J. Korean Soc. Appl. Biol. Chem. **54**(2), 229-236 (2011) **Article**

Biotransformation of N-Heterocyclic Compounds 1-Phenylpyrazole and 1-Phenylpyrrole by *Escherichia coli* (pDTG141) Expressing Naphthalene Dioxygenase of *Pseudomonas* sp. Strain NCIB 9816-4

Cung Nawl Thawng[1†], Ji-Young Ryu[1], Jaehong Han[3], Chang-Jun Cha[3], and Hor-Gil Hur[1,2*]

[1]*School of Environmental Science and Engineering,* [2]*International Environmental Research Center, Gwangju Institute of Science and Technology, Gwangju 500-712, Republic of Korea*

[3]*Department of Biotechnology, Chung-Ang University, Anseong 456-756, Republic of Korea*

Received December 8, 2010; Accepted December 30, 2010

Naphthalene dioxygenase (NDO) from *Pseudomonas* sp. strain NCIB 9816-4 is a multicomponent enzyme system that carries out the initial step in the degradation of naphthalene. This enzyme has broad substrate range and catalyzes several types of reactions including *cis*-dihydroxylation, monooxygenation, and desaturation. 1-Phenylpyrazole and 1-phenylpyrrole were tested for biotransformation by NDO. High-performance liquid chromatography (HPLC) analysis showed two metabolites from each substrate. Liquid chromatography/ Mass Spectroscopy (LC/MS) analysis indicated the molecular weight of metabolites M1 and M2 of 1-phenylpyrazole were increased by 16 and 34, respectively, which could be produced from enzymatic monooxygenation and dioxgenation reactions. HPLC and LC/MS analyses of metabolites M3 and M4 from 1-phenylpyrrole showed increased molecular weights of 16 and 32, as compared to parent compounds, proposing addition of one and two atoms of oxygen to substrate, respectively Metabolite structures were confirmed by ^{1}H- nuclear magnetic resonance (NMR) and ^{13}C-NMR analyses. Metabolites M1 and M2 from 1-phenylpyrazole were determined as 1-phenyl-1*H*-pyrazol-4-ol and (1*H*-pyrazol-1-yl)cyclohexa-4',6'-diene-*cis*-2',3'-diol, respectively. Metabolite M3 from 1-phenylpyrrole was confirmed as (1*H*-pyrrol-1-yl)benzene-2',3'-diol. NDO enzyme could be used to produce a novel synthon from N-heterocylic compounds, useful for synthesis of many biologically active compounds.

Key words: biotransformation, naphthalene dioxygenase, 1-phenylpyrazole, 1-phenylpyrrole, *Pseudomonas* sp.

A diverse array of aromatic compounds exists in nature, primarily as substances released from dead plant materials. In addition to biological sources of aromatic compounds, agricultural and industrial activities introduce a wide variety of xenobiotic aromatic compounds into the biosphere, including herbicides, insecticides, detergents, and industrial wastes. Many of these compounds become environmental pollutants as they contaminate soil and surface water [Berry *et al.*, 1987; Seo *et al.*, 2009b]. These compounds are eventually decomposed by various aromatic compound-metabolizing microorganisms, utilizing various oxygenases.

N-Heterocylic compounds are useful for the synthesis of many biologically active compounds. In the present study, 1-phenylpyrazole and 1-phenlpyrrole, which are N-heterocylic aromatic compounds, were used. These compounds are attractive precursors for synthesis of pharmarceuticals and agrochemicals, including fipronil and ethiprole insecticides, which are one of the most important insecticides for controls of soil insects of corn and fleas of cats and dogs [Meegalla *et al.*, 2002].

Aromatic ring hydroxylating dioxygenases are important as the initial step of bacterial biodegradation of diverse aromatic and polycyclic aromatic hydrocarbons (PAHs), including nitro-, amino-, chlorinated-, heterocyclic-aromatic compounds, and aromatic acids [Boyd *et al.*, 2001; van der Geize *et al*., 2004; Pieper *et al*., 2005]. The oxidation of aromatic compounds to *cis*-dihydrodiols is of special

†Present address: Department of Biotechnology, Chung-Ang University, Anseong 456-756, Republic of Korea

*Corresponding author
Phone: +82-62-715-2437; Fax: +82-62-715-2434
E-mail: hghur@gist.ac.kr

doi:10.3839/jksabc.2011.037

interest for biotechnological as well as chemical applications, because *cis*-dihydrodiols have been shown to be valuable building blocks for stereospecific synthesis of biologically active molecules containing multiple chiral centers [Sheldrake *et al*., 1992]. These ubiquitous enzymes are also of common interest in biotechnology, because their specificity allows the selective oxygenation of organic molecules under environmentally friendly conditions [Alcalde *et al.*, 2006]. Thus, biocatalytic oxygen transfer by the isolated enzymes or whole microbial cells is an elegant and efficient way to achieve selective hydroxylation.

