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Multiple site dioxygenase-catalysed *cis*-dihydroxylation of polycyclic azaarenes to yield a new class of bis-*cis*-diol metabolites

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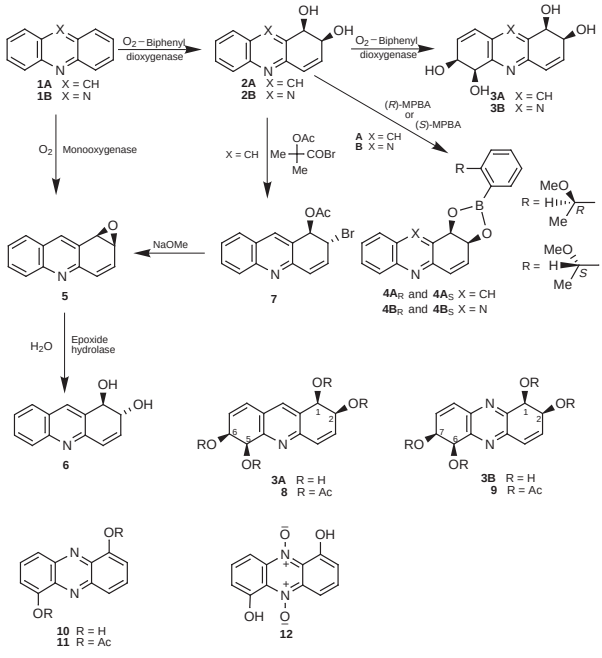
The enhanced stability of new mono-*cis*-dihydrodiol bacterial metabolites of tricyclic azaarenes has facilitated the dioxygenase-catalysed formation and isolation of the corresponding bis-*cis*-dihydrodiols (*cis*-tetraols) and a three step chemoenzymatic route to the derived arene oxide mammalian metabolites.

Dioxygenase-catalysed oxidation of mono- and poly-cyclic arenes by bacteria occurs widely in the environment.[1–3] Dioxygenase enzymes catalyse monohydroxylation (at benzylic and allylic centres), dihydroxylation (at alkene and arene bonds), and a combination of both yielding triol bioproducts (trihydroxylation of alkyl arenes).[1–3] *cis*-Dihydrodiol bioproducts are however very poor substrates for arene dioxygenase enzymes and prior to this communication no report of remote site bis-*cis*-dihydroxylation (tetrahydroxylation) has appeared.

The biodegradation of polycyclic aromatic hydrocarbons (PAHs) in eucaryotic systems, *e.g.* plants, animals and fungi, has frequently been found to proceed *via* monooxygenase-catalysed epoxidation followed by isomerisation to phenols or epoxide hydrolase-catalysed hydrolysis to *trans*-dihydrodiols.[4,5] In a typical example the oxidation of acridine **1A** using rat liver enzymes yielded 2-hydroxyacridine and *trans*-dihydrodiol **6** *via* the arene oxide intermediate **5A** (Scheme 1).[6–8]



Scheme 1

Other studies have shown that remote site oxidation of PAHs can occur with animal liver enzymes to yield combinations of phenol, epoxide and *trans*-diol derivatives in different benzene rings.[9–12] The small quantities of metabolites, *e.g.* bis-*trans*-dihydrodiols, available from such monooxygenase-catalysed (cytochrome-P450) oxidations[9] are generally insufficient to allow rigorous structural or stereochemical analysis.

cis-Dihydrodiol metabolites resulting from oxidation at the 5,6-bond of the bicyclic azaarenes quinoline,[13] 2-chloroquinoline [14] and 2-methoxyquinoline[14] were isolated using a mutant strain (UV4) of the bacterium *Pseudomonas putida* (a source of toluene dioxygenase). These bioproducts were found to be remarkably stable in comparison with their carbocyclic analogues, *e.g.* the 1,2-*cis*-dihydrodiol of naphthalene. On this premise it was anticipated that the corresponding *cis*-dihydrodiol metabolites **2A** and **2B**, if formed from the tricyclic azaarenes acridine **1A** and phenazine **1B**, would be much more stable and consequently could prove to be valuable synthetic intermediates.

