizing strain (NCIMB 8859).
[d]n.a., not applicable.
[e]The isolated yield is reported for dibenzothiophene sulfoxide.

methyl phenyl-, ethyl phenyl-, methyl *p*-tolyl-, *p*-methoxyphenyl methyl-, and methyl *p*-nitrophenyl sulfide to their corresponding *S*-sulfoxides; all the sulfoxides were obtained as the sole products and were of high enantiomeric purity [65] (Table 5). Naphthalene grown cells of *P. putida* strain NCIMB 8859 also form enantiopure *S*-sulfoxides from methyl phenyl sulfide and ethyl phenyl sulfide, suggesting that an oxygenase similar to NDO from *Pseudomonas* sp strain 9816-4 may be responsible for catalysis [4]. Strain NCIMB 8859 also catalyzed the sulfoxidation of 2-methylbenzo-1,3-dithiole to yield both *cis*-(1*R*,2*S*)- and *trans*-(1*R*,2*R*)-sulfoxide isomers of opposite absolute configuration to those formed by the TDO-containing *P. putida* strain UV4 [4,22]. Sulfoxidation of alkyl aryl sulfides by *P. putida* strain UV4 yielded the corresponding *R*-sulfoxides in high ee but in varying yields [4]. Thus, the *S*-sulfoxides formed by NDO were the opposite enantiomers to those formed by the TDO-catalyzed oxidation of either methyl phenyl- or ethyl phenyl-sulfides [4,65]. In contrast, TDO oxidized *para*-substituted methyl phenyl sulfides to *S*-sufoxides but in lower enantiomeric purity and in lower yields than those obtained with NDO [4,65].

The above results suggest that the prochiral aryl alkyl sulfides may occupy a similar position in the NDO active site as the physiological substrate, naphthalene. The high stereoselectivity resulting in the NDO-catalyzed formation of homochiral *S*-sulfoxides is consistent with oxygenation of the pro-*S* lone pair of electrons on the sulfide [65] (Table 5).

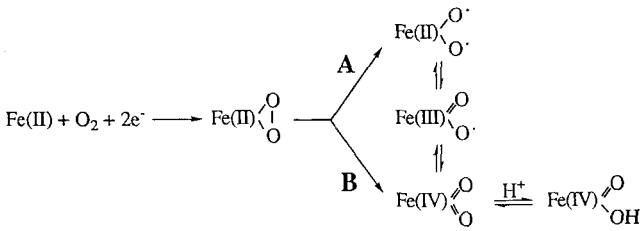
Induced cells of *Pseudomonas* sp 9816/11 oxidized dibenzothiophene to dibenzothiophene sulfoxide (Table 5) [78]. The major product identified was the *cis*-1*R*,2*S*-dihydrodiol (Table 1). The involvement of NDO in both the sulfoxidation and *cis*-dihydroxylation reactions has been confirmed with purified NDO components [78]. NDO encoded by the NAH7 plasmid did not catalyze the sulfoxidation of DBT [85]. The reason for the differences in the reported catabolic activities of NDOs from the NAH7 plasmid and *Pseudomonas* sp 9816-4 is not known at this time.

Oxygen activation

The mechanism of oxygen activation and substrate oxidation has been studied in detail for cytochrome P-450 [42,43], methane monooxygenase [41,71] and other systems involving iron–oxygen interactions [34,76]. There is little experimental evidence however, to support a mechanism for mononuclear iron oxygenases such as NDO. This is partly due to the fact that current spectroscopic techniques are not particularly informative in probing Fe(II)

catalytic centers [76]. The two enzymes that have received the most attention are phthalate dioxygenase [5,35] and 4-methoxybenzoate monooxygenase [99]. Both contain Rieske [2Fe-2S] electron storage centers and oxygen activation occurs at mononuclear iron. Bernhardt and his colleagues have proposed a mesomeric iron peroxo $[FeO_2]^+$ species as the active oxygenase component in 4-methoxybenzoate monooxygenase [99] whereas Ballou and Batie suggested a $[FeO_2H]^+$ intermediate for phthalate 4,5-dioxygenase [5]. Both of the above examples fulfill the requirement for oxygen activation. That is, the binding and reduction of oxygen by an electron-rich species to form an intermediate at the peroxide oxidation level. Oxygen transfer or cleavage could then yield different reaction products depending on the substrate.