In the present study, biotransformation of N-heterocyclic aromatic compounds by naphthalene dioxygenase (NDO) from *Pseudomonas* sp. strain NCIB 9816-4, which has activities of monooxygenation, sulfoxidation, desaturation, dehydrogenation, and *O*- and *N*-dealkylation reactions covering a broad substrate range of more than 90 substrates [Resnick *et al.*, 1996; Lee, 2006], were investigated.

Materials and Methods

Reagents and chemicals. All substrates used in this study were purchased from Sigma Aldrich Chemical Co., and Wako (Osaka, Japan). The high performance liquid chromatography (HPLC)-grade organic solvents such as acetonitrile, ethyl acetate and methanol were purchased from Fisher (Fair Lawn, NJ).

Bacterial strain and culture conditions. *Escherichia coli* JM109 (pDTG141), a recombinant strain expressing NDO from *Pseudomonas* sp. strain NCIB 9816-4 was kindly supplied by Professor Lee Kyoung of Changwon National University, Korea. The strain contains the structural genes (*nahAaAbAcAd*) of NDO from NCIB 9816-4 on plasmid pT7-5. Bacterium was kept on Luria Bertani (LB) agar plates containing 50 μg/mL of ampicillin at 4°C. For the seed culture, *E. coli* JM109 (pDTG141) was grown in 30 mL of LB medium containing 50 μg/mL ampicillin. The seed culture was used to inoculate 3 L of LB medium containing 50 μL/mL ampicillin with shaking at 200 rpm for 18 h at 37°C. The culture broth was centrifuged using a VS-21SMT centrifuge (Vision Scientific Co., Bucheon, Korea) at 6000 rpm for 10 min at 4°C. The cells were washed three times with 20 mM phosphate buffer (pH 7.5) and resuspended in the same buffer, adjusting the optical density at 600 nm to 10 using a UV/visible spectrophotometer (Shimadzu, UV-1601PC, Kyoto, Japan).

Biotransformation reactions and metabolites extraction. For the whole cell biotransformation reactions, all the respective substrate chemicals were prepared in methanol as stock solution (1 M) and added to the resuspended cells to a final concentration of 1 mM. Glucose as an energy source for the reaction was added to each culture to a final concentration of 1 mM. The reaction mixture was incubated on a shaker at 200 rpm 37°C for 18 h and extracted with three volumes of ethyl acetate. The ethyl acetate extract was evaporated to dryness with a rotary evaporator (Eyela, Tokyo, Japan) under vacuum. The residue was dissolved in methanol and filtered by using 0.2-μm Whatman PVDF syringe filters for further purification.

High performance liquid chromatography (HPLC). For the analysis of metabolites, analytical HPLC was performed using a Varian ProStar HPLC equipped with a photodiode array (PDA) detector (Varian, Walnut Creek, CA) and a reverse phase C18 Spherisorb (Waters, Milford, MA) column (5 μm particle size, 4.6 mm×25 cm). The mobile phase consisted of acetonitrile and water containing 0.1% (v/v) formic acid, and the gradient was programmed as follows: 10% acetonitrile at 0 min, 40% acetonitrile at 15 min, and 90% acetonitrile at 25 min, holding for 15 min. Ten microliter-aliquot of each sample was injected at a flow rate of 1 mL/min. UV detection was performed at 250 nm. For the large-scaled purification of metabolites, a Varian semi-prep HPLC equipped with a Rainin C18 ODS column (10 μm particle size, 21.4 mm× 25 cm, Varian Associates, Walnut Creek, CA) and a UV detector was used. The mobile phase consisted of acetonitrile and water containing 0.1% (v/v) formic acid, and the gradient program was the same as above. The flow rate was 15 mL/min with the same method of detection as above.

Liquid chromatography/mass spectrometry analysis (LC/MS). LC/MS was performed by coupling Alliance 2695 (Waters) with a Quattro LC triple quadruple tandem mass spectrometer (Waters) in an electrospray ionization (ESI) mode. The same mobile phase and flow rate were used as for HPLC. The flow rate was set at 0.2 mL/min and UV detection was performed with photo diode array detector 2996 (Waters). Injection volume was 10 μL. The source temperature, desolvation temperature, cone and capillary voltages, and electron multiplier voltage, were set at 150, 350°C, 15, 20, 25, 35 V, and 2.0 kV, respectively, and 700 V for ESI (-) and 650 V for ESI (+). Nitrogen gas set at 30 L/h was used as nebulizer gas, and ultra pure nitrogen set at 500 L/h was used as desolvation gas.

