Stereospecific Oxidation of (*R*)- and (*S*)-1-Indanol by Naphthalene Dioxygenase from *Pseudomonas* sp. Strain NCIB 9816-4

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A recombinant *Escherichia coli* strain which expresses naphthalene dioxygenase (NDO) from *Pseudomonas* sp. strain NCIB 9816-4 oxidized (*S*)-1-indanol to *trans*-(1*S*,3*S*)-indan-1,3-diol (95.5%) and (*R*)-3-hydroxy-1-indanone (4.5%). The same cells oxidized (*R*)-1-indanol to *cis*-1,3-indandiol (71%), (*R*)-3-hydroxy-1-indanone (18.2%), and *cis*-1,2,3-indantriol (10.8%). Purified NDO oxidized (*S*)-1-indenol to both *syn*- and *anti*-2,3-dihydroxy-1-indanol.

In recent years there has been an increased demand for optically pure compounds with biological activity. Multicomponent bacterial dioxygenases, for example, toluene dioxygenase (4, 8) and naphthalene dioxygenase (NDO) (7), have been utilized to produce more than 120 *cis*-1,2-diols (1, 10, 15), several of which have been used as chiral synthons for the synthesis of biologically active products and value-added chemicals (2, 3, 6, 14, 20). The present study was initiated by the observation that NDO oxidizes (*S*)-1-indanol to a compound tentatively identified as *trans*-1,3-indandiol (5). This provided the opportunity to examine the effect of substrate stereochemistry on the reactions catalyzed by NDO. We now report the absolute configurations of the 1,3-indandiol formed from (*S*)- and (*R*)-1-indanol and describe novel triol metabolites formed by this versatile bacterial dioxygenase.

Biotransformation of (*S*)-1-indanol. *Escherichia coli* JM109 (DE3)(pDTG141) (16), which contains the cloned *nahAaAbAcAd* genes encoding the NDO components (Reductase_{NAP}, Ferredoxin_{NAP}, and Iron-sulfur protein, ISP_{NAP}) from *Pseudomonas* sp. strain NCIB 9816-4, was used for biotransformations. Cells were grown in a medium containing 1% brain heart infusion, 0.5% yeast extract, 1% NaCl, 0.01% Fe(NH₄)₂(SO₄)₂ · 6H₂O, and 0.01% sodium ampicillin. Expression of the genes was induced by the addition of isopropyl-β-D-galactopyranoside (IPTG) (final concentration, 200 μM) followed by incubation at 27°C for 3 h. Harvested cells were stored at -70°C. Frozen cells (1.0 g) were resuspended in 100 ml of 50 mM sodium phosphate buffer (pH 6.8) containing 0.5% sodium pyruvate, 5% methanol, and 25 or 100 mg of (*S*)-1-indanol (99% purity) (Aldrich). Two substrate concentrations were used to facilitate identification of the intermediates and end products formed during the sequential oxidation of each indanol enantiomer. Reactions were conducted in 2.8-liter Fernbach flasks at 28°C with shaking at 240 rpm for 20 h, at which time cells were removed by centrifugation and 20 ml of a saturated NaCl solution was added to the supernatant solutions. Each supernatant was extracted as described previously (13). Gas chromatography-mass spectrometry (GC-MS) analysis (13) showed that IPTG-induced cells of JM109(DE3)(pDTG141) oxidized 25 mg of (*S*)-1-indanol to *trans*-1,3-indandiol and a small amount of 3-hydroxy-1-indanone (Table 1). Both of these compounds, in addition to small amounts of

1-indenol and 1-indanone, were also formed from 100 mg of (*S*)-1-indanol. The identities of 1-indenol, 1-indanone, 3-hydroxy-1-indanone, and *trans*-1,3-indandiol were confirmed by showing that their retention times and mass spectra were identical to those of authentic compounds (5, 13, 19). *trans*-1,3-Indandiol was isolated (17.6 mg; *R_f* = 0.35) by preparative thin-layer chromatography (TLC) with a developing solvent of methanol-chloroform (1:9) (13). An analysis of its proton nuclear magnetic resonance (¹H-NMR) spectrum (13) is shown in Table 2. The specific rotation (13) of [α]_D + 29.2° (c 1.3, methanol) and the known configuration of the 1-indanol substrate confirmed its structure as (+)-*trans*-(1*S*,3*S*)-indan-1,3-diol.