Acridine **1A** was biotransformed using a mutant strain of the bacterium *Sphingomonas yanoikuyae* B8/36 (a source of biphenyl dioxygenase, BPDO) following the reported procedure.[15] After removal of the bacterial cells the bioproducts were then extracted with EtOAc to yield a relatively polar compound (R_f 0.2, 5% MeOH in $CHCl_3$) which was identified by spectral methods (NMR, MS) and elemental microanalysis as dihydrodiol **2A**. ^{1}H NMR spectroscopy established that *cis*-dihydroxylation had occurred exclusively at the 1,2-position ($J_{1,2}$ 4.7 Hz). Reaction of *cis*-diol **2A** ($[\alpha]_D$ +72, MeOH) with (*R*)-(+)- and (*S*)-(−)-2-(1-methoxyethyl)phenylboronic acid (MPBA) yielded the boronate derivatives **4A**$_R$ and **4A**$_S$ respectively. ^{1}H NMR analyses of the boronates confirmed that *cis*-diol **2A** was enantiopure (> 98% ee); the absolute configuration was determined as (*1R,2S*) by application of the empirical ^{1}H NMR rule earlier established for a series of MPBA derivatives from other *cis*-dihydrodiol metabolites of PAHs [15, 16] (Table 1). The (*1R,2S*) configuration for *cis*-diol **2A**

Table 1 Yields, optical rotations and absolute configurations for metabolites **2A**, **2B**, **3A** and **3B** obtained using *S. yaniokuyae* B8/36, and derivatives **8**, **9** and **5**

Compound	Isolated yield (%)	$[\alpha]_D/10^{-1}$ deg cm^2 g^{-1} (solvent)	Absolute configuration
2A	50–55	+72 (MeOH)	1*R*,2*S*
2B	40	+102 (MeOH)	1*R*,2*S*
3A	12	+266 (Pyridine)	1*R*,2*S*,5*R*,6*S*
3B	15	+180 (MeOH)	1*R*,2*S*,6*R*,7*S*
8	95	+63 ($CHCl_3$)	1*R*,2*S*,5*R*,6*S*
9	95	+83 ($CHCl_3$)	1*R*,2*S*,6*R*,7*S*
5	55	+30 ($CHCl_3$)	1*R*,2*S*

was independently confirmed by a stereochemical correlation process involving oxidative degradation of the derived 1,2-diacetoxy-1,2,3,4-tetrahydroacridine to give (2*S*,3*S*)-(−)-dimethyl (2,3-diacetoxy)adipate of known configuration.[17]

In later biotransformation studies of acridine **1A**, total removal of water from the centrifuged culture medium at 35–40 °C under reduced pressure, followed by extraction of the semi-solid residue with EtOAc–MeOH (9:1) yielded a mixture of *cis*-diol **2A** and a more polar metabolite (R_f 0.15, 12% MeOH in $CHCl_3$) which was identified as the bis-*cis* diol **3A** (Table 1) on the basis of ^{1}H NMR (COSY, NOE) and MS data and formation of tetraacetate **8**. The chirality of the bis-*cis*-diol **3A** suggested that it was formed by initial *cis*-dihydroxylation of acridine **1A** at the 1,2-bond on the *Si*:*Si* face of the molecule followed by further *cis*-dihydroxylation at the 5,6-bond again on the *Si*:*Si* face to yield the (*1R,2S,5R,6S*) enantiomer exclusively. Confirmation that the bis-*cis*-diol **3A** had been derived from the mono-*cis*-diol **2A** was obtained by its addition as substrate to *S. yaniokuyae* B8/36. The samples of bis-*cis*-diol **3A**, isolated from metabolism of either acridine **1A** or the mono-*cis*-diol **2A**, were found to be indistinguishable.