Nuclear magnetic resonance spectrometry. NMR measurements for all metabolites were performed on a Varian VNMRS 600 MHz spectrometer equipped with a Varian carbon-enhanced cryogenic probe. CD_3OD was used as a solvent, and chemical shifts for proton and carbon were measured in parts per million (ppm) relative

to TMS. One-dimensional ^{1}H and ^{13}C-NMR spectra were obtained under standard conditions using Varian software, VNMRJ v. 2.3. For all two-dimensional NMR experiments, the ^{1}H and the ^{13}C spectral widths were set to 6,000 Hz and 25.6 kHz for gHSQC, respectively, and to 36.2 KHz for gHMBC. Two-dimensional gHMBC and gCOSY experiments were run under absolute-value mode, whereas gHSQC was operated under phase-sensitive mode. The data for the gCOSY spectra were acquired in a 2048×128 matrix, followed by linear prediction up to two times in F1 data points and zero-filling to 2,048 data points before applying a window function. The data for the gHMBC spectra were acquired in a 1,024×400 matrix, predicted linearly up to four times in F1 data points and zero-filled to 2,048 data points. The squared sine-bell window functions in the F1 and F2 dimensions were used for gCOSY data. The data for gHSQC experiments were acquired in a 1,024×256 matrix, linear-predicted up to four times in F1 data points and zero-filled to 2,048 data points. Gaussian window functions in F1 and F2 dimensions were used for gHSQC before the final Fourier transform. The gHSQC and gHSQC experiments were optimized for a one-bond coupling constant of 140 Hz, and for a for long-range coupling constant of 8 Hz respectively.

IUPAC nomenclatures of structures. IUPAC nomenclatures of the structures discussed were obtained by the Chembiodraw ultra 11.0 software (CambridgeSoft, Cambridge, MA).

Biotransformation kinetics. Kinetic of biotransformation of 1-phenylpyrazole and 1-phenylpyrrole were performed with the resting culture ($OD_{600\,nm}$=10) of *E. coli* JM109 (pDTG141). The bacterial cells were incubated in a medium with final concentrations of 1 mM of 1-phenylpyrazole and 1 mM of 1-phenylpyrrole at 37°C and 200 rpm. After extraction of the cells (2 mL) with 5 mL of ethyl acetate, the remaining mixture was evaporated *in vacuo.* The residue was dissolved in 0.5 mL of methanol. Quantification was done by camparison of peak area with that of authentic compounds. All experiments were performed in triplicate.

Results and Discussion

Biotransformation of 1-phenylpyrazole and 1-phenylpyrrole was carried out with *E. coli* JM109 (pDTG141) expressing NDO from *Pseudomonas* sp. strain NCIB 9816-4. Extraction from the culture after 18 h incubation was analyzed using HPLC chromato-graphy with a PDA detector at 250 nm. Each two metabolites from 1-phenylpyrazole and 1-phenylpyrrole were eluted at 8.2 and 16.3 min, and 13.3 and 20.8 min, respectively (Fig. 1A, Fig. 2A). However, no metabolite appeared at the

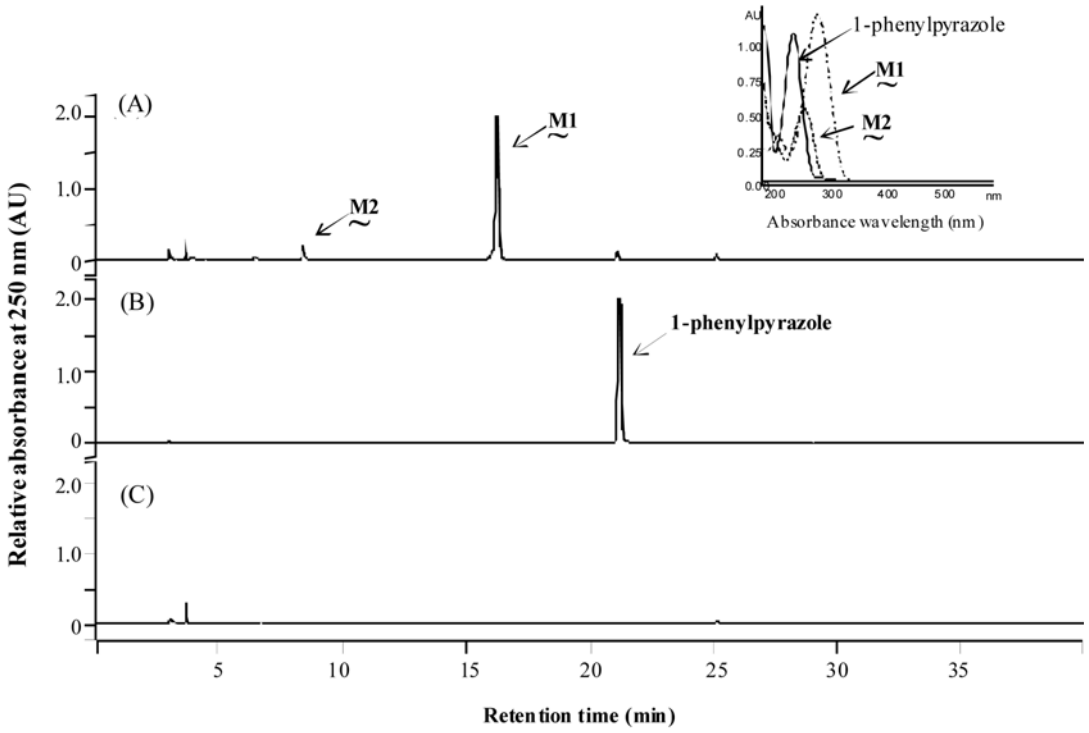

Fig. 1. HPLC elution profiles of the metabolites (M1) and (M2) produced from 1-phenylpyrazole by *E. coli* JM109 (pDTG141) expressing NDO. *E. coli* JM109 (pDTG141) with 1-phenylpyrazole (A), 1 mM of authentic 1-phenylpyrazole alone (B), and *E. coli* JM109 (pDTG141) without 1-phenylpyrazole (C). Insert: UV spectra of 1-phenylpyrazole, and metabolites M1 and M2.

