Dioxygenase-catalysed *cis*-dihydrodiol formation in the carbo- and hetero-cyclic rings of quinolines

D. R. Boyd,*[a]† N. D. Sharma,[a] J. G. Carroll,[a] J. F. Malone,[a] D. G. Mackerracher[a] and C. C. R. Allen[b]

[a] *The School of Chemistry, The Queen's University of Belfast, Belfast, UK BT9 5AG*
[b] *The QUESTOR Centre, The Queen's University of Belfast, Belfast, UK BT9 5AG*

Evidence of enzyme-catalysed *cis*-dihydroxylation in the pyridine (3,4-bond) and benzene rings (5,6- and 7,8-bonds) of quinoline and 2-substituted quinolines is examined in light of the isolation of a heterocyclic *cis*-diol derivative of 2-quinolone, as a single enantiomer of opposite absolute configuration to that found for the enantiopure carbocyclic *cis*-diol metabolites from quinolines.

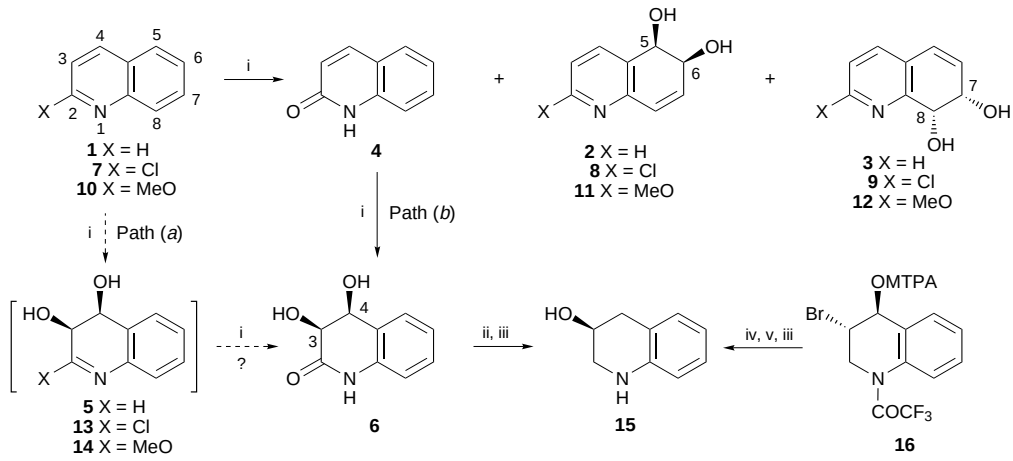
Dioxygenase-catalysed *cis*-dihydroxylation of arenes to yield the corresponding carbocyclic *cis*-dihydrodiol metabolites is well-established in the mono- and poly-cyclic aromatic hydrocarbon series.[1] Recently the first *cis,trans*-dihydrodiol metabolites from a heterocyclic aromatic ring, *e.g.* thiophene, benzothiophene and benzofuran, were isolated using the toluene-dioxygenase (TDO) system present in a mutant strain of the bacterium *Pseudomonas putida* UV4.[2,3] To date, however, no direct evidence for the formation of a *cis*-dihydrodiol metabolite from a pyridine ring has been reported. Indeed, the mechanism of bacterial metabolism of pyridine rings is not clearly understood.[4]

As part of an earlier study of the bacterial metabolism of bicyclic azaarenes using *P. putida* UV4, *cis*-dihydroxylation of the carcinogen quinoline **1** was observed to occur at the carbocyclic ring to yield *cis*-dihydrodiols **2** and **3** in very low yields (Scheme 1).[5] The major metabolites formed in the pyridine ring of **1** were 3-hydroxyquinoline and anthranilic acid. The phenolic metabolite was postulated to be derived from the initially formed unstable *cis*-dihydrodiol **5** of unknown absolute configuration,[5] while anthranilic acid could have been derived from either **5** or **4**. Previous studies[6] of the metabolism of **1** with different strains of *P. putida, P. fluorescens* and a *Rhodococcus* species did not indicate the formation of any *cis*-dihydrodiols, *e.g.* **2** or **3**, but reported oxidation of the pyridine ring to yield **4**.

