Regio- and stereo-selective dioxygenase-catalysed *cis*-dihydroxylation of fjord-region polycyclic arenes

Derek R. Boyd,*[a] Narain D. Sharma,[a] John S. Harrison,[a] Martina A. Kennedy,[a] Christopher C. R. Allen[b] and David T. Gibson[c]

[a] *School of Chemistry, The Queen's University of Belfast, Belfast, UK BT9 5AG*
[b] *QUESTOR Centre, The Queen's University of Belfast, Belfast, UK BT9 5AG*
[c] *Department of Microbiology and the Center for Biocatalysis and Bioprocessing, The University of Iowa, Iowa City, IA 52242*

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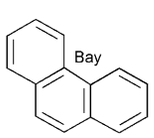
Bacterial dioxygenase-catalysed *cis*-dihydroxylation of the tetracyclic arenes benzo[*c*]phenanthrene **2**, and the isosteric compounds benzo[*b*]naphtho[1,2-*d*]furan **8**, and benzo[*b*]naphtho[1,2-*d*]thiophene **9**, has been found to occur exclusively at fjord-region bonds. The resulting *cis*-dihydrodiols **7**, **10** and **11** were found to be enantiopure and of similar absolute configuration. *cis*-Dihydroxylation was also observed in the pseudo-fjord region of the 8,9,10,11-tetrahydro-precursors (**12** and **13**) of benzo[*b*]naphtho[1,2-*d*]furan **8**, and benzo[*b*]naphtho[1,2-*d*]thiophene **9**, to yield the corresponding enantiopure hexahydro *cis*-diols **14** and **15**. A novel tandem *cis*-dihydroxylation and bis-desaturation of the tetrahydro-substrate, tetrahydrobenzo[*b*]naphtho[1,2-*d*]thiophene **13**, catalysed by biphenyl dioxygenase, was found to yield the fjord-region *cis*-dihydrodiol **17** of benzo[*b*]naphtho[1,2-*d*]thiophene **9**.

Introduction

Polycyclic aromatic hydrocarbons (PAHs), *e.g.* phenanthrene **1** and benzo[*c*]phenanthrene **2**, and the corresponding aza-, oxa- and thia-heterocyclic analogues are ubiquitous in the environment as a result of partial combustion of organic material including wood and fossil fuels. Their biodegradation in animals, plants and fungi by eucaryotic monooxygenases generally involves arene oxides, *e.g.* **3** and **4**, and in the presence of epoxide hydrolases these are in turn converted to *trans*-dihydrodiols, *e.g.* **5** and **6**, Scheme 1.[1] Bacteria commonly utilize a complementary biodegradation pathway for arenes, relative to animals, plants and fungi, involving *cis*-dihydrodiols as initial metabolites and procaryotic dioxygenases as biocatalysts, Scheme 1.[2–4] Although bacterial biodegradation has been identified as a major method for the removal of arenes from the environment, the majority of procaryotic metabolism reports have focused on the smaller members, *e.g.* mono-, bi-, and tri-cyclic arenes.[2–4] Studies of the bacterial biodegradation of larger PAHs, *e.g.* tetra- and penta-cyclic arenes are of importance in view of their increased recalcitrance and threat to health.



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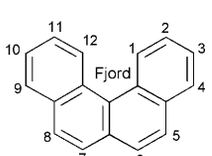

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Angular members of the PAH series may contain both bay (*cf.* phenanthrene, **1**) and fjord (*cf.* benzo[*c*]phenanthrene, **2**) regions. While the bacterial biotransformation of larger PAHs and their heterocyclic analogues, containing one or more bay regions has been investigated,[3,4] studies of arenes with fjord or pseudo-fjord regions (Schemes 1 and 2) have not previously been reported. The low solubility of larger PAHs in water, allied to the inability of the more widely used dioxygenase enzymes to accept such substrates, *e.g.* toluene dioxygenase (TDO) and naphthalene dioxygenase (NDO), are assumed to be limiting factors; isosteric heteroarenes being more water-soluble than carbocyclic arenes are expected to biodegrade more efficiently.