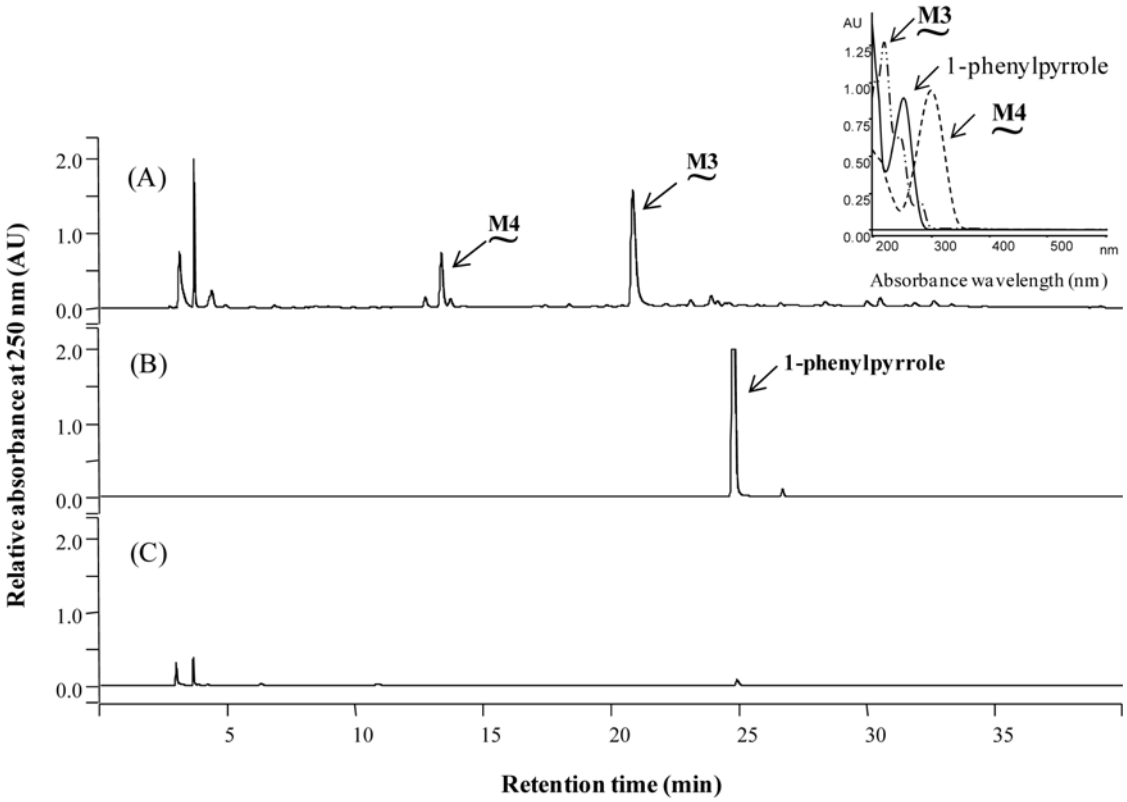

Fig. 2. HPLC elution profiles of the metabolites (M3) and (M4) produced from 1-phenylpyrrole by *E. coli* JM109 (pDTG141) expressing NDO. *E. coli* JM109 (pDTG141) with 1-phenylpyrrole (A), 1 mM of authentic 1-phenylpyrrole alone (B), and *E. coli* JM109 (pDTG141) without 1-phenylpyrrole (C). Insert: UV spectra of 1-phenylpyrrole, and metabolites M3 and M4.

control experiments of 1 mM of either authentic 1-phenylpyrazole or 1-phenylpyrazole alone (Fig. 1B and 2B), as well as in *E. coli* JM109 (pDTG141) with the parent compounds (Fig. 1C and 2C).

