Bacterial Aromatic Hydroxylation: *cis*-Dihydrodiol Metabolites and their Possible Role in the 'NIH Shift'

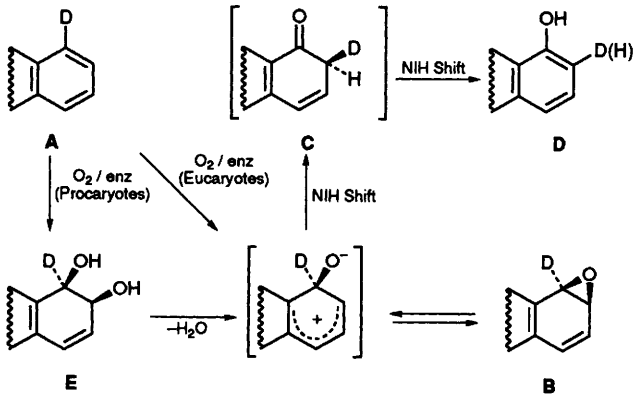
Stephen A. Barr,[a] Derek R. Boyd,*[a] Narain D. Sharma,[a] Lynne Hamilton,[a] R. Austin S. McMordie[a] and Howard Dalton*[b]

[a] *School of Chemistry, The Queen's University of Belfast, Belfast, UK BT9 5AG*
[b] *Department of Biological Sciences, The University of Warwick, Coventry, UK CV4 7AL*

cis-Dihydrodiol and arene oxide metabolites of carbocyclic and heterocyclic arenes yield the corresponding phenols *via* an 'NIH Shift' mechanism.

A mutant strain (UV4) of the soil bacterium *Pseudomonas putida* has yielded both *cis*-dihydrodiol and phenolic metabolites from a range of bicyclic arenes including quinoline,[1] isoquinoline,[1] quinoxaline,[1] benzofuran,[2] and benzothiophene.[3] Aromatic hydroxylation occurred in either the heterocyclic (3-hydroxyquinoline,[1] 2- and 4-hydroxyisoquinoline,[1] 2-hydroxyqinoxaline[1]), or the carbocyclic (8-hydroxyquinoline,[1] 5- and 8-hydroxyisoquinoline,[1] 6-hydroxybenzofuran[2] and 5-hydroxybenzothiophene[3]) rings. The isolation of *cis*-dihydrodiol and phenol metabolites from *P. putida* UV4, after biotransformation of monocyclic arene substrates, bearing electron donating substituents (*e.g.* 2-thiomethoxytoluene), has also recently been observed.[3] Bacterial oxidation of other arenes to the corresponding phenols has been widely reported in the literature.[4–6] While many bicyclic *cis*-dihydrodiol metabolites proved to be relatively stable, a number rapidly aromatised to phenolic products during the biotransformation or attempted isolation procedure. The remarkable difference in the stability, of *cis*-dihydrodiol derivatives of monosubstituted benzene substrates, under acid conditions (>10^6-fold),[7] suggests that some bacterial (procaryotic) aromatic hydroxylations may proceed *via* spontaneous dehydration of unstable *cis*-dihydrodiol intermediates which are formed by dioxygenase-catalysed oxidation.

The aromatic hydroxylation of polycyclic arenes in eucaryotic systems (*e.g.* fungi, plants, animals) generally occurs by monooxygenase-catalysed oxidation. Thus, arene oxide intermediates,[8] *e.g.* of naphthalene[9] and quinoline,[10] have been intercepted during liver microsomal oxidation of arenes. The spontaneous isomerization of arene oxide intermediates has been widely used to explain the migration to and retention of a hydrogen isotope (or a halogen atom), at a neighbouring position (relative to the labelled position in the arene substrate **A**), during aromatic hydroxylation.[8] The latter phenomenon is described as the 'NIH shift' (an example of a typical 'NIH Shift' is shown in Scheme 1) and has been observed during many enzyme-catalysed aromatic hydroxylations (≫100 examples).[8] While the spontaneous isomerisation of arene oxide intermediates **B** to phenols **D** is consistent with the 'NIH shift' mechanism, such intermediates are not obligatory as an identical result can also be obtained by direct loss of a proton from a β-hydroxy carbocation (zwitterion intermediate).[11] Kinetic studies of the acid-catalysed dehydration of unlabelled *cis*-dihydrodiol derivatives of monosubstituted benzenes[7] are consistent with reaction *via* a carbocation intermediate, but fail to distinguish deprotonation of the carbocation from rearrangement to a ketodiene, *e.g.* **C** (*via* an 'NIH shift') as alternative pathways to the phenolic product. This communication now provides the required experimental evidence of phenol formation from labelled *cis*-dihydrodiol intermediates **E** *via* an 'NIH shift' mechanism.



