Dioxygenase-catalysed formation of *cis*/*trans*-dihydrodiol metabolites of mono- and bi-cyclic heteroarenes

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A new range of heterocyclic ring *cis*/*trans*-dihydrodiol derivatives (1B, 3B–8B) obtained from metabolism of monocyclic (1A, 3A) and bicyclic heteroarenes (4A–8A) by *Pseudomonas putida* UV4, has been isolated and stereochemically assigned.

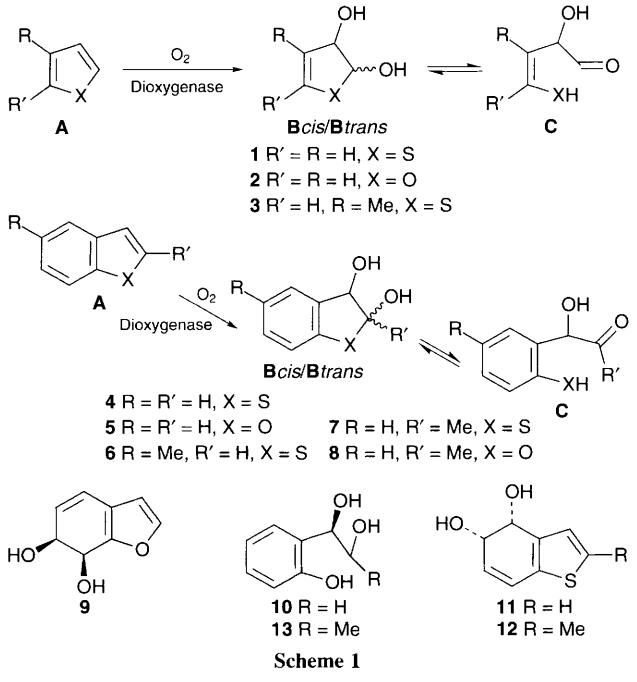
The metabolism of mono- and poly-cyclic arenes by the bacterium *Pseudomonas putida* is known to occur *via* dioxygenase-catalysed asymmetric *cis*-dihydroxylation. Prompted by the earlier observation[1] that toluene dioxygenase-catalysed dihydroxylation (using *P. putida* UV4) was found to occur in the heterocyclic ring of benzothiophene **4A**, to yield *cis*-2,3-dihydroxy-2,3-dihydrobenzothiophene $\mathbf{4B}_{cis}$ in equilibrium with the *trans* isomer ($\mathbf{4B}_{trans}$), a systematic search was undertaken to find further evidence for *cis*/*trans*-dihydrodiol metabolites of both monocyclic (*e.g.* **1A–3A**) and bicyclic oxa- and thia-arenes (*e.g.* **5A–8A**).

Addition of thiophene **1A** as the substrate to growing cultures of *P. putida* UV4, using a similar procedure to that reported for benzothiophene **4A**[1] yielded mainly sulfoxidation products (28% yield).[2] A minor metabolite (*ca.* 1% yield) proved to be a non-crystalline isomeric mixture of *cis*- and *trans*-2,3-dihydroxy-2,3-dihydrothiophene, $\mathbf{1B}_{cis}$ and $\mathbf{1B}_{trans}$. To our knowledge this is the only reported example of a *cis*- or *trans*-dihydrodiol metabolite from an aromatic monoheterocycle and show typical cyclic thiohemiacetal properties including equilibration (mutarotation) *via* an undetected open chain aldehyde isomer **1C**. The *cis*/*trans*-dihydrodiol $\mathbf{3B}_{cis}/\mathbf{3B}_{trans}$ was isolated in higher yield (11%) from *P. putida* UV4 metabolism of 3-methylthiophene **3A** (Scheme 1) in addition to sulfoxidation products.[2] Similar attempts to isolate the corresponding *cis*/*trans* isomer $\mathbf{2B}_{cis}/\mathbf{2B}_{trans}$ as a bacterial metabolite from furan **2A** were unsuccessful.