LC/MS spectra of the metabolites **M1** and **M2** compared to the parent material (Fig. 3A) were characterized by $[M+1]^+$ at *m/z* 160.87 (Fig. 3B) and 178.69 (Fig. 3C). Because the molecular ion peak of 1-phenylpyrazole was at *m/z* 144.94, the increased number of *m/z* by sixteen from the parent compound to metabolite **M1** can be explained by monooxygenation of substrate. Metabolite **M2** showed molecular ion of $[M+1]^+$ at *m/z* 178.69, indicating the introduction of two hydroxyl groups into the substrate as expected from the dioxygenase reaction. The ^{13}C NMR spectrum of **M1** showed seven signals including two peaks at 117.5 and 129.5 ppm, assigned for the symmetry-related phenyl carbon atoms at 2', 6' and 3', 5' positions, respectively (Table 1, Fig. 4A). The spectral changes were not observed from the phenyl ring moiety, suggesting the reaction occurred at pyrazole moiety of 1-phenylpyrazole substrate. The ^{1}H-NMR spectrum also showed one of pyrazolic H atom was missing. No correlation was found in C-4 position of the HMQC spectrum, and, as expected, the HMBC spectrum showed correlations of C-4/H-3 and C-4/H-5, because hydroxyl group is attached to C-4 position. Finally, the COSY spectrum found no H-H correlations between H-4 and H atoms at C-3 and C-5, which confirmed the hydroxylation at C-4 position. Based on the interpretation of HMBC, COSY, and HMQC spectra, the ^{13}C and ^{1}H chemical shifts were assigned (Table 1). Therefore, the metabolite **M1** was identified as a compound with IUPAC name of 1-Phenyl-1*H*-pyrazol-4-ol (Fig. 4A). The ^{13}C-NMR spectrum of **M2** of 1-phenylpyrazole showed nine signals with the new ^{13}C-NMR signals at 68.39 and 72.29 ppm, suggesting two hydroxyl groups attached to carbon-carbon single bonds. The signals in HMQC spectrum showed all eight protons from ^{1}H-NMR spectrum are directly attached to each corresponding carbons. The position of two hydroxyl groups on C-2' and C-3' were confirmed as follows: correlations of H-3'/H-2' and H-2'/H-3' in the COSY spectrum showed they are neighbors. The correlations of H-4'/H-5' and H-5'/H-6' confirmed their neighboring. Finally, HMQC spectrum of C-2'/H-2' and C-3'/H-3' confirmed the position of dioxygenation at C-2' and C-3'. Based on the interpretation of HMBC, HMQC, and COSY, the ^{13}C-NMR and ^{1}H-NMR chemical shifts were assigned (Table 2). Configuration of dihydrodiol

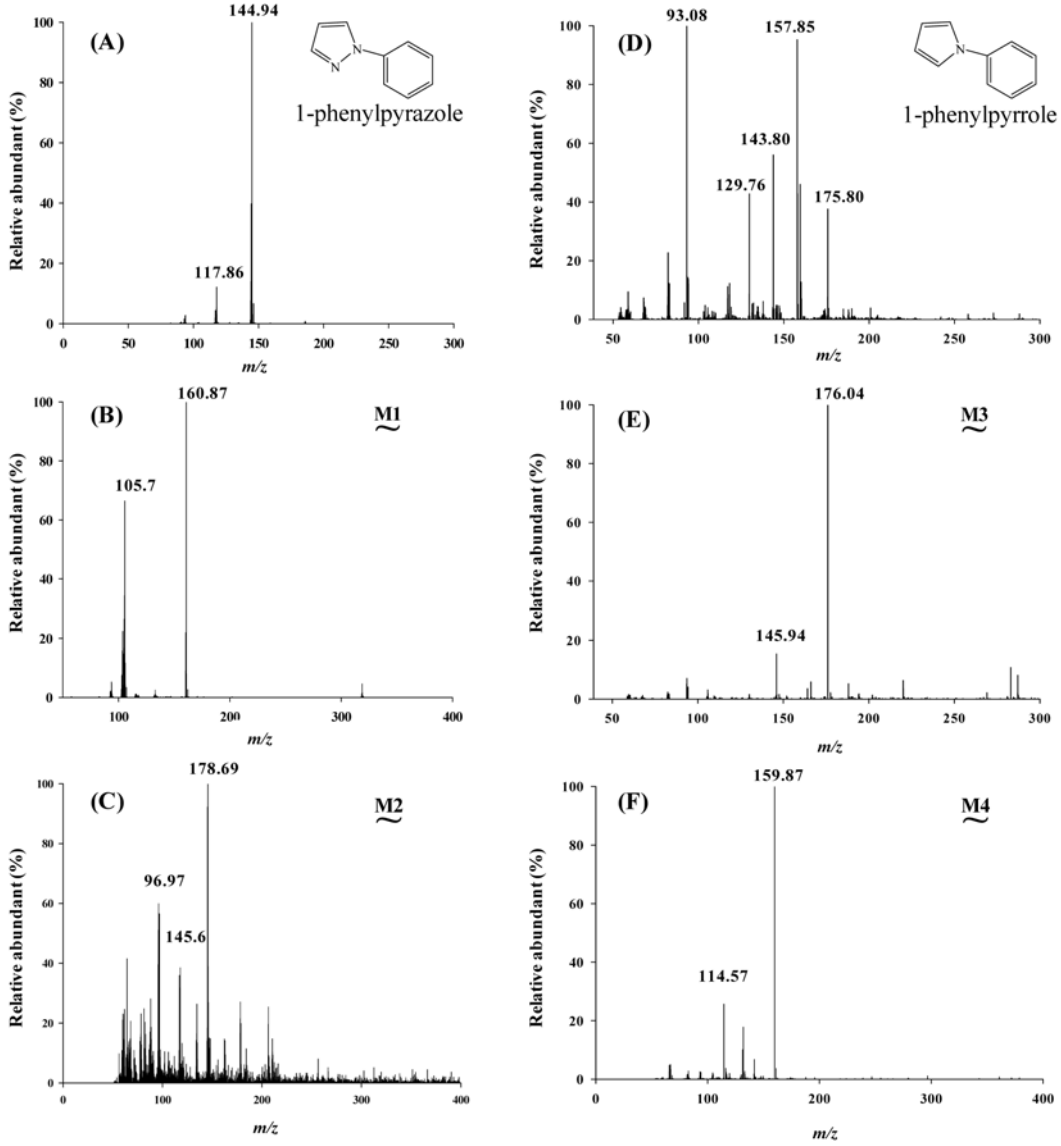

Fig. 3. LC/MS spectra of parent compounds 1-phenylpyrazole (A), and 1-phenylpyrrole (D), and bacterial metabolites M1 (B), M2 (C), M3 (E), and M4 (F).

