Dioxygenase-catalysed dihydroxylation of arene *cis*-dihydrodiols and acetonide derivatives: a new approach to the synthesis of enantiopure tetraoxygenated bioproducts from arenes

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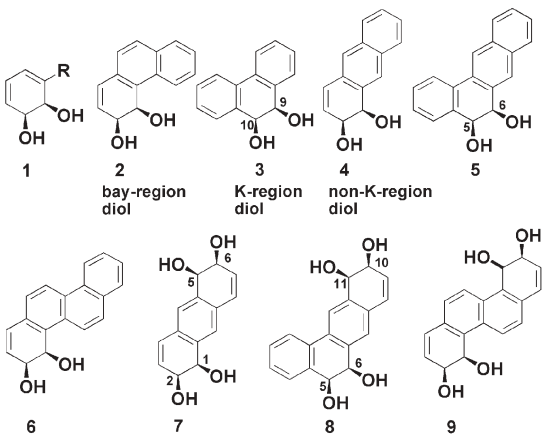
Received (in Cambridge, UK) 24th August 2006, Accepted 12th September 2006
First published as an Advance Article on the web 5th October 2006
DOI: 10.1039/b612191h

cis-Dihydrodiols of anthracene and benz[*a*]anthracene, and acetonide derivatives of the _cis_-dihydrodiols of benzene, fluorobenzene, biphenyl and phenanthrene have been identified as substrates for dioxygenase enzymes, yielding the corresponding enantiopure arene bioproducts, bis(_cis_-dihydrodiol)s and _cis_-diol acetonides respectively.

Mutant bacterial strains containing dioxygenases, *e.g.* toluene and biphenyl dioxygenases (TDO and BPDO), but lacking in the corresponding diol dehydrogenase enzymes, have been used to produce *cis*-dihydrodiol metabolites (*cis*-diols), from substituted monocyclic, *e.g.* **1** using TDO, and polycyclic arenes, *e.g.* **2**, **4** and **6** with BPDO, which have in turn been used widely in synthesis.[1a–f]



BPDO-catalysed *cis*-dihydroxylations of polycyclic aromatic hydrocarbons (PAHs), were mainly achieved using the B8/36 mutant strain of *Sphingomonas yaniokuyae* (originally described as a *Beijerinckia* strain).[2a–h] The active site in this type of dioxygenase was of sufficient size to accommodate larger PAH substrates including anthracene,[2a,2b] phenanthrene,[2a,2c] benz[*a*]anthracene,[2d,2e] and chrysene.[2f–2h] Thus, using *S. yanoikuyae* B8/36 whole cells, it was possible to obtain *cis*-diol metabolites, *e.g.* **2**, **4** and **6** from the corresponding PAHs. Regioselectivity during BPDO-catalysed arene *cis*-dihydroxylation was strongly favoured at relatively hindered arene bonds proximate to a bay region, *e.g.* diols **2** and **6** from phenanthrene and chrysene respectively. Conversely, no *cis*-dihydroxylation was observed at the more accessible regions of highest electron density *i.e.* the K-regions of phenanthrene (9,10 bond to give *cis*-diol **3**) or benz[*a*]anthracene (5,6 bond to give *cis*-diol **5**). *cis*-Diols **1**(R = H, F, Ph), **2** and **4** are among the bacterial metabolites isolated earlier and examined as substrates in the current study. The K-region *cis*-dihydrodiols **3** and **5**, synthesised by chemical dihydroxylation ($RuCl_3/NaIO_4$) of phenanthrene and benz[*a*]anthracene, were also tested. Using chrysene or *cis*-diol metabolite **6**, as substrates for *S. yanoikuyae* B8/36, revealed the presence of a second, less stable bioproduct. This was identified as bis(*cis*-dihydrodiol) **9**, the first member of a new family of tetraol metabolites.[2h] It was assumed that the presence of two favoured bay regions in chrysene was an important factor in the formation of bis(*cis*-diol) **9**.