Bacterial metabolism of the carcinogenic angular PAHs benz[*a*]anthracene,[5,6] chrysene[7,8] and benzo[*a*]pyrene[5] and the heterocyclic analogues benzo[*b*]naphtho[2,1-*d*]thiophene[8] and benzo[*c*]phenanthridine[8] has been studied in our laboratories using *Sphingomonas yanoikuyae* B8/36, a mutant strain containing biphenyl dioxygenase (BPDO) and deficient in *cis*-diol dehydrogenase activity. These studies have confirmed that bacterial dioxygenase-catalysed *cis*-dihydroxylation of angular PAHs occurs in a regioselective manner to yield bay-region *cis*-dihydrodiols as major,[5,6] or exclusive,[5,7,8] bioproducts. A second sequential *cis*-dihydroxylation was also found to occur in the tetracyclic arenes chrysene and benzo[*b*]naphtho[2,1-*d*]-thiophene at the other bay region in these substrates.[8]

Angular PAHs, containing a fjord region, are more congested compared with arenes containing a bay region, and thus some are found to be non-planar in the crystalline state. This study indicates that bacterial *cis*-dihydroxylation of tetracyclic arenes and tetrahydroarenes occurs with optimal regio- and stereo-selectivity within the congested fjord (arenes **2**, **8**, and **9**) or pseudo-fjord (arenes **12** and **13**) regions using biphenyl dioxygenase (BPDO) from *S. yanoikuyae* B8/36 or a naphthalene dioxygenase (NDO) found to be present in a mutant strain (9816/11) of *Pseudomonas putida*. Furthermore, a novel tandem biotransformation pathway involving bis-desaturation and *cis*-dihydroxylation of the tetrahydrothiaarene **13** was found to yield a fjord-region *cis*-dihydrodiol regioisomer **17** which was not detected during biotransformation of benzo[*b*]naphtho-[1,2-*d*]thiophene **9**.

Results and discussion

Benzo[*c*]phenanthrene, **2**, is the smallest member of the PAH series to contain a fjord region. Its metabolism in animal liver systems, occurs *via* monooxygenase (CYP-450)-catalysed epoxidation to yield the 3,4- and 5,6-arene oxides (**4** and **3**) followed by epoxide hydrolase-catalysed hydrolysis to give

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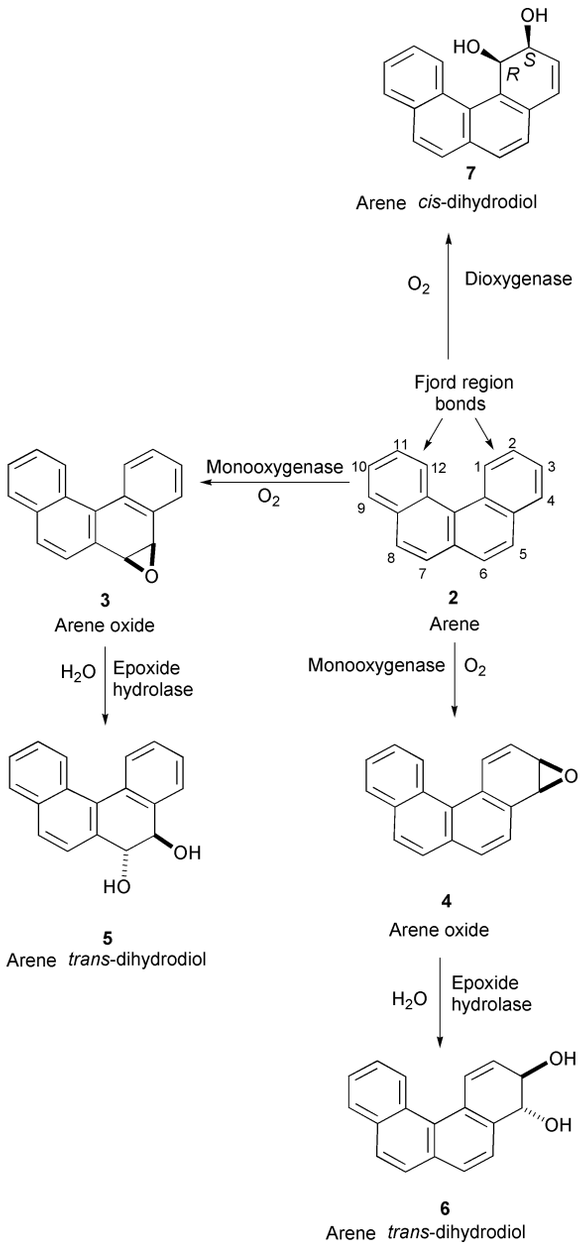
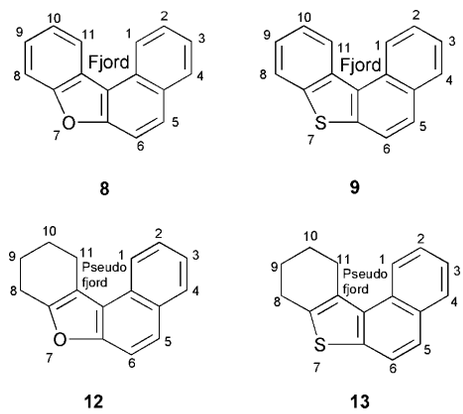
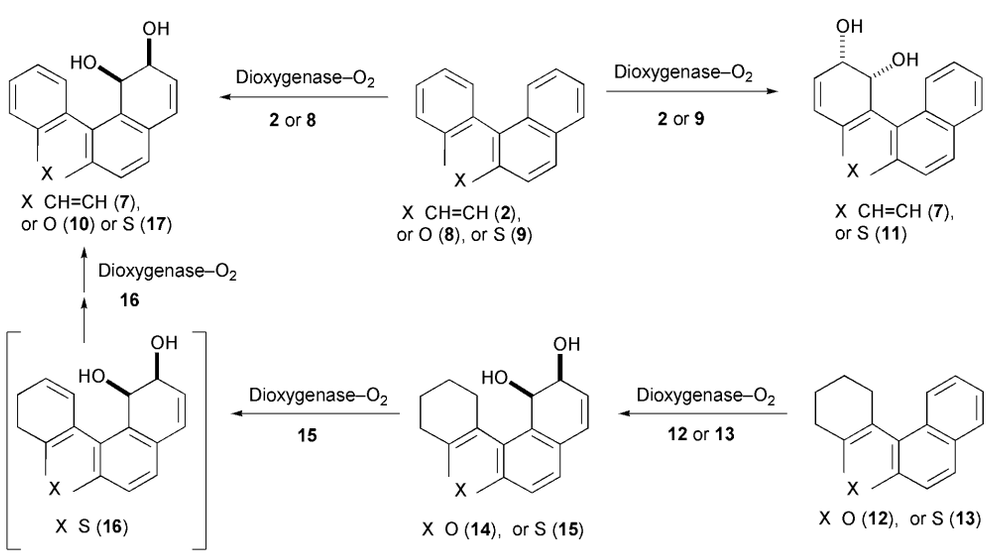