Earlier studies[3] of the metabolism of benzo[*b*]furan **5A** led to the isolation of *cis*-dihydrodiol **9** and to the proposal that the *cis*/*trans*-dihydrodiol $\mathbf{5B}_{cis}/\mathbf{5B}_{trans}$ could also be present as undetected transient metabolic precursors of the phenolic diol **10**.[3] It has now been possible to intercept and characterise the previously undetected, but relatively stable, *cis*/*trans*-dihydrodiols $\mathbf{5B}_{cis}/\mathbf{5B}_{trans}$ (17% yield). This appears to be an unprecedented example of a *cis*/*trans*-dihydrodiol in a furan ring resulting from dioxygenase-catalysed oxidation. Direct oxidation of 2,3-disubstituted benzo[*b*]furans by dimethyldioxirane to yield the corresponding arene oxides, followed by hydrolysis, has also been found to yield racemic substituted *cis*/*trans*-diols.[4] As anticipated, when the *cis*/*trans*-dihydrodiol metabolite $\mathbf{5B}_{cis}/\mathbf{5B}_{trans}$ from benzo[*b*]furan was added as a substrate to *P. putida* UV4, it was found to be rapidly reduced to the phenolic diol **10** (presumably *via* aldehyde **5C**). No similar thiophenolic diol metabolites were found during benzo[*b*]thiophene metabolism by *P. putida* UV4. The latter observations suggest that dihydrodiol derivatives of furans are more susceptible to ring opening and aldehyde reduction than their thiophene analogues. Failure to extract the *cis*/*trans*-dihydrodiol $\mathbf{2B}_{cis}/\mathbf{2B}_{trans}$ as a metabolite of furan **2A** in *P. putida* UV4, could thus have been due to formation of aldehyde **2C** in low yield followed by reduction to water-soluble bioproducts.

A reinvestigation[1] of the metabolism of benzo[*b*]thiophene **4A** in *P. putida* UV4 showed that, in addition to the heterocyclic *cis*/*trans*-dihydrodiol $\mathbf{4B}_{cis}/\mathbf{4B}_{trans}$ (15% yield) and the carbocyclic *cis*-dihydrodiol **11** (9% yield), the products of sulfoxidation (7% yield) were also present.[2] The effect of methyl substitution upon the regioselectivity of dioxygenase-catalysed asymmetric dihydroxylation was demonstrated by addition of 5-methylbenzo[*b*]thiophene **6A** as substrate. Dihydroxylation then occurred exclusively in the heterocyclic ring to give *cis*/*trans*-diol $\mathbf{6B}_{cis}/\mathbf{6B}_{trans}$ in high yield (79%). When a methyl group was present at the C-2 position of benzo[*b*]thiophene (**7A**), dihydroxylation occurred to a reduced degree at the substituted C-2–C-3 bond ($\mathbf{7B}_{cis}/\mathbf{7B}_{trans}$, 3% yield) relative to the unsubstituted C-4–C-5 bond (**12**, 25% yield). A similar biotransformation of the furan ring was observed when 2-methylbenzo[*b*]furan **8A** was used as substrate *i.e.* diol $\mathbf{8B}_{cis}/\mathbf{8B}_{trans}$ (8% yield) and the derived phenolic diol **13** (*ca.* 1% yield) were isolated. Despite comprehensive studies of the toluene dioxygenase (TDO)-catalysed dihydroxylation of methyl-substituted benzene substrates in these and other laboratories, dihydrodiols $\mathbf{7B}_{cis}/\mathbf{7B}_{trans}$ and $\mathbf{8B}_{cis}/\mathbf{8B}_{trans}$ appear to be the only examples of bioproducts resulting from dihydroxylation at a methyl-substituted arene bond.

Following the isolation of the new series of *cis*/*trans*-dihydrodiol metabolites in heterocyclic rings ($\mathbf{1B}_{cis}/\mathbf{1B}_{trans}$, $\mathbf{3B}_{cis}/\mathbf{3B}_{trans}$–$\mathbf{8B}_{cis}/\mathbf{8B}_{trans}$) a study of their relative and absolute



Scheme 1

stereochemistry was undertaken. The assignment of relative stereochemistry of the *cis*/*trans*-isomers **4B**$_{cis}$/**4B**$_{trans}$ and **5B**$_{cis}$/**5B**$_{trans}$ (Table 1) was based upon a comparison of NOE values between the proximate vicinal protons H-2 and H-3. Using the GOESY technique (±0.2%) the larger NOE values (2.1–3.5%) were attributed to the *cis*-isomers (**4B**$_{cis}$ and **5B**$_{cis}$) and the smaller values (1.1–1.6%) to the *trans*-isomers (**4B**$_{trans}$ and **5B**$_{trans}$). The validity of the NOE method was confirmed using *cis*-1,2-dihydroxyindane (1.7%) and *trans*-1,2-dihydroxyindane (0.2%) as model compounds.