Biotransformation of (*R*)-1-indanol. Biotransformation experiments identical to those described above were conducted with (*R*)-1-indanol. GC-MS analysis of the products formed from 25 mg of substrate (Table 1) revealed the presence of 3-hydroxy-1-indanone and two unidentified metabolites with molecular ions at *m/z* 150 (compound I) and *m/z* 148 (compound II). The same compounds were formed from 100 mg of substrate, together with minor amounts of 1-indenol, 1-indanone, and *cis*-1,2-indandiol (Table 1). Compound I was isolated (7.5 mg; *R_f* = 0.38) by preparative TLC and gave mass and UV absorption spectra similar to those given by *trans*-1,3-indandiol. ¹H-NMR analysis of compound I (Table 2) showed the presence of diastereotopic protons H_a and H_b at the C-2 position which gave signals distinct from those given by the homotopic protons (H_a) at the same position in *trans*-(1*S*,3*S*)-indan-1,3-diol. This property and the absence of optical activity identified compound I as the *meso* compound *cis*-1,3-indandiol.

Oxidation products formed from (*R*)-1-indanol were also derivatized with phenylboronic acid (PBA) (17) prior to GC-MS analysis. Three derivatives were detected, with the retention times listed in Table 1. The compounds eluting at 20.53 and 20.15 min each gave molecular ions at *m/z* 236 and were identified as the PBA derivatives of *cis*-1,2- and *cis*-1,3-indandiol, respectively. In contrast, the third PBA derivative (retention time, 21.98 min) gave a molecular ion at *m/z* 252, and its presence coincided with the loss of compound II from the GC elution profile. These results suggested that compound II was a triol which dehydrated in the GC inlet to give a compound with a molecular ion at *m/z* 148 (Table 1). The putative triol was isolated (3.0 mg; *R_f* = 0.31) by preparative TLC. High-resolution MS of its PBA derivative gave a molecular weight of 252.0935 (C₁₅H₁₃O₃B requires a molecular weight of

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TABLE 1. Relative yields of products formed from (*S*)- and (*R*)-1-indanol by *E. coli* JM109(DE3)(pDTG141) expressing NDO^a

Product	<i>m/z</i> of molecular ion (<i>M</i> ⁺)	Retention time (min)	% product formed from the indicated amt (mg/100 ml) of:			
			(<i>S</i>)-1-Indanol ^b		(<i>R</i>)-1-Indanol ^c	
			100	25	100	25
1-Indanol (substrate)	134	9.76	55.5	— ^d	33.4	—
1-Indenol	132	9.45	0.7	—	1.5	—
1-Indanone	132	10.40	5.6	—	8.3	—
<i>cis</i> -1,2-Indandiol	150	12.35 (20.53) ^e	—	—	0.2	—
Compound I ^f	150	12.42 (20.15)	—	—	46.0	71.0
<i>trans</i> -1,3-Indandiol	150	12.53	35.3	95.5	—	—
3-Hydroxy-1-indanone	148	12.84	2.9	4.5	7.5	18.2
Compound II ^g	148	14.02 (21.98)	—	—	3.1	10.8

^a Biotransformations were conducted as described in the text. Yields were determined by integration of ion current peak areas under the GC-MS conditions. The identities of compounds I and II are shown in Table 2.

^b The amounts of crude material recovered from transformations with 100 and 25 mg of (*S*)-1-indanol were 72 and 18 mg, respectively.

^c The amounts of crude material recovered from transformations with 100 and 25 mg of (*R*)-1-indanol were 81 and 20 mg, respectively.

^d —, not detected.

^e Values in parentheses show the retention time of the PBA derivative.

^f *m/z* of fragmentation ions (percentage of base peak): for the underivatized product, 150 (29), 132 (97), 104 (100), 91 (14), 77 (81), and 51 (46); for the PBA derivative, 236 (100), 192 (41), 132 (60), 115 (46), 104 (43), and 77 (37).

^g *m/z* of fragmentation ions (percentage of base peak): for the underivatized product, 148 (100), 131 (22), 119 (67), 105 (43), 91 (83), 77 (41), and 65 (26); for the PBA derivative, 252 (51), 208 (31), 175 (100), 148 (66), 131 (94), 105 (48), and 91 (60).

252.0958). ¹H-NMR analysis of compound II (Table 2) showed the presence of three hydroxymethine protons at 4.30 and 4.83 ppm and a single coupling constant (*J*_{ab} = 4.6 Hz), which identified the metabolite as *cis*-1,2,3-indantriol (*syn*-2,3-dihydroxy-1-indanol). In control experiments, cells of *E. coli* JM109 (DE3) oxidized 25 mg of (*S*)- and (*R*)-1-indanol to 1-indanone in yields of 13 and 0.4%, respectively.