Scheme 1



the corresponding *trans*-dihydrodiols (**5** and **6**, Scheme 1).[9] Further enzyme-catalysed epoxidation of the 1,2-bond in *trans*-dihydrodiol **6** yielded the fjord-region *anti*-3,4-dioxo-3,4-dihydrobenzo[*c*]phenanthrene 1,2-oxide. This metabolite, showing an extremely high level of *in vitro* DNA binding, was identified as the ultimate carcinogen from benzo[*c*]phenanthrene **2** and was reported to have the highest tumour-initiating activity of known PAH diol-epoxides.[10,11] Monooxygenase-catalysed epoxidation has not been reported at the 1,2- (fjord-region) bond of benzo[*c*]phenanthrene **2**.

Bacterial oxidation, catalysed by BPDO using intact cells of *S. yanoikuyae* B8/36 cultured under reported conditions,[8] has now been found to occur exclusively at the 1,2-bond to yield a single *cis*-dihydrodiol metabolite **7** (Scheme 1). The structure of the fjord-region *cis*-dihydrodiol **7** was deduced by MS and NMR analyses. The NOE enhancement (7%) between protons H-1 and H-12 and the coupling constant for $J_{1,2}$ (6 Hz) provided evidence of *cis*-dihydroxylation occurring at the 1,2-bond with proton H-1 being in a pseudoaxial conformation due to steric congestion in the fjord region. *cis*-Dihydrodiol **7** was found to be a single enantiomer (12% isolated yield, $[a]_D$ −222, $CHCl_3$, >98% ee) by reaction with (*S*)- and (*R*)-2-(1-methoxyethyl)benzeneboronic acid (MEBBA) and ^{1}H-NMR analysis of the corresponding diastereomeric boronate esters $\mathbf{18}_S$ and $\mathbf{18}_R$. Formation of the MEBBA derivatives $\mathbf{18}_S$ and $\mathbf{18}_R$ also allowed the absolute configuration (1*R*,2*S*) of *cis* diol **7** to be assigned (Table 1). This absolute configuration assignment method has also been used for bay-region *cis*-dihydrodiol derivatives in the polycyclic arene series including phenanthrene[7] and chrysene.[12] Thus, the ^{1}H-NMR spectrum of the (*S*)-MEBBA derivative of a bay-region *cis*-dihydrodiol, having a benzylic (*R*) config-

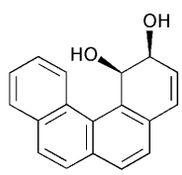


Scheme 2

Table 1 Chemical shift values (δ_{MeO}) for the (*S*)-MEBBA (**18**$_S$–**23**$_S$) and (*R*)-MEBBA (**18**$_R$–**23**$_R$) derivatives of the *cis*-dihydrodiol metabolites **7**, **10**, **11**, **17**, **14**, and **15**