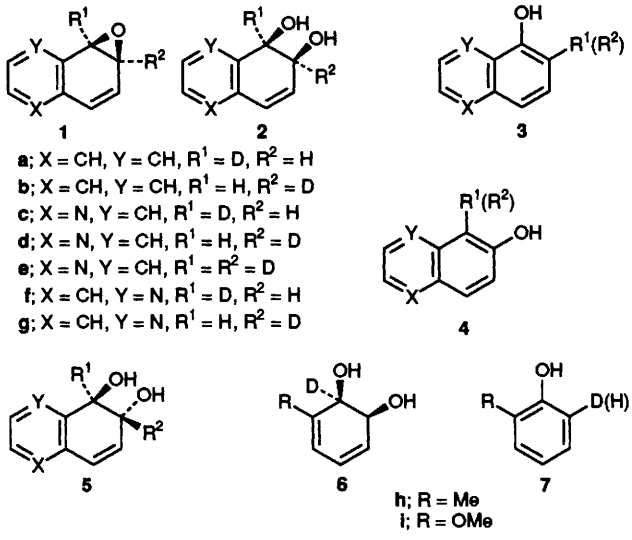
Scheme 1

Phenols have been isolated as bacterial metabolites of naphthalene and quinoline,[1–4] and thus a series of specifically labelled arene oxide **1a–1g** and *cis*-dihydrodiol **2a–2e** derivatives of naphthalene and quinoline were synthesised for this study. The arene oxides **1c–1g** were obtained by reported chemical synthetic procedures,[12] using specifically D-labelled dihydroarene precursors. The 'NIH shift' mechanism during aromatisation of an arene oxide derivative of a heterocyclic arene had not previously been established. The results shown in Table 1 are compiled from GC–MS analysis of the trimethyl silyl ethers formed from the main phenolic products, and are consistent with earlier evidence[13] for an 'NIH shift' mechanism during aromatisation of labelled naphthalene-1,2-oxides **1a** and **1b**, but have now been extended to neutral aqueous (80 and 81% D, respectively), neat thermal (73% D) and acidic (49% D) conditions. Aromatisation of the arene oxides of quinoline **1f** and **1g** under acidic conditions, to yield mainly phenol **3f**, also showed a significant degree of migration and retention of label (79, 53% D, respectively). As protonation of the nitrogen atom readily occurs under acidic conditions aromatisation of the quinoline derivatives was generally carried out thermally (sealed tube, 120–180 °C). the monodeuteriated arene oxides **1f** and **1g** again showed the 'NIH shift' but with lower % D values (56–71 and 42–46,

Table 1 Percentage deuterium retention in aromatised phenols

Substrate	Product	Conditions	D Retention (%)
1a	**3a**	Δ (H$^+$)	73,[a] 80[b] (49[c])
1b	**3a**	Room temp.	81[b]
1c	**3c**	Δ	43,[d] 50[e]
1d	**3c**	Δ	69[d]
1e	**3c**	Δ	60–70[f]
1f	**3f**	Δ (H$^+$)	56,[d] 71[e] (79[g])
1g	**3f**	Δ (H$^+$)	42,[d] 46[e] (53[g])
2a	**3a**	Δ (H$^+$)	73[a] (45[c])
2b	**3a**	Δ	53[h]
2c	**4c**	Δ	12[i]
2d	**4c**	Δ	15[i]
2e	**4c**	Δ	45[i]
5f	**4c**	Δ	51[i]
5g	**4c**	Δ	49[i]
6h	**7h**	Δ	34[a]
6i	**7h**	Δ	28[a]

Aromatization conditions: [a] 150 °C, 5 min; [b] pH 7 buffer; [c] pH 1.8 buffer; [d] 130 °C, 10 min; [e] 120 °C, 15 min; [f] 130–180 °C, 5–45 min; [g] CF_3CO_2H; [h] 150 °C, 15 min; [i] 180 °C, 60 min



respectively). The thermal isomerisation of the dideuteriated arene oxide **1e** resulted mainly in the formation of phenol **3c** the % D content of which was reduced from the expected value of 100% to *ca.* 60–70%. The loss of deuterium was assumed to be due to an exchange process since the unlabelled phenols **3a** and **3c** were found to show a significant degree of D incorporation when heated in the presence of a trace of D_2O. The differences in % D retained between experiments may be accounted for by differing contributions from this exchange mechanism.

The specifically labelled *cis*-dihydrodiol derivatives of naphthalene **2a** and **2b** and quinoline **2c**, **2d** and **2e** were synthesised by bacterial metabolism of the corresponding D-labelled arene substrates using *P. putida* UV4. The *cis*-dihydrodiol metabolites of naphthalene **2a** and **2b** were found to yield mainly 1-naphthol **3a** by dehydration and showed clear evidence of the 'NIH shift' under both acidic (45% D) and neutral (75, 53% D) conditions. Aromatisation of the *cis*-dihydrodiol metabolites of quinoline **2c** and **2d** under thermal conditions, to yield the phenol **4c** as the major product, also showed the 'NIH shift' but with a lower retention of the D label (12, 15% D, respectively). The reduced values must again be due to an exchange process since thermal dehydration of the dideuteriated *cis*-dihydrodiol **2e** similarly yielded phenol **4c** but with D_2 content reduced to *ca.* 45%. Owing to the instability and thus unavailability of *cis*-7,8-dihydroxy-7,8-dihydroquinoline, in sufficient quantities, from *P. putida* UV4 biotransformations, chemically synthesised[12] samples of the corresponding specifically labelled *trans*-dihydrodiols **5f** and **5g** were instead thermally dehydrated. As anticipated, the 'NIH shift' was observed (51, 49% D, respectively) in the major phenolic product **4c**. Although dehydration of *trans*-dihydrodiols can also involve the 'NIH shift',[14] the reaction is generally much slower than for the corresponding *cis*-dihydrodiols, and may therefore be a less common pathway in the production of phenols during enzyme-catalysed oxidation of arenes. Dehydration of dihydrodiols **2c**, **2d**, **2e**, **5f** and **5g** to yield largely the common phenolic product **4c** is assumed to occur by a similar procedure to that shown for the formation of the isomeric phenol in Scheme 1.