In the case of cytochrome P-450 a considerable body of literature suggests that a ferric peroxo species $[FeO_2]^+$, undergoes heterolytic cleavage to yield a high valent iron-oxo, Fe(V)=O, intermediate which can account for the diverse range of reactions catalyzed by this enzyme [42,43]. Any mechanism proposed for NDO must account for the enantiospecific *cis*-dihydroxylation of π bonds as well as monohydroxylation, desaturation, sulfoxidation, *N*- and *O*-dealkylation reactions all of which, with the exception of *c*is-dihydroxylation, are also reactions catalyzed by cytochrome P-450. An analogous reaction to that proposed in the cytochrome P-450 cycle could yield a ferrous peroxo intermediate which could adopt an end-on or side-on configuration. Many different reactive oxygen species can be derived from either of these putative intermediates. For example, homolytic (A) or heterolytic (B) cleavage of a side-on peroxo intermediate would yield the species shown below.



Both cleavage mechanisms would yield intermediate species capable of catalyzing the enantiospecific *cis*-dihydroxylation of naphthalene and it is of interest to note that, as mentioned above, the O=Fe(IV)—OH species has been proposed as an intermediate in *cis*-dihydrodiol formation by phthalate 4,5-dixoygenase [5]. The species shown above are at a high enough oxidation level to account for the diverse cytochrome P-450-type reactions catalyzed by NDO. Of the proposed peroxo species, the dioxo-Fe(IV) form resembles the classical chemical catalysts, potassium permanganate ($KMnO_4$) and osmium tetroxide (OsO_4), which are used in the preparation of *cis*-diols. Further speculation is not warranted at this time and the proposed active oxygen species illustrate the need for detailed kinetic and spectroscopic studies to clarify the mechanism of action of multicomponent oxygenases that utilize mononuclear iron at their active sites. More than 20 of these enzymes have been identified either by protein purification and/or sequence analysis. They have been classified according to the number of protein components and the properties of their redox centers [7]. In most cases, the substrate specificity of these enzymes has not been examined in detail. The results presented in this short review suggest that these oxygenases may represent a new source of chiral intermediates for the synthesis of biologically active compounds of commercial interest.

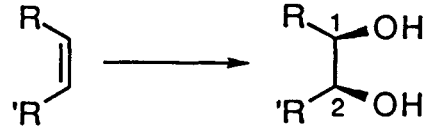
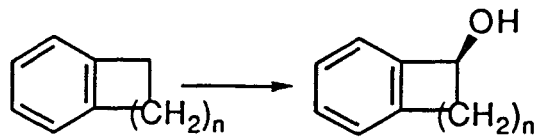
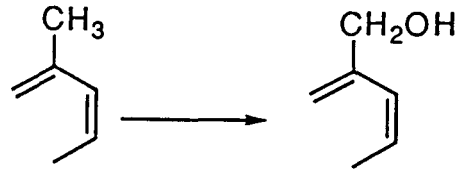
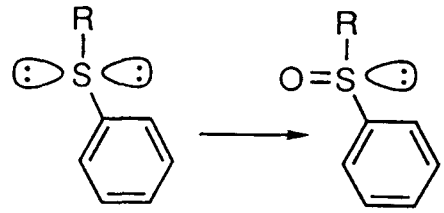
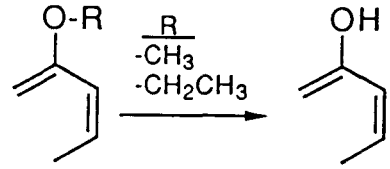
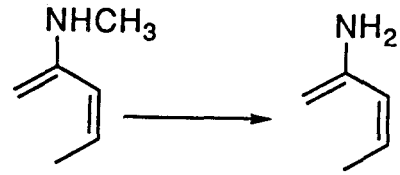
Conclusions

The ability of bacterial dioxygenases, such as NDO, to hydroxylate a wide range of aromatic hydrocarbons and related molecules has important implications for two different areas of scientific endeavor. These are bioremediation and the production of chiral precursors for the synthesis of specialty chemicals. For example, most of the products formed by NDO from the substrates shown in Tables 1–5 do not serve as growth substrates for *Pseudomonas* sp 9816-4. They could however, serve as sources of carbon and energy for many of the organisms present in soil and other environments. Studies on the structure and mechanism of action of NDO and related enzymes could lead to the development of new catalytic capabilities for the oxidation of compounds of environmental concern. Such studies provide an important aspect of the scientific basis for the development of bioremediation technology.