Encouraged by the recent isolation of *cis*-dihydrodiol metabolites of five-membered aromatic heterocycles[2,3] and of 3-hydroxyquinoline (from bacterial metabolism of **1**),[5] a new approach to obtain direct evidence of *cis*-diol formation in a pyridine ring was sought. It was assumed that our earlier, unsuccessful, efforts to isolate the *cis*-diol **5** from **1** was due to its instability, and to competition from oxidation at position 2 in the pyridine ring yielding **4** and anthranilic acid. To test this assumption the alternative approach of using 2-substituted quinolines **7** and **10** as substrates was adopted. It was hoped that the chloro and methoxy groups present at the 2-position might be readily replaced by OH groups as a result of spontaneous hydrolysis of the initial unstable *cis*-dihydrodiol metabolites **13** and **14**, respectively, to yield the more stable *cis*-diol **6** (Scheme 1). Furthermore it was anticipated that substitution would block oxidation at the 2-position and formation of **4**, thus increasing the yields of other metabolites. Oxidation at C-2 is a common initial step in the bacterial metabolism of quinoline *via* either the 'coumarin' or '*meta*-cleavage' pathways.[4,6]

Addition of **7** to growing cultures of *P. putida* UV4 yielded two carbocyclic *cis*-dihydrodiols **8** (R_f 0.3, 8%) and **9** (R_f 0.45, 30%) as major metabolites (Scheme 1) which were separated using PLC (MeOH–$CHCl_3$, 7 : 93). Formation of the cyclic boronate derivatives using (*S*)-(−)- and (*R*)-(+)-2-(1-ethoxyethyl)phenylboronic acid (MPBA)[7] and their ^{1}H NMR analysis proved that *cis*-dihydrodiols **8** and **9** were both enantiopure, *i.e.* ⩾98% ee. The absolute configurations of the *cis*-dihydrodiols **8** (5*R*,6*S*) and **9** (7*S*,8*R*) were established by comparison of CD spectra and by stereochemical correlation with the corresponding *cis*-dihydrodiols **2** and **3** of known configuration[5] (Table 1).

On the basis of NMR, mass and IR spectroscopy and elemental microanalysis, the least abundant (2%) and most polar metabolite **6** (R_f 0.14) proved to be a *cis*-diol ($J_{3,4}$ = 3.7 Hz) within a δ-lactam ring. The enantiopurity (⩾98% ee) of diol **6** was determined by formation of a monocamphanate derivative from esterification of the hydroxy group at C-4. The structure, relative configuration and absolute configuration of



Scheme 1 *Reagents and conditions*: i, *P. putida* UV4, O_2; ii, TBDMS OTf; iii, $LiAlH_4$; iv, Ac_2O, AgOAc; v, NaOH, MeOH

Table 1 Data for *cis*-diol metabolites and derivatives from **7** and **10**

Compound	$[\alpha]_D$ (MeOH)/ 10^{-1} deg cm^2 g^{-1}	Configuration
8	+140	5*R*,6*S*
9	+148	8*R*,7*S*
11	+8	5*R*,6*S*
12	+20	8*R*,7*S*
6	+6[a]	3*S*,4*S*
15	+39	3*S*