Many arene *cis*-diol metabolites (>300) have been isolated,[1a–f] often in relatively large quantities (10–100 g). However, until the present, bis(*cis*-dihydrodiol) **9** was the sole representative member of this family of PAH metabolites. As part of our quest to find further members of this elusive family of metabolites, a series of monocyclic *cis*-diols **1** (R = H, F, Ph) and the bicyclic *cis*-diol from naphthalene, all obtained earlier in good yields using *P. putida* UV4 (a source of TDO), were examined as substrates for the same mutant strain. Similarly *cis*-dihydrodiols **2–6**, derived from the corresponding PAHs, were also tested as substrates for the same mutant strain containing BPDO. No evidence of bis(*cis*-dihydrodiol) formation was found using monocyclic *cis*-diols **1** (R = H, F, Ph) or PAH *cis*-diols of naphthalene and phenanthrene (**2** or **3**).

The bis(*cis*-diol) metabolite **7** was, however, obtained from further biotransformation of the non-K-region *cis*-diol of anthracene **4**, using *S. yanoikuyae* B8/36 under conditions reported earlier.[2f,2g] Metabolite **7** was isolated by PLC (20% yield, $[\alpha]_D$ +136, MeOH, >98% ee). Based on spectroscopic (NMR, MS) and stereochemical correlation studies, its absolute configuration was assigned as (1*R*,2*S*,5*R*,6*S*). Under similar conditions, the racemic K-region *cis*-diol **5** yielded a bis(*cis*-dihydrodiol) metabolite **8** after PLC purification (7% yield, $[\alpha]_D$ +106, MeOH, >98% ee). The recovered sample of *cis*-dihydrodiol **5** was found to be slightly enriched in one enantiomer (60% recovered yield, $[\alpha]_D$ −8, THF, 6% ee) whose (5*R*,6*S*) absolute configuration had been assigned earlier.[3] The exclusive BPDO-catalysed *cis*-dihydroxylation of the (5*S*,6*R*) enantiomer of *cis*-diol **5**, allied to spectroscopic studies of the metabolite and its derivatives, allowed the (5*S*,6*R*,10*S*,11*R*) absolute configuration to be assigned to bis(*cis*-dihydrodiol) **8**. It is noteworthy that all members of the PAH bis(*cis*-dihydrodiol)

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family (tetraols **7–9**) had similar relative and absolute configurations; this resulted from the second *cis*-dihydroxylation occurring on a benzene ring separated by one or two other rings from, and *syn* to, the existing *cis*-diol moiety.

The observation that metabolism of *cis*-diols **4** and **5** yields bis(*cis*-diols) **7** and **8**, but that *cis*-diols of benzene **1** (R = H), substituted benzene **1** (R = F, Ph), naphthalene and phenanthrene (**2** and **3**) fail to form this type of metabolite, merits explanation. The relatively hydrophilic nature of the smaller *cis*-diol precursors **1** (R = H, F, Ph) and those from naphthalene could be an important factor. These *cis*-diols may already be sufficiently water-soluble to satisfy the mineralisation/detoxification requirements of the bacterial cells. While the relative water solubilities of the *cis*-diol substrates from PAHs are currently unknown, they may parallel those of the parent PAHs whose values (mg L^{-1}) followed the sequence naphthalene (31.7) > biphenyl (7.0) > phenanthrene > (1.29) ≫ anthracene (0.073) ≫ benz[*a*]anthracene (0.014).[4] On this basis, the formation of the bis(*cis*-diols) of anthracene **7** and benz[*a*]anthracene **8** in low yields may be due to the lower water solubilities of *cis*-diol substrates **4** and **5**. Further factors for failure may include (i) the inability of TDO or BPDO to catalyse *cis*-dihydroxylations on adjacent PAH rings, *e.g. cis*-diols of naphthalene and phenanthrene **3**, (ii) bay region steric congestion, *e.g. cis*-diol **2** and (iii) instability of the bis(*cis*-diols).