There is considerable interest in the use of chiral cyclohexadiene diols as precursors for the enantiospecific synthesis of natural and biologically active products. Most of the work to date has been conducted with the diverse range of diols formed by TDO [20,21,87,101]. 1-Chloro-(2*S*,3*S*)-dihydroxycyclohexa-4,6-diene (*cis*-chlorobenzene dihydrodiol) [104], first used to synthesize both enantiomers of erythrose [48], has proved to be an extremely versatile chiral synthon [50]. Recent reviews have documented the use of *cis*-chlorobenzene dihydrodiol in the synthesis of chiral products [20,49]. The results obtained with NDO extend the range and number of chiral diols (Table 1) that may find use in organic synthesis. In addition, NDO and TDO sometimes form opposite enantiomers of the same products when both enzymes oxidize the same substrates. The example mentioned previously of (1*S*)- and (1*R*)-indanol formed from indan by NDO and TDO, respectively, illustrates the possible role of these enzymes in generating both stereoisomers of chiral oxidation products. Other examples can be seen in Tables 1, 2 and 5. Thus, NDO has the potential to produce various oxygenated metabolites of the opposite stereochemistry to the enantiomers formed by TDO and, in addition, may prove useful in the preparation of synthons from polycyclic ring systems which are not favored by TDO. Biocatalytic asymmetric *cis*-dihydroxylation by NDO (or TDO [3]) may in some cases provide a practical alternative to osmium-assisted chemical asymmetric dihydroxylation methods which are not applicable to unsubstituted arenes and lack desired stereospecificity with *cis*-olefins [60].

Table 6 lists the different reactions catalyzed by NDO from *Pseudomonas* sp 9816-4. The observed trends in the stereochemical oxidations catalyzed by NDO with aromatic

Table 6 Summary of reactions catalyzed by NDO

Reaction type		Substrate	Product	Product stereochemistry
1.	Aromatic and olefin *cis*-dihydroxylation			1*R*,2*S*
2.	Benzylic hydroxylation			*S*[a]
3.	Methyl group hydroxylation			n.a.[b]
4.	Oxygen-dependent desaturation			n.a.
5.	Sulfoxidation			*S*[c]
6.	*O*-Dealkylation			n.a.
7.	*N*-Dealkylation			n.a.

[a]The *S*-stereochemistry has been observed for the benzylic alcohols formed from indan and indene (n = 2), however, a racemic alcohol was formed when n = 1 (Table 2).
[b]n.a., not applicable.
[c]The *S*-stereochemistry has been observed when R = -CH_3 or -CH_2CH_3 and where the aryl-group of the sulfoxide has higher priority than the R-group as determined by the Prelog–Cahn–Ingold sequence rules.

and benzocyclic aromatic substrates are summarized as: i) the dihydroxylation reactions with substrates containing a prochiral aromatic or olefinic double bond result in the formation of *cis*-diols where the hydroxyl groups have a '1*R*,2*S*' absolute configuration (in cases where the assignment is not at the 1,2-position [ie bay region *cis*-3,4-diols, minor *cis*-dihydrodiols, or *cis*-diols numbered as the starting substrate], the stereochemistry at the hydroxyl-bearing carbon adjacent the bridgehead carbon is of *R*-configuration). Similar conclusions with respect to the *cis*-diols formed from benzocycloalkenes were reported by Boyd and associates [3]; ii) benzylic monohydroxylation reactions are often catalyzed with facial selectivity and involve oxidation of the pro-*S* hydrogen to yield secondary

alcohols of *S*-configuration; and iii) sulfoxidation of prochiral aryl alkyl sulfides occurs at the pro-*S* lone pair of electrons to yield chiral sulfoxides of *S*-configuration. The ability of NDO to oxidize only the side-chain substituents of alkyl and *O*-, *S*- and *N*-alkyl substituted benzenes is consistent with the proposed requirement of at least one aromatic ring for substrate binding [64]. This hypothesis is supported by the reactions summarized in Tables 1–5; all substrates contain one or more aromatic ring(s) which are not the site of enzymatic oxidation. The trends observed in the regiospecific and stereospecific oxidations catalyzed by NDO provide a basis for predicting the stereochemistry of chiral oxidation products formed from prochiral substrates.

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