[a] Pyridine solvent

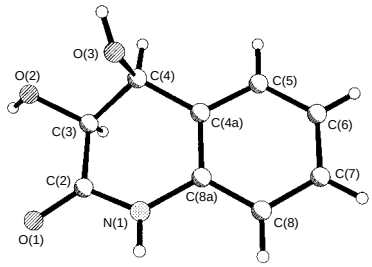


Fig. 1 Crystal structure of metabolite **6**

cis-diol **6** was established by X-ray crystal structure analysis.‡ The preferred conformation of **6** in the crystalline state (Fig. 1) contains the OH groups at C-3 and C-4 in pseudo-equatorial and -axial positions, respectively. The (3*S*,4*S*) absolute configuration deduced from the X-ray study was confirmed by stereochemical correlation with **15** (Scheme 1) which, in turn, has been correlated to the known configuration of the 2-methoxy-2-trifluoromethyl-2-phenylacetate (MTPA) ester derivative **16**.[8] In solution the OH group at C-3 could not be readily converted to a TBDMS ether or camphanate ester derivative, suggesting that *intramolecular* H-bonding to the amide C=O might be present. In the solid state, however, only *intramolecular* H-bonding was observed, each molecule being involved in a total of six H-bonding interactions to three different neighbours. Diol **6** proved to be remarkably stable compared with *cis*-dihydrodiols **2**, **3**, **8** and **9**. Thus while the latter compounds were found to aromatise under acidic conditions (dilute HCl), **6** remained unchanged. An earlier report on the bacterial metabolism of **7** described only the formation of a single *cis*-dihydrodiol **9** in the carbocyclic ring of unspecified ee and absolute configuration.[9]

Addition of **10** as substrate to *P. putida* UV4 also yielded two carbocyclic *cis*-dihydrodiols, **11** (R_f 0.30, 2%) and **12** (R_f 0.4, 7%) (Scheme 1). Similar stereochemical analysis methods to those used for **8** and **9** (NMR and CD spectroscopy) again showed that single enantiomers of configuration indicated in Table 1 [(5*R*,6*S*) and (8*R*,7*S*), respectively] had been formed. The more abundant (13%) and more polar *cis*-diol metabolite from **10** was found to be of identical structure, ee and absolute configuration [(3*S*,4*S*)] to **6** derived from **7**. The optical rotations and absolute configurations of **6**, **8**, **9**, **11** and **12**, and the derived monol **15**, are shown in Table 1.

The formation of the stable (3*S*,4*S*) enantiomer of *cis*-diol **6** as a bacterial metabolite from both **7** and **10** may be explained by a metabolic sequence involving (i) stereoselective *cis*-dihydroxylation to yield the unstable diols **13** and **14**, respectively, and (ii) hydrolysis to yield the stable diol **6** [Scheme 1, path (*a*)]. However, past work on the dioxygenase-catalysed *cis*-dihydroxylations of a range of carbocyclic and five-membered heterocyclic arenes had shown an exclusive or marked preference for the opposite absolute configuration (*e.g.* *cis*-diols **8**, **9**, **11** and **12**), which could be considered as the normal absolute configuration for arene *cis*-diols.

Based on several additional observations an alternative sequence involving partial hydrolysis of substrates **7** and **10** to yield **4**, followed by dioxygenase-catalysed *cis*-dihydroxylation to yield *cis*-diol **6**, appears to be more plausible [Scheme 1, path(*b*)]. Thus, traces of **4** were detected (using GC–MS and ^{1}H NMR analysis) during the biotransformation of **10**, and when **4** was added to *P. putida* UV4 under the normal biotransformation conditions, *cis*-diol **6** (10% isolated yield) of identical ee and absolute configuration to that obtained from **7** and **10** was isolated as a metabolite.

The (3*S*,4*S*) absolute configuration of *cis*-diol **6** derived from the TDO-catalysed dihydroxylation of the pyridine ring in **4**, **7** and **10** seems to be abnormal when compared with that found during TDO-catalysed *cis*-dihydroxylation of arenes in general. The opposite absolute configuration had been observed for the *cis*-diol metabolites of a series of benzocycloalkenes, *e.g.* 1,2-dihydronaphthalene, and the heterocyclic analogues, *e.g.* chromene and thiochromene, using the TDO biocatalyst.[1] The (3*S*,4*S*) configuration of *cis*-diol **6** would be expected if the substrates **7** and **10** were to undergo partial hydrolysis to yield **4**, and if it were to be accepted as a benzocycloalkene-type substrate by the TDO system.