The vicinal coupling constants for the *cis*-isomers **4B**$_{cis}$ and **5B**$_{cis}$ are larger ($J_{2,3}$ 4.1–5.1 Hz) compared to the *trans*-isomers **4B**$_{trans}$ and **5B**$_{trans}$ (< 1–1.6 Hz). On the basis of the larger NOE and coupling constant values associated with the *cis*-isomers **4B**$_{cis}$ and **5B**$_{cis}$, it is assumed that the comparable heterocyclic *cis*-isomers **1B**$_{cis}$, **3B**$_{cis}$ and **6B**$_{cis}$ will also have larger vicinal coupling constants. While the earlier[1] assignment of absolute stereochemistry of the diol **4B**$_{cis}$/**4B**$_{trans}$ was correct, based on the new GOESY and coupling constant data, the relative stereochemistry for the major isomer in $CDCl_3$ (Table 1) should be *cis*. A similar comparison of vicinal coupling constants was not possible for *cis*- and *trans*-diols **7B** and **8B** due to the presence of a methyl group at C-2. From a consideration of non-bonding interactions in molecular models, a *cis* configuration between the vicinal hydroxy groups of diols **7B** and **8B** would appear preferable. ^{1}H and ^{13}C NMR spectra of the diols in $CDCl_3$ solution showed the presence exclusively (**8B**) or mainly (>90%, **7B**) of one isomer which was assumed to have a *cis* configuration. The initially isolated metabolite **7B**$_{cis}$/**7B**$_{trans}$ showed a slight excess (9%) of the 3*R* enantiomer (Table 1) but X-ray crystallographic analysis confirmed that the diol metabolite **7B** had crystallised in the racemic *cis* form. The asymmetric unit consists of two independent molecules but they do not differ significantly in any detail of geometry or conformation. One molecule is shown in Fig. 1.†

Since the phenolic diols **10** and **13** could be obtained both from $NaBH_4$ reduction and enzyme-catalysed reduction of the corresponding diols **5B**$_{cis}$/**5B**$_{trans}$ and **8B**$_{cis}$/**8B**$_{trans}$, it was assumed that the *cis*- and *trans*-isomers of diols **1B**, **3B–8B** equilibrate *via* the corresponding acyclic isomers (**1C**, **3C–8C**). However, no direct evidence could be found for the characteristic aldehyde signals of compounds **1C**, **3C–6C** from ^{1}H and ^{13}C NMR or IR spectroscopy in $CDCl_3$ solution. On the basis of the spectral data presented, and the X-ray crystal structure analysis on the bis(methoxytrifluoromethylphenylacetate) [bis(MTPA)] esters of dihydrodiol **4B**$_{cis}$ and **5B**$_{trans}$ (unpublished data from these laboratories), the products resulting from dioxygenase-catalysed dihydroxylation at the C-2–C-3 bonds of the heteroarenes **1A**, **3A–8A** are clearly mixtures of the corresponding *cis*- and *trans*-isomers with very minor (as yet undetected) contributions from the corresponding aldehydes **1C** and **3C–6C** or ketones **7C** and **8C**. The proportion of each heterocyclic *cis*/*trans*-diol isomer **1B**, **3B–6B**, was found to be solvent dependent. Thus, the *trans* geometry was favoured (80–100%) in hydroxylic solvents (D_2O or CD_3OD) while in non-hydroxylic solvents ($CDCl_3$) the *cis*-isomer was dominant (Table 1).