Having established that the 'NIH shift' mechanism occurs during the dehydration of *cis*-dihydrodiol metabolites of bicyclic arenes, two samples of monodeuteriated monocyclic arene derivatives were included for examination. Thermal dehydration of the *cis*-dihydrodiols, obtained by dioxygenase-catalysed oxidation of 2D-toluene **6h** and 2D-anisole **6i** in *P. putida* UV4, showed a significant degree of migration and retention of label (34 and 28%, respectively, after correction for the presence of only one D atom at the two *ortho* positions) in the corresponding major phenols **7h** and **7i**.

The main conclusions of this study are: (i) the 'NIH shift' may occur during dehydration of a *cis*-dihydrodiol intermediate (obtained as a result of dioxygenase-catalysed oxidation of an arene) or rearrangement of an arene oxide intermediate or dehydration of a *trans*-dihydrodiol (formed as a result of monooxygenase-catalysed oxidation of an arene) *via* a common ketodiene intermediate. This means that the migration and retention of label during aromatic hydroxylation cannot be used to distinguish between monooxygenase- and dioxygenase-catalysed oxidation pathways; and (ii) the 'NIH shift' mechanism can be observed during aromatisation of arene oxide and dihydrodiol metabolites of heterocyclic as well as carbocyclic arenes. In some cases, however, the contribution of this mechanism may be significantly underestimated owing to exchange processes.

We thank Dr P. Porter for assistance with preliminary studies, the D.E.N.I. for postgraduate studentships (to L. H. and R. A. S. McM.) and the Biotechnology Directorate of the SERC for support (to S. A. B. and N. D. S.).

Received, 8th June 1994; Com. 4/03475I

References

1 D. R. Boyd, N. D. Sharma, M. R. J. Dorrity, M. V. Hand, R. A. S. McMordie, J. F. Malone and H. P. Porter, *J. Chem. Soc., Perkin Trans. 1*, 1993, 1065.
2 D. R. Boyd, N. D. Sharma, R. Boyle, J. F. Malone, J. Chima and H. Dalton, *Tetrahedron Asymmetry*, 1993, **4**, 1307.
3 D. R. Boyd, N. D. Sharma, B. T. McMurray and H. Dalton, unpublished data.
4 C. E. Cerniglia, C. van Baalen and D. T. Gibson, *J. Gen. Microbiol.*, 1980, **116**, 485.
5 (*a*) K. Ballschmiter, C. Umglert and P. Heinzmann, *Angew. Chem. Int. Ed. Engl.*, 1977, **16**, 64; (*b*) K. Ballschmiter and C. Scholtz, *Angew. Chem. Int. Ed. Engl.*, 1981, **20**, 955.
6 G. M. Whited and D. T. Gibson, *J. Bacteriol.*, 1991, **173**, 3010.
7 D. R. Boyd, J. Blacker, B. Byrne, H. Dalton, M. V. Hand, S. C. Kelly, R. A. More O'Ferral, S. N. Rao, N. D. Sharma and G. N. Sheldrake, *J. Chem. Soc., Chem. Commun.*, 1994, 313.
8 D. R. Boyd and D. M. Jerina in *Small Ring Heterocycles. Part 3*, ed. A. Hassner, *Chemistry of Heterocyclic Compounds*, vol. 42, Wiley, New York 1985, 197.
9 D. M. Jerina, J. W. Daly, B. Witkop, P. Zaltzman-Nirenberg and S. Udenfriend, *Biochemistry*, 1970, **9**, 147.
10 S. K. Agarwal, D. R. Boyd, H. P. Porter, W. B. Jennings, S. J. Grossman and D. M. Jerina, *Tetrahedron Lett.*, 1986, **26**, 4253.
11 F. P. Guengerich, *Crit. Rev. Biochem. Mol. Biol.*, 1990, **25**, 97.
12 S. K. Agarwal, D. R. Boyd, R. J. H. Davies, L. Hamilton, D. M. Jerina, J. J. McCullough and H. P. Porter, *J. Chem. Soc., Perkin Trans. 1*, 1990, 1969.
13 D. R. Boyd, J. W. Daly and D. M. Jerina, *Biochemistry*, 1972, **11**, 1961.
14 D. M. Jerina, J. W. Daly and B. Witkop, *J. Am. Chem. Soc.*, 1967, **89**, 5488.