was determined as *syn* configuration by the coupling constant (6.26 Hz) between two protons at C-2' and C-3' [Seo *et al.*, 2010]. Further study is needed for absolute configuration of the compound such as the specific rotation or circular dichroism to confirm enantiopurity of **M2**, (1*H*-pyrazol-1-yl)cyclohexa-4',6'-diene-*cis*-2',3'-diol (Fig. 4B) [Seo *et al.*, 2010].

LC/MS spectra of the metabolites **M3** and **M4** from 1-phenylpyrrole (Fig. 3D) were characterized by $[M+1]^+$ at *m/z* 176.04 (Fig. 3E) and 159.87 (Fig. 3F). Because the molecular ion peak of 1-phenylpyrrole was found at *m/z* 143.80, the molecular ion of $[M+1]^+$ at *m/z* 176.04 from the metabolite **M3** indicated introduction of two oxygen atoms into the substrate. Metabolite **M4** has the molecular ion of $[M+1]^+$ at *m/z* 159.87, proposing the insertion of single oxygen atom. The pathway could be epoxidation or monoxygenation. The ^{13}C spectrum of **M3** showed eight signals including two peaks at 109.50 and 122.76 ppm, the symmetry-related pyrrole signals of C-3 and C-4, and C-2 and C-5, respectively. Because the phenyl group of **M3** showed six signals instead of four symmetry-related ones, the oxygenation reaction was suggested to occur in the phenyl group of 1-phenylpyrrole. The ^{13}C signals at 140.20 and 147.97 ppm could be the hydroxylation positions as they are on the most downfield signals. There is no proton signal detected for C-2' and C-3' position in ^{1}H spectrum nor any correlation found in COSY, HMBC, and HMQC spectra. It was confirmed that two hydroxyl groups are attached to C-2' and C-3' positions. Based on the interpretation of HMBC, HMQC, and COSY, the ^{13}C and ^{1}H NMR chemical shifts of **M3** were assigned (Table 3). The **M3** metabolite was identified

Table 1. Assignments of the ^{1}H and ^{13}C chemical shifts for the metabolite M1 from 1-phenylpyrazole

Assignment	δ of ^{13}C	CHn	δ of ^{1}H	COSY	HMBC
3	131.01	d	7.349 (s)		C-3/H-5
4	143.90	s			C-4/H-3,C-4/H-5
5	112.44	d	7.93 (d,0.6 Hz)		C-5/H-3,C-5/H-2',6'
1'	140.11	s			
2',6'	117.29	d	7.71 (d,d,d,1.2,7.8,1.2Hz)	H-2',6'/H-3',5'	C-2',6'/H-4',C-2',6'/H-3',H-5'
3',5'	129.43	d	7.43 (d,d,16.2,1.2 Hz)	H-3',5'/H-2',6'	
4'	125.29	d	7.21 (t,7.2 Hz)		C-4'/H-2',6'

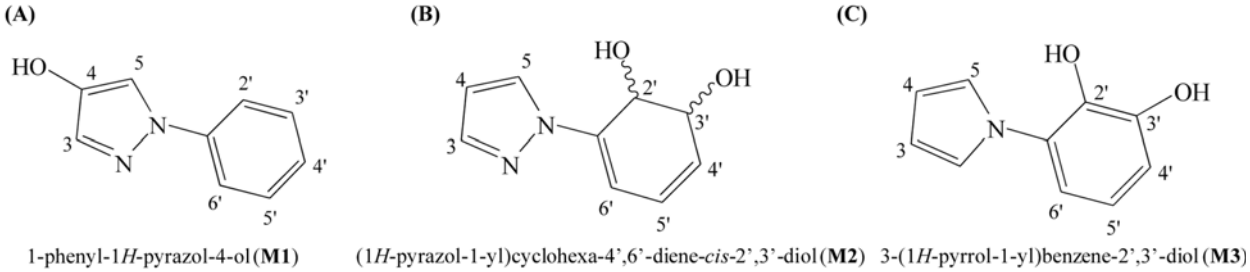

Fig. 4. Structures and IUPAC nomenclatures of the metabolites M1 (A) and M2 (B) from 1-phenylpyrazole, and the metabolite M3 (C) from 1-phenylpyrrole by whole cell culture of *E. coli* JM109(pDTG141) expressing NDO of *Pseudomonas* sp. strain NCIB 9816-4.

