

Biotransformation of Unsaturated Heterocyclic Rings by *Pseudomonas putida* to Yield *cis*-Diols

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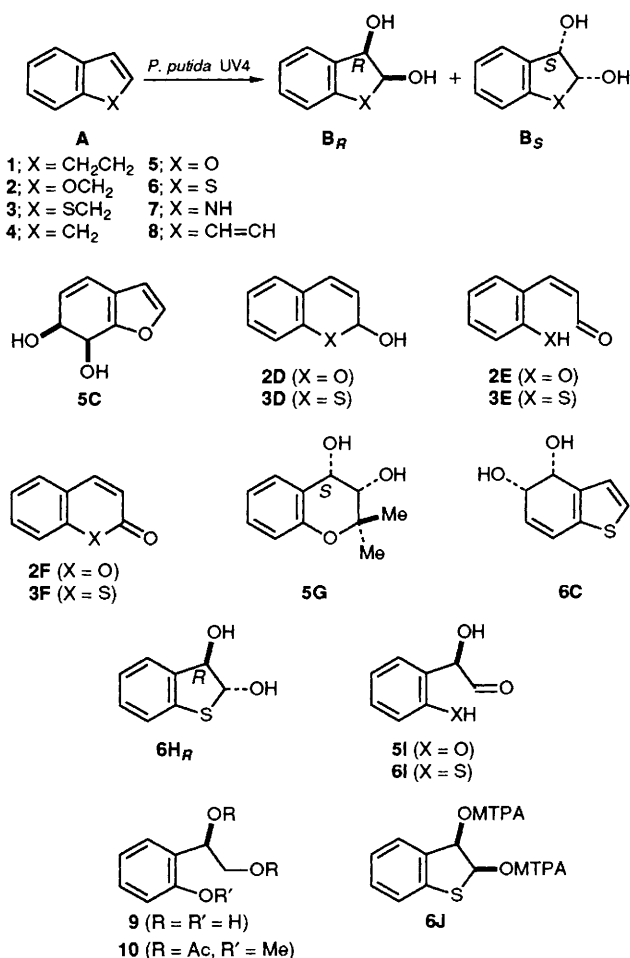
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New *cis*-diol metabolites of both aromatic and non-aromatic heterocyclic rings have been isolated from growing cultures of a mutant strain of the soil bacterium *Pseudomonas putida* (UV4) and stereochemically assigned; a novel heterocyclic *cis*-diol of benzothiophene, 2,3-dihydroxy-2,3-dihydrobenzothiophene is found to exist exclusively in the *cis*-configuration in water but equilibrates readily with the *trans*-isomer.

The bacterial metabolism of arenes and cyclic alkenes has been reported¹ to yield a large number (>120) of *cis*-diol products, but to our knowledge there is no report in the literature of the isolation of this type of metabolite from the oxidation of a heterocyclic ring. Metabolism of azaarenes (quinoline,² isoquinoline,² quinoxaline² and quinazoline²) oxaarenes (benzofuran³ and dibenzofuran⁴) and thioarenes (dibenzothiophene⁵) using the bacterium *Pseudomonas putida* (or a *Beijerinckia* species^{4,5}) has yielded isolable *cis*-dihydrodiol products only from the carbocyclic ring of the substrates. In this note we present results which show that using *Pseudomonas putida* UV4 *cis*-diol metabolites are formed in the heterocyclic ring of both arenes and cyclic alkenes.

We have previously reported the formation of *cis*-diol metabolites (**1B_S**, **4B_R/4B_S**, **5C** and **8B_R**) during oxidation of the carbocyclic rings of the corresponding bicyclic arenes *i.e.* 1,2-dihydronaphthalene **1A**,⁶ indene **4A**,⁶ benzofuran **5A**³ and naphthalene **8A**.² Similar biotransformation procedures,^{1-3,6} in the present study using *P. putida* UV4, yielded a series of new *cis*-diol metabolites of heterocyclic substrates: chromene **2A**, thiochromene **3A** and benzothiophene **6A**.

cis-Diols **2B_S** and **3B_S** were the more abundant enantiomers (>99%, benzylic-*S*), which are directly comparable to *cis*-diol metabolite **1B_S** obtained from 1,2-dihydronaphthalene (again >99% benzylic-*S*).⁶ The *cis*-diol metabolite **4B_R/4B_S** showed an enantiomeric excess of 20% in favour of the **4B_S** enan-



tiomer.⁶ These observations show a consistent stereochemical trend for *cis*-diol metabolites derived from cyclic alkenes. In the earlier studies^{3,6} the opposite absolute configuration (benzylic-*R*) was consistently observed for *cis*-dihydrodiol metabolites of carbocyclic arenes.