Reduction of the hydrophilicity of the monocyclic *cis*-dihydrodiols **1** (R = H, F, Ph), was achieved by protection as the corresponding acetonide derivatives. The biotransformation (*P. putida* UV4) of *meso*-acetonide **10**, using reported conditions,[5] resulted in an *anti*-*cis*-dihydroxylation to give (1*S*,2*R*,3*S*,4*S*)-*cis*-diol acetonide $\mathbf{11_S}$, (80% yield, $[\alpha]_D$ +152, $CHCl_3$, >98% ee) as the sole metabolite (Scheme 1).

Metabolite $\mathbf{11_S}$ is a valuable secondary synthon which is normally difficult to obtain. The other enantiomer of this acetonide, $\mathbf{11_R}$, is available from osmylation and dehalogenation of the *cis*-dihydrodiol acetonides of bromo- or iodobenzene (Scheme 1). Compound $\mathbf{11_R}$ has been widely used as a precursor of pinitols, conduritols and inositols and other natural products.[1a–f] In addition to its potential use in the synthesis of the opposite enantiomers of natural products, using literature procedures, further applications are currently in progress as part of our continuing programme into the synthesis of unnatural products *e.g.* carbasugars.[6]

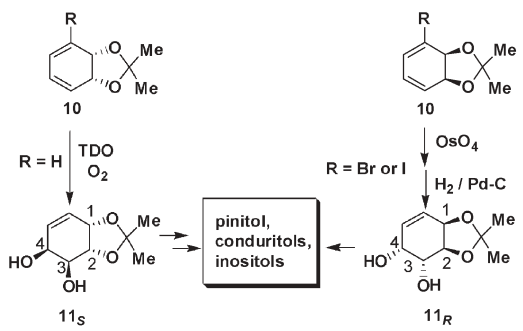
The new concept of converting a *cis*-diol into the less hydrophilic acetonide derivative, to enhance its acceptability as a substrate, was then tested on two other monocyclic arene *cis*-diol metabolites. Thus, TDO-catalysed dihydroxylation (*P. putida* UV4) of fluorobenzene yielded a mixture of *cis*-diol enantiomers $\mathbf{1_S}$ and $\mathbf{1_R}$ (*ca.* 80 : 20, R = F). This mixture was converted into the corresponding acetonide enantiomers $\mathbf{12_S}$ and $\mathbf{12_R}$ (*ca.* 80 : 20, 90% yield). *anti*-*cis*-Dihydroxylation was found to occur exclusively on one acetonide enantiomer $\mathbf{12_R}$, to yield the *cis*-diol acetonide (1*R*,2*R*,5*S*,6*S*)-$\mathbf{13_R}$ (10% yield, $[\alpha]_D$ +77, $CHCl_3$, >98% ee, Scheme 2) as the sole identified metabolite with a large proportion of residual substrate. The yield of *cis*-diol acetonide $\mathbf{13_R}$ was much lower than that of metabolite $\mathbf{11_S}$. This was assumed to be due to the restricted capacity of the TDO active site to accommodate larger substrates.

The more stable acetonide derivative **14** of the (1*S*,2*R*)-cis-diol of biphenyl **1** (R = Ph), was used as a substrate for the BPDO enzyme (*S. yanoikuyae* B8/36). Recovered substrate (30% yield) and a single *cis*-diol acetonide metabolite (1*S*,2*R*,1′*S*,2′*R*)-**15** (20% yield, $[\alpha]_D$ +280, >98% ee) were obtained as a result of *cis*-dihydroxylation of the phenyl ring. The enantiopurity and absolute configuration were established by spectroscopic analyses of the metabolite **15** and bis(acetonide) derivative **16** (90% yield, $[\alpha]_D$ +204, $CHCl_3$, >98% ee), which showed C_2 symmetry (Scheme 3).