Table 2. Assignments of the ^{1}H and ^{13}C chemical shifts for the metabolite (M2) from 1-phenylpyrazole

Assignment	δ of ^{13}C	CHn	δ of ^{1}H	COSY	HMBC
3	142.11	d	7.65 (d,1.8 Hz)		
4	108.31	d	6.45 (t,2.4 Hz)		C-4/H-3,C-4/H5
5	129.31	d	8.0 (d,1.8 Hz)	H-5/H-4	C-5/H-4,C-5/H-3
1'	139.70	s			C-1'/H-2',C-1'/H-5',C-1'/H-6'
2'	68.39	d	4.71 (d,6.26 Hz)	H-2'/H-3'	C-2'/H-3',C-2'/H-4',C-2'/H-6'
3'	72.29	d	4.59 (m)	H-3'/H-2',H-3'/H-4'	C-3'/H-2',C-3'/H-4',C-3'/H-6'
4'	131.79	d	5.83 (m)	H-4'/H-5'	C-4'/H-3',C-4'/H-2',C-4'/H-6'
5'	122.98	d	6.03 (m)	H-5'/H-3',H-5'/H-4',H-5'/H-6'	C-5'/H-3'
6'	112.64	d	6.40 (d,6.0 Hz)	H-6'/H-5',H-6'/H-4',H-6'/H-3',H-6'/H-5'	

Table 3. Assignments of the ^{1}H and ^{13}C chemical shifts for the metabolite (M3) from 1-phenylpyrrole

Assignment	δ of ^{13}C	CHn	δ of ^{1}H	COSY	HMBC
2,5	122.76	d	7.01 (t,1.8 Hz)	H-2,5/H-3,4	C-2,5/H-3,4
3,4	109.50	d	6.19 (t,1.8 Hz)	H-3,4/H-2,4	C-3,4/H-2,5
1'	130.77	s			
2'	140.20	s			
3'	147.97	s			
4'	114.09	d	6.70-6.74 (m)	H-4'/H-4',5',6'	C-4'/H-4',5',6'
5'	120.58	d	6.70-6.74 (m)	H-4'/H-4',5',6'	C-4'/H-4',5',6'
6'	117.22	d	6.70-6.74 (m)	H-4'/H-4',5',6'	C-4'/H-4',5',6'

as (1*H*-pyrrol-1-yl)benzene-2',3'-diol (Fig. 4C). Although the LC/MS analysis provided the evidence that the metabolite was monooxygenated, the chemical structure elucidation of **M4** is still in progress.

Figure 5A and B show the biotransformation kinetic of 1-phenylpyrazole and 1-phenylpyrrole, respectively, by whole cells expressing NDO of *Pseudomonas* sp. strain NCIB 9816-4. The concentrations of the metabolites **M1**, **M2**, **M3**, and **M4**, were calculated by comparing with concentration of the parent substrates measured at

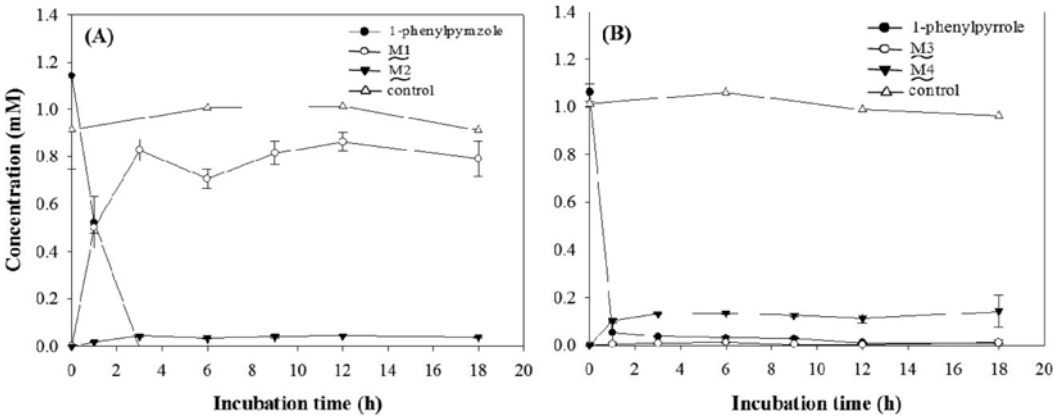

Fig. 5. Biotransformation kinetics of 1-phenylpyrazole (A) and 1-phenylpyrrole (B) by whole cell culture of *E. coli* JM109 (pDTG141) expressing NDO of *Pseudomonas* sp. strain NCIB 9816-4. The control experiment was *E. coli* JM109 (pUC18). All experiments were performed in triplicates.

250 nm. 1-Phenylpyrazole was completely transformed into **M1** (at 828 mM) and **M2** (at 42 mM) after 3 h incubation. 1-Phenylpyrrole was completely metabolized into **M3** (at 2 mM) and **M4** (at 113 mM) after 12 h incubation with no stoichiometric reactions. However, whole cells of *E. coli* JM109 (pUC18) did not transform 1-phenylpyrazole and 1-phenylpyrrole.