Enzyme-catalysed oxidation of pyridine rings containing alkyl,[4] aryl[8] and thioalkyl substituents[10] has generally been found to occur at the exocyclic substituents, indicating that *cis*-dihydroxylation of a pyridine ring is not a preferred metabolic step, and hence the formation of the *cis*-diol **6** appears to be unusual. Although the formation of compound **6** is still consistent with either TDO-catalysed *cis*-dihydroxylation of the 2-substituted quinoline substrates **7** and **10** [Scheme 1, path (*a*)] or the derived 2-quinolone **4** [Scheme 1, path (*b*)], the currently available evidence is strongly in favour of the latter pathway.

We thank the BBSRC (N. D. S.) and the Queen's University Environmental Science and Technology Research (QUESTOR) Centre (C. C. R. A., J. C.) for financial support, and Eric Becker (Mannheim) and David Clarke (QUESTOR) for valuable assistance with the biotransformations.

Notes and References

† E-mail: dr.boyd@qub.ac.uk

‡ *Crystal data* for **6**: $C_9H_9NO_3$, M = 179.2, monoclinic, $P2_1$, a = 5.453(4), b = 11.039(8), c = 6.762(5) Å, β = 100.14(6)°, V = 400.7(5) Å^3, Z = 2, D_c = 1.485 g cm^{-3}, $F(000)$ = 188, μ(CuKα) = 0.95 mm^{-1}, 828 unique data (and Friedel opposites) (θ_{max} = 50°), 735 with $I > 2\sigma(I)$, R_1 = 0.057, wR_2 (all data) = 0.097, GOF = 0.99, absolute structure parameter −0.3(4). Data were collected on a Siemens P3 diffractometer at 293 K using Cu-Kα radiation, λ = 1.5418 Å; structure determination and refinement using SHELXS-86 and SHELXL-93, respectively; full-matrix least-squares refinement with allowance for anisotropic thermal parameters for non-hydrogen atoms; hydrogens included as riding atoms at positions calculated from the geometry of the molecule, except for the hydrogens of the OH groups, which were included at positions located in a difference Fourier map and refined as free atoms. CCDC 182/770

1 D. R. Boyd and G. N. Sheldrake, *Nat. Prod. Rep.*, 1998, in the press.
2 D. R. Boyd, N. D. Sharma, R. Boyle, B. T. McMurray, T. A. Evans, J. F. Malone, H. Dalton, J. Chima and G. N. Sheldrake, *J. Chem. Soc., Chem. Commun.*, 1993, 49.
3 D. R. Boyd, N. D. Sharma, I. N. Brannigan, D. A. Clarke, H. Dalton, S. A. Haughey and J. F. Malone, *Chem. Commun.*, 1996, 2361.
4 J.-P. Kaiser, Y. Feng and J.-M. Bollag, *Microbiol. Rev.*, 1996, 483.
5 D. R. Boyd, N. D. Sharma, M. R. J. Dorrity, M. V. Hand, R. A. S. McMordie, J. F. Malone, H. P. Porter, J. Chima, H. Dalton and G. N. Sheldrake, *J. Chem. Soc., Perkin Trans. 1*, 1993, 1065.
6 G. Schwartz, R. Bauder, M. Speer, T. O. Rommel and F. Lingens, *Biol. Chem. Hoppe-Seyler*, 1989, **370**, 1183.
7 S. M. Resnick, D. S. Torok and D. T. Gibson, *J. Org. Chem.*, 1995, **60**, 3546.
8 D. R. Boyd, N. D. Sharma and J. F. Malone, unpublished data.
9 S. Fetzner, B. Vogler and F. Lingens, *FEMS Microbiol. Lett.*, 1993, **112**, 151.
10 D. R. Boyd, H. Dalton, N. D. Sharma, S. A. Haughey, R. A. S. McMordie, B. T. McMurray, K. Sproule and G. N. Sheldrake, *J. Chem. Soc., Chem. Commun.*, 1995, 119.

Received in Cambridge, UK, 22nd December 1997; 7/09138I