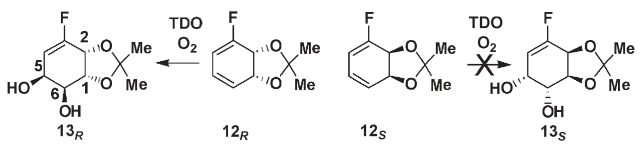
Finally, having established that acetonide derivatives of *cis*-diols, from the monocyclic arene series, can provide single enantiomer tetraoxygenated bioproducts, using both TDO ($\mathbf{11_S}$ and $\mathbf{13_R}$) and BPDO (**15**), the possibility of a similar process occurring in a member of the corresponding PAH series was then investigated. Acetonide **17**, from the bay region (3*S*,4*R*)-*cis*-diol **2**, was selected as a substrate of BPDO due to its structural similarity to acetonide **14** and produced two *cis*-diol acetonides (**18** and **19**). These were separated by HPLC (250 × 10 mm Primespher 5 C-18 column and 20% MeCN in H_2O as eluent).

The minor metabolite was easily identified as (3*S*,4*R*,5*R*,6*S*)-*cis*-diol **18** (3% yield, $[\alpha]_D$ +130, $CHCl_3$, >98% ee). Treatment with DMP–TsOH yielded the (3*S*,4*R*,5*R*,6*S*)-bis(acetonide) **20** (90% yield, $[\alpha]_D$ +16, $CHCl_3$). ^{1}H-NMR analysis of compound **20** showed that it also had C_2 symmetry. The formation of the minor metabolite *cis*-diol acetonide **20** was remarkable, since it must have resulted from BPDO-catalysed *anti*-*cis*-dihydroxylation in the sterically hindered bay region.

The absolute configuration of the major bioproduct **19** (15% yield, $[\alpha]_D$ +90, $CHCl_3$) was established as (3*S*,4*R*,7*S*,8*R*) through a stereochemical correlation sequence involving catalytic hydrogenation of the alkene bonds (Pd/C, H_2; 95% yield) followed by treatment with DMP–TsOH to give the bis(acetonide) **21** (97%



Scheme 1



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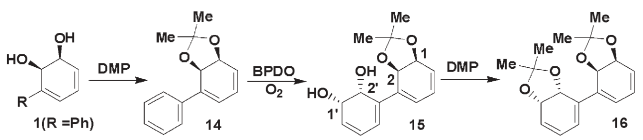
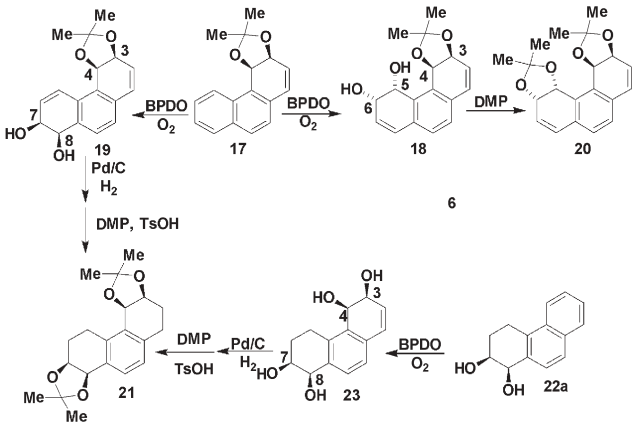