Oxidation of 1-indanol and 1-indenol by purified NDO. The involvement of NDO in the reactions catalyzed by JM109 (DE3)(pDTG141) was shown by incubating purified NDO components with the substrates as described previously (5). Purified NDO oxidized (*S*)-1-indanol to *trans*-1,3-indandiol (major product), 1-indenol, and 1-indanone in the presence of

excess substrate (5). Under identical conditions, NDO oxidized (*R*)-1-indanol to *cis*-1,3-indandiol (15.6%), 1-indenol (3.6%), and 1-indanone (2.2%). 3-Hydroxy-1-indanone and *cis*-1,2,3-indantriol were detected when the reaction mixture contained excess NADH. These results indicated that 1-indanone and 1-indenol are intermediates in the formation of 3-hydroxy-1-indanone and *cis*-1,2,3-indantriol, respectively (Fig. 1). GC-MS analysis showed that NDO oxidized (*S*)-1-indenol (88% enantiomeric excess) (5) to *cis*-1,2,3-indantriol (20%) and another product (13%) eluting at 15.00 min with a molecular ion at *m/z* 148. The PBA derivative of this new compound eluted at 22.56 min with a molecular ion at *m/z* 252, and its fragmentation pattern was essentially identical to that of *cis*-1,2,3-indantriol.

TABLE 2. ¹H-NMR chemical shifts and coupling constants of *trans*-(1*S*,3*S*)-indan-1,3-diol, compound I, and compound II formed from 1-indanols by NDO^a

Compound	Structure	Proton	Chemical shift(s) (ppm) ^b	Coupling constant(s) (Hz)
<i>trans</i> -(1 <i>S</i> ,3 <i>S</i>)-Indan-1,3-diol		H _a	2.26 (t, 2H)	<i>J</i> _{ab} , 5.4
		H _b	5.32 (t, 2H)	
		Aromatic	7.30–7.33 (m, 2H)	
		Aromatic	7.39–7.41 (m, 2H)	
I		H _a	1.73 (dt, 1H)	<i>J</i> _{ab} , 12.6; <i>J</i> _{ac} , 7.5 <i>J</i> _{bc} , 7.0 <i>J</i> _{c(ab)} , 7.2
		H _b	2.92 (dt, 1H)	
		H _c	4.96 (t, 2H)	
		Aromatic	7.29–7.33 (m, 2H)	
II		H _a	4.30 (t, 1H)	<i>J</i> _{ab} , 4.6
		H _b	4.83 (d, 2H)	
		Aromatic	7.31–7.34 (m, 2H)	
		Aromatic	7.37–7.41 (m, 2H)	

^a Spectra were recorded at 360 MHz in methanol-*d*₄ [*trans*-(1*S*,3*S*)-indan-1,3-diol and compound I] or acetonitrile-*d*₃ (compound II).

^b Chemical shifts are referenced to tetramethylsilane. Abbreviations: s, singlet; d, doublet; t, triplet; dt, doublet of triplets; and m, multiplet.

FIG. 1. Proposed reactions involved in the oxidation of (*R*)- and (*S*)-1-indanol by NDO. Reactions: A, monohydroxylation; B, desaturation; C, *cis*-dihydroxylation; D, oxygen- and NADH-dependent alcohol oxidation. The absolute configuration of *trans*-(1*S*,3*S*)-indan-1,3-diol was deduced from the stereochemistry of the substrate. Major and minor reactions are represented by thick and thin arrows, respectively. The yields of the oxidation products are given in Table 1.

These results indicated that the new product was another stereoisomer of 1,2,3-indantriol. This triol was not formed from (*R*)-1-indanol under the conditions where *cis*-1,2,3-indantriol was formed (Table 1). Thus, it can be concluded that NDO oxidizes (*S*)-1-indenol to *cis*-1,2,3-indantriol (*syn*-2,3-dihydroxy-1-indanol) and *anti*-2,3-dihydroxy-1-indanol (Fig. 1).

Conclusions. The reactions catalyzed by NDO with both enantiomers of 1-indanol as substrates are shown in Fig. 1. The major products formed from (*R*)- and (*S*)-1-indanol were *cis*-1,3-indandiol and *trans*-(1*S*,3*S*)-indan-1,3-diol, respectively. The monohydroxylation, desaturation, dihydroxylation, and alcohol oxidation reactions observed in the oxidation of (*R*)- and (*S*)-1-indanol by NDO (Fig. 1) have been previously demonstrated (5, 9, 11, 18, 19). In addition, the results obtained from a series of studies on the NDO-catalyzed oxidations of indan and related benzocyclic compounds indicate that the reactive oxygen species can catalyze both the dihydroxylation of π bonds to form *cis*-1,2-diols with the *R* configuration at the benzylic position and benzylic *S*-monohydroxylation (reviewed in reference 12). Exceptions to these trends are observed in the oxidation of 1-indanone (13) and (*S*)-1-indenol by NDO, indicating that both the regio- and stereochemistry of the substrate can influence the stereoselectivity of NDO. Therefore, the formation of different 1,3-diol and 1,2,3-triol stereoisomers from (*R*)- and (*S*)-1-indanol by NDO stems from the preexisting stereochemistry of the substrate and the inherent stereospecificity in the benzylic monooxygenation and double-bond dioxygenation reactions catalyzed by NDO.

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