Biotransformation of phenazine **1B** with *S. yaniokuyae* B8/36 or *Pseudomonas putida* 9816/11 (a source of naphthalene dioxygenase, NDO), and the normal extraction procedure yielded, in both cases, a mono-*cis*-dihydrodiol (R_f 0.45, 10% MeOH in $CHCl_3$, 5% yield from NDO and 40% yield from BPDO) which was identified as *cis*-1,2-dihydroxy-1,2-dihydrophenazine **2B** from ^{1}H NMR ($J_{1,2}$ 4.3 Hz) and MS analyses. Formation of MPBA derivatives **4B**$_R$ and **4B**$_S$ of the mono-*cis*-diol **2B** and their ^{1}H NMR analyses established that it was enantiopure (> 98% ee) and of (*1R,2S*) configuration from both bacterial mutant strains. Application of the improved extraction procedure (EtOAc–MeOH after removal of water from the centrifuged bioextracts) led to the isolation of a mixture of (*1R,2S*)-mono-*cis*-diol **2B** with a second metabolite (R_f 0.12, 15% MeOH in $CHCl_3$) from the *S. yaniokuyae* B8/36 biotransformation. This very polar bioproduct was identified as the phenazine bis-*cis*-dihydrodiol **3B** from NMR, MS and CD spectral data and formation of tetraacetate **9**; the structure was confirmed by aromatisation (thermal dehydration) and acetylation of the resulting bis-phenol **10** to yield 1,6-diacetoxyphenazine **11**.[18]

When (*1R,2S*)-mono-*cis*-diol **2B** was added as substrate to *S. yaniokuyae* the bis-*cis*-diol **3B** was isolated as the sole metabolite. The CD spectra of the bis-*cis*-diols **3A** and **3B** were found to be very similar, as anticipated. Thus the absolute configurations **(***1R,2S,5R,6S***)** and **(***1R,2S,6R,7S***)** were assigned for metabolites **3A** and **3B**, respectively**.** 1,6-Dihydroxyphenazine **10**, obtained by dehydration of the metabolite bis-*cis*-dihydrodiol **3B**, and the derived 1,6-dihydroxyphenazine 5,6-dioxide (iodinin) **12** have also been isolated from among a range of phenazine antibiotics produced as secondary metabolites in other bacterial systems.[19]

A further manifestation of the stability of the mono-*cis*-dihydrodiol **2A** became apparent from the reaction with 2-acetoxyisobutyryl bromide. It was anticipated that the resulting product, 1-acetoxy-2-bromo-1,2-dihydroacridine **7**, would aromatise spontaneously. However, compound **7** proved to be sufficiently stable to be isolated and identified by ^{1}H NMR analysis (crude yield *ca.* 80%) prior to treatment with NaOMe to yield (*1R,2S*)-(+)-1,2-epoxy-1,2-dihydroacridine (acridine 1,2-oxide, **5**). Thus the eucaryotic metabolite **5**, derived from acridine **1A**, was obtained as a single enantiomer in two steps with an overall yield of *ca.* 55% from the procaryotic metabolite **2A**. This procedure compares favourably with our earlier method for the synthesis of enantiopure acridine 1,2-oxide **5**, an eight step synthesis with an overall yield of 18%.[4,8] It also represents a significant improvement over an earlier five step method for the synthesis of enantiopure arene oxides of PAHs from the corresponding *cis*-dihydrodiols.[20]

Preliminary studies have indicated that the two-step synthetic procedure (**2A**→**7**→ **5**) used for the arene oxide synthesis is also applicable to other relatively stable *cis*-dihydrodiol metabolites of bi- and tri-cyclic azaarenes, *e.g.* the *cis*-dihydrodiols of 2-chloroquinoline (5,6- and 7,8-).[14] All arene oxide derivatives of azaarenes (*e.g.* acridine 1,2-oxide **5**) were found to hydrolyse, under aqueous conditions, to the corresponding *trans*-dihydrodiols (*e.g.* **6**) by exclusive nucleophilic attack at the allylic position.[7,8] The *cis*-dihydrodiol **2A** was also a minor hydrolysis product of arene oxide **5**.[8]

Recent studies of the bacterial metabolism of tetracyclic arene substrates, each containing two bay regions (chrysene and benzo[*b*]naphtho[2,1-*d*]thiophene), using *S. yanoikuyae* B8/36, have shown the formation of relatively unstable bis-*cis*-dihydrodiols as minor metabolites (0.3 and 3% yield, respectively).[21] Thus the new family of enantiopure arene tetraol metabolites arising from sequential *cis*-dihydroxylation on the arene *Si*:*Si* face is not confined to the linear azaarene series and more examples are anticipated.

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