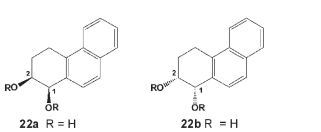
Scheme 3



Scheme 4

yield, $[\alpha]_D$ +72, $CHCl_3$, Scheme 4). To assign the relative and (3*S*,4*R*,7*S*,8*R*) absolute configuration of bis(acetonide) **21** (Scheme 4), a chemical resolution method for racemic *cis*-1,2,3,4-tetrahydrodiol **22a**–**22b** was developed. This was achieved by fractional crystallisation of the corresponding dicamphanate esters $\mathbf{22a_{cam}}$ and $\mathbf{22b_{cam}}$ formed from (1*S*)-camphanic chloride. Diastereoisomer $\mathbf{22a_{cam}}$ ($[\alpha]_D$ −140, $CHCl_3$) was crystallised from EtOH while a pure sample of the more soluble residual compound $\mathbf{22b_{cam}}$ ($[\alpha]_D$ +106, $CHCl_3$) was obtained by crystallisation from a mixture (3 : 2) of EtOH–$CHCl_3$. The absolute configuration of dicamphanate $\mathbf{22b_{cam}}$ was established as (1*S*,2*R*) by X-ray crystallography. It had been expected that the configuration would be determined relative to the known configuration of the camphanate groups but the fortuitous crystallisation of isomer $\mathbf{22b_{cam}}$ as the $CHCl_3$ solvate led to the independent determination of configuration as (1*S*,2*R*) from the anomalous X-ray scattering of the solvent Cl atoms† (Fig. 1).

Hydrolysis of dicamphanate $\mathbf{22a_{cam}}$ gave the (1*R*,2*S*) enantiomer of the tetrahydro-phenanthrene *cis*-diol **22a** ($[\alpha]_D$ −100, $CHCl_3$); it was then used as a substrate with *S. yanoikuyae* B8/36. *syn-cis*-Dihydroxylation occurred at the pseudo bay region and the (3*S*,4*R*,7*S*,8*R*) tetraol **23** was isolated as the sole metabolite (20% yield, $[\alpha]_D$ −123, MeOH). On hydrogenation, followed by reaction with DMP–TsOH, bis(acetonide) **21** (95% yield, $[\alpha]_D$ +66, $CHCl_3$), of identical absolute configuration to that formed from metabolite **19**, was obtained. Thus, the stereochemical correlation sequence (Scheme 4) established the absolute configuration of bis(acetonide) **21** as (3*S*,4*R*,7*S*,8*R*) with >98% ee. The major metabolite, *cis*-diol acetonide **19**, therefore, should also have been formed *via* a BPDO-catalysed *syn-cis*-dihydroxylation of acetonide **17**. The recovered sample, after addition of racemic *cis*-diol (**22a**–**22b**) as substrate, was found to be enantioenriched in enantiomer **22b**, indicating that kinetic resolution had occurred during BPDO-catalysed dihydroxylation.



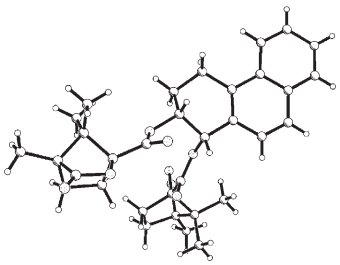


Fig. 1 Crystal structure of one of the two crystallographically independent molecules of (+)-*cis*-(1*S*,2*R*)-1,2,3,4-tetrahydro-phenanthrene dicamphanate $\mathbf{22b_{cam}}$.

In conclusion, this communication provides preliminary evidence of dioxygenase-catalysed *cis*-dihydroxylation to yield (i) enantiopure bis(*cis*-diol) metabolites of PAHs (**7**, **8**) at non-K region and bay region positions using BPDO, (ii) enantiopure *cis*-diol acetonides of monocyclic arenes ($\mathbf{11_S}$, $\mathbf{13_R}$, **15**) and tricyclic PAHs (**18**, **19**), using TDO and BPDO enzymes respectively.

We thank CenTACat and HEA NS Programme and Science Foundation Ireland (NDS) and the European Social Fund (TB, SMcG) for financial support. We also wish to acknowledge receipt of the *P. putida* UV4 and *S. yanoikuyae* B8/36 strains from the laboratories of Dr R. Holt (robert.holt(@)npilpharma.co.uk) and Prof. R. Parales (rparales@ucdavis.edu) respectively.

Notes and references

† CCDC reference number 618516. For crystallographic data in CIF or other electronic format see DOI: 10.1039/b612191h

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