Recrystallization from hydroxylic solvents yielded pure samples of **4B**$_{trans}$ and **6B**$_{trans}$ while pure samples of **4B**$_{cis}$, **6B**$_{cis}$ and **7B**$_{cis}$ were crystallized from non-hydroxylic solvents. In order to circumvent the problem of optical rotation measurements of equilibrating *cis*/*trans* mixtures of isomers of the chiral heterocyclic diols **1B**, **3B–8B**, the *cis*-isomers were exclusively converted to and characterised as the corresponding phenylboronate derivatives (Table 1). The enantiomeric excess values were determined by formation of the corresponding MTPA esters and 2-(1-methoxyethyl)phenylboronates (MPB).[5] It is noteworthy that the heterocyclic dihydrodiol metabolites obtained in the present study **1B**$_{cis}$/**1B**$_{trans}$ and **3B**$_{cis}$/**3B**$_{trans}$–**8B**$_{cis}$/**8B**$_{trans}$ were of variable enantiopurity (9–98% ee). The possibility of racemisation of the diol metabolites **1B**, **3B–8B** occurring *via* the corresponding aldehyde (**1C**, **3C–6C**) or ketone isomers (**7C**, **8C**) during the biotransformation cannot be excluded. The absolute configurations were determined by comparison of circular dichroism spectra (**4B**$_{trans}$, **6B**$_{trans}$), by ^{1}H NMR analysis of the MTPA and MPB derivatives (**1B**, **3B–8B**) and by X-ray analysis of the bis(MTPA) esters of **4B**$_{cis}$ and **5B**$_{trans}$.

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Table 1 Isolated yields, optical rotations, *cis*:*trans* ratio in $CDCl_3$, enantiomeric excess values and absolute configurations of dihydrodiols **1B**$_{cis}$/**1B**$_{trans}$, **3B**$_{cis}$/**3B**$_{trans}$–**8B**$_{cis}$/**8B**$_{trans}$ from *P. putida* UV4

Compound	Yield (%)	$[\alpha]_D$[a]	Ratio	% Ee	Absolute configuration
1B$_{cis}$/**1B**$_{trans}$	*ca.* 1	−4	60:40	43	3*R*
3B$_{cis}$/**3B**$_{trans}$	11	−15	60:40	48	3*S*
4B$_{cis}$/**4B**$_{trans}$	15	+152	80:20	>98	3*R*
5B$_{cis}$/**5B**$_{trans}$	17	−34	60:40	55	3*R*
6B$_{cis}$/**6B**$_{trans}$	79	+237	78:22	>98	3*R*
7B$_{cis}$/**7B**$_{trans}$	3	+1	90:10	9	3*R*
8B$_{cis}$/**8B**$_{trans}$	8	+201	100:0	80	3*S*

[a] Determined as the *cis*-phenylboronate derivative.

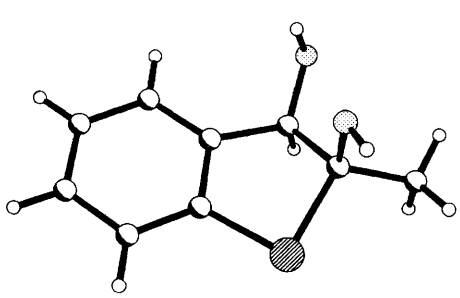

Fig. 1 Crystal structure of **7B**

Footnote

† *Crystal data* for **7B**$_{cis}$: $C_9H_{10}O_2S$, M = 182.2, monoclinic, space group $P2_1/n$, a = 5.539(2), b = 22.371(12), c = 14.120(8) Å, β = 101.98(3)°, U = 1717.6(2.0) Å^3, Z = 8, D_c = 1.409 Mg m^{-3}, μ(Mo-Kα) = 0.33 mm^{-1}, F(000) = 768, colourless block, 0.54 × 0.36 × 0.14 mm; Siemens P4 diffractometer; 2213 independent data collected at 153 K in 2θ range 5–45°, $0 \leq h \leq 5$, $0 \leq k \leq 24$, $-15 \leq l \leq 15$, direct methods solution (SHELXS-86) and full-matrix least-squares refinement on F^2 (SHELXL-93), anisotropic temperature factors for non hydrogens; hydrogens located but refined using riding model, $R1$ = 0.086, $wR2$ = 0.227 for 1340 data with $F_o > 4\sigma$ (F_o), G_oF = 0.97. Atomic coordinates, bond lengths and angles and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre (CCDC). See information for authors, Issue No. 1. Any request to the CCDC for this material should quote the full literature citation and the reference number 182/225.

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