The *cis*-diols **2B_S** and **3B_S** obtained from substrates **2A** and **3A**, respectively, were accompanied by metabolites resulting from oxidation at the allylic position. Racemic lactol **2D**, the open-chain aldehyde isomer **2E**, and the further oxidation product coumarin **2F**, were thus detected as minor products (37% of total metabolites) from chromene **2A**. Similar allylic oxidation products **3E** and **3F** (but not the lactol **3D**), derived from thiochromene **3A**, were also isolated and identified. Oxidation of chromenes at the allylic position is often prevented in natural products by the presence of a *gem*-dimethyl group. Taking our cue from nature we added 2,2-dimethylchromene as substrate for *P. putida* UV4. The *cis*-diol **5G** {18% isolated yield, [α]_D -15.2 (CHCl₃), >98% enantiomeric excess (e.e.)} was obtained as the sole metabolite in this biotransformation and was assigned a 4*S*,3*S*-configuration (by stereochemical correlation).

Metabolism of indole **7A** by *P. putida* has previously been postulated to occur *via* a transient *cis*-diol intermediate **7B_R** (or **7B_S**), which dehydrates to yield the phenol indoxyl.⁷ The spontaneous formation of indigo dye from indole in *P. putida* is used as a spectrophotometric assay for dioxygenase enzymes⁸ and may also have commercial potential.⁷ All attempts to isolate or detect the heterocyclic *cis*-diol intermediate **7B_R** (or **7B_S**) in this commercially important biotransformation process have been unsuccessful. When benzothiophene **6A** was used as substrate with *P. putida* UV4, ethyl acetate extraction gave two separable crystalline products (preparative TLC, silica gel, diethyl ether-pentane, 1:1). The low *R_f* metabolite (0.20) was the *cis*-diol, **6C** of 4*R*,5*S*-configuration

Table 1 Yield, optical rotation, enantiomeric excess value and absolute configuration

Compound	Yield (%) ^a	[α] _D ^b	e.e. (%) ^c	Configuration
2B_S	20	+64 ^d	>98	4 <i>S</i> ,3 <i>R</i> ^e
3B_S	40	+53	>98	4 <i>S</i> ,3 <i>S</i> ^f
6C	9	+98	>98	4 <i>R</i> ,5 <i>S</i> ^f
6B_R	15 ^g	-97 ^h	>98	3 <i>R</i> ,2 <i>S</i> ^f
6H_R	15 ^g	+117 ⁱ	>98	3 <i>R</i> ,2 <i>R</i> ^c

^a Isolated yield after chromatographic purification. ^b CHCl₃ solvent. ^c From ¹H NMR analysis of the di-MTPA ester. ^d THF solvent. ^e By stereochemical correlation and X-ray crystallographic analysis. ^f By X-ray crystallographic analysis of a di-MTPA ester derivative. ^g Isolated yield of diol **6B_R** (which equilibrates with **6H_R**). ^h MeOH solvent (>99% **6B_R**). ⁱ In CHCl₃ solvent, >99% **6H_R** present.

resulting from oxidation at the 4,5-bond (Table 1). Oxidation at the 6,7-position of benzofuran to yield *cis*-diol **5C** of 6*S*,7*S*-configuration, under similar conditions, had already been reported.³

The high *R_f* (0.50) metabolite exhibited the properties of a *vic*-diol. In neutral aqueous or MeOH solution it was found to exist as a single isomer and was identified as *cis*-diol **6B_R** from the ¹H NMR data in D₂O (*J*_{2,3} 1.6 Hz). Under acidic conditions (D₂O containing a trace of CF₃CO₂H) a significant proportion (40%) of the *trans*-diol **6H_R** was formed (*J*_{2,3} 4.5 Hz). Evaporation of the neutral aqueous solution gave the pure *cis*-diol **6B_R** as a crystalline solid {[α]_D -97 (MeOH)}. When the *cis*-diol **6B_R** was dissolved in CDCl₃ solvent the *trans*-isomer **6H_R** was dominant (*ca.* 80%) with a minor contribution (*ca.* 20%) of the *cis*-isomer **6B_R**. Recrystallization of the diol from dichloromethane-hexane yielded pure *trans*-diol **6H_R**. When the *trans*-diol isomer was dissolved in acid-free CHCl₃ and immediately examined by polarimetry, an optical rotation value of [α]_D +117 (CHCl₃) was obtained without evidence of equilibration.

