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

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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 reported[1] 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] quinoxaline[2] and quinazoline[2]) oxaarenes (benzofuran[3] and dibenzofuran[4]) and thioarenes (dibenzothiophene[5]) using the bacterium *Pseudomona 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-

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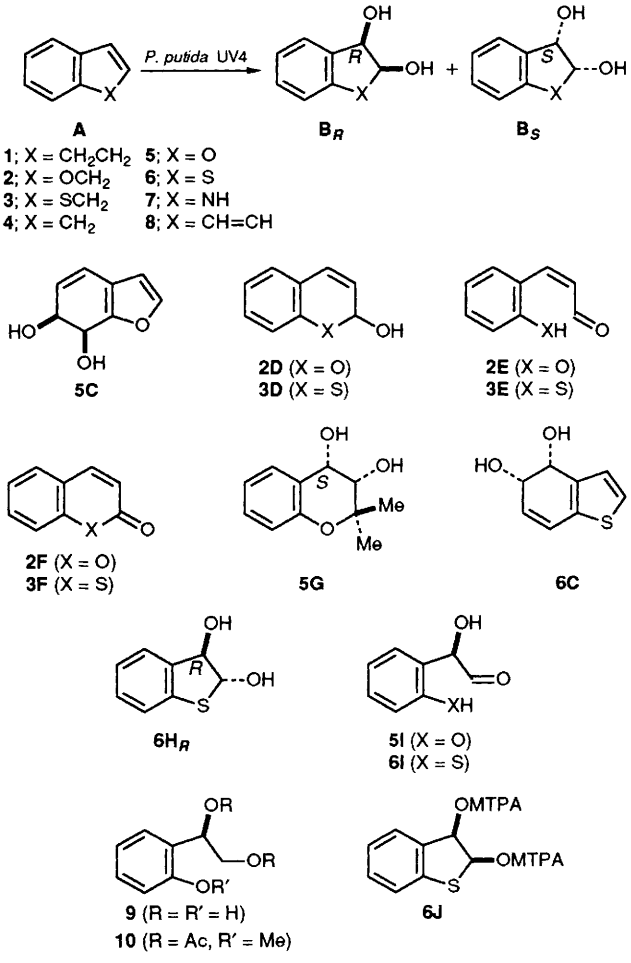

tiomer.[6] These observations show a consistent stereochemical trend for *cis*-diol metabolites derived from cyclic alkenes. In the earlier studies[3,6] the opposite absolute configuration (benzylic-*R*) was consistently observed for *cis*-dihydrodiol metabolites of carbocyclic arenes.

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, $[\alpha]_D$ −15.2 ($CHCl_3$, >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 enzymes[8] 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]	$[\alpha]_D$[b]	e.e. (%)[c]	Configuration
2B$_S$	20	+64[d]	>98	4*S*,3*R*[e]
3B$_S$	40	+53	>98	4*S*,3*S*[f]
6C	9	+98	>98	4*R*,5*S*[f]
6B$_R$	15[g]	−97[h]	>98	3*R*,2*S*[f]
6H$_R$	15[g]	+117[i]	>98	3*R*,2*R*[e]

[a] Isolated yield after chromatographic purification. [b] $CHCl_3$ solvent. [c] From ^{1}H 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 $CHCl_3$ 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 ^{1}H NMR data in D_2O ($J_{2,3}$ 1.6 Hz). Under acidic conditions (D_2O containing a trace of CF_3CO_2H) 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 {$[\alpha]_D$ −97 (MeOH)}. When the *cis*-diol **6B**$_R$ was dissolved in $CDCl_3$ 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 $CHCl_3$ and immediately examined by polarimetry, an optical rotation value of $[\alpha]_D$ +117 ($CHCl_3$) 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, $[\alpha]_D$ −24 (MeOH)} was assigned an *R*-configuration at the benzylic chiral centre (50% e.e) through stereochemical correlation with *S*-glyceric acid by RuO_4 oxidative cleavage of

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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 postulated[7] 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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