Biotransformation of 1-phenylpyrazole and 1-phenylpyrrole by the whole cells of *E. coli* expressing NDO has many benefits compared to the *in vitro* purified enzyme. Whole cell biotransformation method requires neither isolation and purification steps which may cause rapid loss of enzyme activity nor expensive *in situ* regeneration of cofactor such as NADH or NADPH [Duetz *et al.*, 2001]. Both 1-phenylpyrazole and 1-phenylpyrrole were converted into two metabolites by NDO. The chemical structures of metabolites **M1** and **M2** from 1-phenylpyrazole were confirmed as 1-phenyl-1*H*-pyrazol-4-ol and (1*H*-pyrazol-1-yl)cyclohexa-4',6'-diene-*cis*-2',3'-diol, respectively. The metabolite **M1** was same as the metabolite produced by the modified BDO; however, **M2** could not be produced by BDO [Misawa *et al.*, 2002]. Metabolite **M3** from 1-phenylpyrrole was identified as (1*H*-pyrrol-1-yl)benzene-2',3'-diol, which could be formed by dehydrogenation of the corresponding *cis*-diol produced by the modified BDO.

In conclusion, NDO in *E. coli* produced two metabolites **M1** and **M2** from 1-phenylpyraozle, whereas modified BDO produced only one metabolite **M1** at 47% conversion ratio. **M2** produced by NDO has a great potential of being used as a chiral synthon for the synthesis of various therapeutic agents and agrochemicals, because it contains *N*-heterocycle with chiral centers. NDO produced two metabolites, **M3** and **M4**, from 1-phenylpyrrole, whereas modified BDO was reported to convert the same substrate completely into only dihydrodiol product [Misawa *et al.*, 2002]. Results of the present study show that versatile dioxygenase NDO can catalyze N-heterocylic compounds into diol structures, which are useful for the synthesis of many biologically active compounds.

Acknowledgment. This work was supported by the Korea Research Foundation Grant funded by the Korean Government (MOEHRD, Basic Research Promotion Fund KRF-2006-311-F00053).

References

Alcalde M, Ferrer M, Plou FJ, and Ballesteros A (2006) Environmental biocatalysis: from remediation with enzymes to novel green processes. *Trends Biotechnol* **24**, 281-287.

Berry DF, Francis AJ, and Bollagi JM (1987) Microbial metabolism of homocyclic and heterocyclic aromatic compounds under anaerobic conditions. *Microbiol Rev* **51**, 43-59.

Boyd DR, Sharma ND, and Allen CC (2001) Aromatic dioxygenases: molecular biocatalysis and applications. *Curr Opin Biotechnol* **12**, 564-573.

Duetz WA, van Beilen JB, and Witholt B (2001) Using proteins in their natural environment: potential and limitations of microbial whole-cell hydroxylations in applied biocatalysis. *Curr Opin Biotechnol* **12**, 419-425.

Lee K (2006) *p*-Hydroxylation reactions catalyzed by naphthalene dioxygenase. *FEMS Microbiol Lett* **255**, 316-320.

Meegalla SK, Doller D, Liu R, Sha D, Soll RM, and Dhanoa DS (2002) Efficient syntheses of 2-(2,6-dichloro-4-triuoromethyl-phenyl)tetrahydrocyclopenta, tetrahydro-thiopyrano, hexahydrocycloheptapyrazoles, and tetrahydro-indazoles. *Tetrahedron Lett* **43**, 8639-8642.

Misawa N, Shindoc K, Takahashid H, Suenagae H, Iguchid K,

Okazakia H, Harayamab S, and Furukawae K (2002) Hydroxylation of various molecules including heterocyclic aromatics using recombinant *Escherichia coli* cells expressing modified biphenyl dioxygenase genes. *Tetrahedron* **58**, 9605-9612.

Pieper DH (2005) Aerobic degradation of polychlorinated biphenyls. *Appl Microbiol Biotechnol* **67**, 170-191.

Resnick SM, Lee K, and Gibson DT (1996) Diverse reactions catalyzed by naphthalene dioxygenase from *Pseudomonas* sp strain NCIB 9816. *J Ind Microbiol Biotechnol* **17**, 438-457.

Seo J, Kang SI, Ryu JY, Lee YJ, Park KD, Kim M, Won D, Park HY, Ahn JH, Chong Y, Kanaly RA, Han J, and Hur HG (2009a) Location of flavone B-ring controls regioselectivity and stereoselectivity of naphthalene dioxygenase from *Pseudomonas* sp. strain NCIB 9816-4. *Appl Microbiol Biotechnol* **86**, 1451-1462.

Seo JS, Keum YS, and Li QX (2009b) Bacterial degradation of aromatic compounds. *Int J Environ Res Public Health* **6**, 278-309.

Sheldrake GN (1992) In *Biologically derived arene* cis-*dihydrodiols as synthetic building blocks.* John Wiley & Sons Ltd., Chichester, UK.

van der Geize R, and Dijkhuizen L (2004) Harnessing the catabolic diversity of rhodococci for environmental and biotechnological applications. *Curr Opin Microbiol* **7**, 255-261.