Mutarotation of the *cis*-diol **6B_R** and *trans*-diol **6H_R** isomers was evident in the presence of acid or base (presumably *via* the undetected open-chain aldehyde **6I**).

The di-MTPA esters [MTPA = α-methoxy-α-(trifluoromethyl)phenylacetic acid] of the *cis*-**6B_R** and *trans*-**6H_R** isomers were found to be readily separable by PLC (silica gel, diethyl ether-pentane, 1:9). An X-ray crystal structure analysis of the high *R_f* di-MTPA ester **6J** [obtained using (+)-MTPA acid] confirmed the relative configuration as *cis* and unequivocally established an *R*-configuration at the benzylic chiral centre of the parent diol **6B_R**. It thus follows that the *trans*-diol formed by equilibration *via* aldehyde **6I** will also have a benzylic-*R* configuration **6H_R**. It is probable that the initially formed product from enzyme-catalysed oxidation of benzothiophene **6A** has the *cis*-configuration **6B_R** (since no *trans*-diol products have been previously isolated from metabolism of alkenes or arenes by *P. putida*). These observations do not allow the intermediacy of a *trans*-isomer **6H_R** to be totally excluded. Formation of 3-hydroxybenzothiophene on acid-catalysed dehydration of both *cis*- and *trans*-diols **6B_R** and **6H_R** demonstrated that phenols could be derived from diols formed from heterocyclic rings.

The successful isolation of the novel *cis*-**6B_R** and *trans*-**6H_R** diol products from bacterial biotransformation of benzothiophene **6A** prompted a reinvestigation of the metabolites derived from benzofuran **5A**. In addition to the *cis*-diol **5C**³ a further metabolite (38% of total metabolites), which proved to be more polar and water-soluble, was found after repeated extraction of the culture medium with ethyl acetate. This elusive bioproduct was identified as the phenolic diol **9** (which was isolated as a metabolite of flavone from the cultures of the fungus *Rhizopus nigricans*).⁹ Metabolite **9** {32% isolated yield, [α]_D -24 (MeOH)} was assigned an *R*-configuration at the benzylic chiral centre (50% e.e) through stereochemical correlation with *S*-glyceric acid by RuO₄ oxidative cleavage of

the protected metabolite **10**. Metabolite **9** could have been derived from the initially formed *cis*-diol **5B_R** by a two-step reaction sequence involving: (i) spontaneous ring opening to yield the transient aldehyde **5I** and (ii) reduction of the aldehyde. If the above metabolic sequence is assumed for diol **9** then the heterocyclic *cis*-diol metabolites of benzothiophene **6A** and benzofuran **5A** may both show a preference for the benzylic-*R* configuration (>99% **6B_R** and 75% **5B_R**).

The dioxygenase enzyme system in *P. putida* UV4 appears to have the capacity for distinguishing between the 'arene bond' at the 2,3-position of benzothiophene (and possibly benzofuran) yielding a *cis*-diol of benzylic-*R* configuration and the 'alkene bond' of carbocyclic analogue indene, (forming preferentially a *cis*-diol of benzylic-*S* configuration).⁶

The following conclusions can be made from the present study. (i) Isolated *cis*-diol metabolites from a heterocyclic ring have an excess of the benzylic-*S* configuration from an alkene bond (chromene, thiochromene) and the benzylic-*R* configuration from an arene bond (benzothiophene). (ii) The *cis*-diol metabolite in the heterocyclic ring of benzothiophene **6B_R** can readily isomerize *via* the corresponding open-chain aldehyde form **6I** and hence the possibility of *trans*-diol **6H_R** involvement in metabolism cannot be excluded. (iii) The postulated⁷ formation of transient *cis*-**7B_R** (or **7B_S**) diols as initial metabolites of indole **7A** in *P. putida* followed by their spontaneous dehydration to yield indoxyl, is strongly supported by these studies.

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