cis- Dihydrodiol	δ_{MeO} (*S*-MEBBA)	δ_{MeO} (*R*-MEBBA)	Absolute configuration
7	3.25 (**18**$_S$)	3.32 (**18**$_R$)	(1*R*,2*S*)
10	3.01 (**19**$_S$)	3.17 (**19**$_R$)	(1*R*,2*S*)
11	3.14 (**22**$_S$)	3.23 (**22**$_R$)	(11*R*,10*S*)
17	3.15 (**21**$_S$)	3.21 (**21**$_R$)	(1*R*,2*S*)
14	3.16 (**20**$_S$)	3.22 (**20**$_R$)	(1*R*,2*S*)
15	3.19 (**23**$_S$)	3.23 (**23**$_R$)	(1*R*,2*S*)

uration, shows a MeO signal with smaller δ values relative to the corresponding (*R*)-MEBBA derivative. The absolute configuration of *cis*-dihydrodiol **7** was confirmed by a circular dichroism study (exciton chirality) of the phenylboronate derivative of the *cis*-1,2,3,4-tetrahydrodiol obtained by catalytic hydrogenation of *cis*-dihydrodiol **7**.



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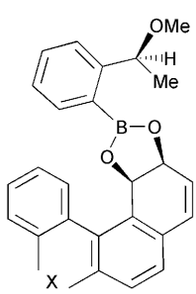
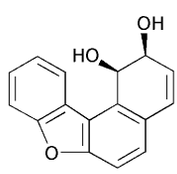


X CH=CH (**18**$_S$), or O (**19**$_S$), or S (**21**$_S$)

When benzo[*c*]phenanthrene **2** was added as substrate to *Pseudomonas putida* 9816/11, a mutant strain containing NDO with the *cis*-dihydrodiol dehydrogenase activity blocked, *cis*-dihydrodiol **7** of identical enantiopurity and absolute configuration was again obtained as the sole metabolite. This observation is noteworthy since earlier biotransformation studies have suggested that NDO has a smaller active site compared with BPDO and did not accept a tetracyclic arene substrate.[2,3] While the yield obtained using NDO was much lower (*ca.* 1%), addition of the surfactant Triton X100 (2 mM) during the biotransformation of benzo[*c*]phenanthrene **2** using *P. putida* 9816/11 improved the mass transfer process and increased the yield of *cis*-dihydrodiol **7** by a factor of seven. Unfortunately Triton X100 proved to be toxic to *S. yanoikuyae* B8/36 cells containing BPDO.[13] Naturally occurring surfactants (biosurfactants, *e.g.* glycolipids or lipopeptides) may play a similar role in increasing the efficiency of bacterial biodegradation of PAHs in the soil.

It is evident that benzo[*c*]phenanthrene **2**, and presumably other recalcitrant PAHs, can be removed from the environment by bacterial biodegradation involving *cis*-dihydroxylation at a fjord region which prevents the formation of carcinogenic fjord-region diol epoxides in mammals. In order to extend the study to heteroarenes, the heterocyclic arenes benzo[*b*]naphtho[1,2-*d*]furan **8** and benzo[*b*]naphtho[1,2-*d*]thiophene **9**, having fjord regions and the corresponding tetrahydroarenes **12** and **13** with pseudo-fjord regions, were examined as substrates.

Unlike benzo[*c*]phenanthrene **2**, benzo[*b*]naphtho[1,2-*d*]furan **8** is unsymmetrical and has two non-equivalent fjord-region bonds (1,2- and 10,11-) which are associated with naphthalene and benzene rings each fused to the furan ring. When used as a substrate for *S. yanoikuyae* B8/36, benzo[*b*]naphtho[1,2-*d*]furan **8** yielded the fjord-region *cis*-dihydrodiol **10** (14% yield, $[a]_D$ +42, $CHCl_3$) as the sole metabolite. The regioselectivity of dioxygenase-catalysed dihydroxylation of benzo[*b*]naphtho[1,2-*d*]furan **8** was confirmed by ^{1}H-NMR spectral analysis including NOE. Thus, irradiation of the H-1 and H-4 signals individually showed enhancements at H-11 (4%) and H-5 (3%) respectively. The relative stereochemistry of the OH groups at C-1 and C-2 was found to be *cis* from the *vicinal* coupling constant ($J_{1,2}$ 5.4 Hz). Formation of the corresponding (*S*)- and (*R*)-MEBBA derivatives **19**$_S$ and **19**$_R$, followed by NMR analysis in the manner described for *cis*-dihydrodiol **7**, indicated that diol **10** was enantiopure (98% ee) and also of (1*R*,2*S*) configuration (Table 1). Biotransformation of substrate **8** using *P. putida* 9816/11 also gave *cis*-dihydrodiol **10** (11% yield) of identical enantiomeric excess (98% ee) and (1*R*,2*S*) absolute configuration. Using both BPDO and NDO enzymes *cis*-dihydroxylation had thus occurred exclusively at the 1,2-fjord-region bond in the furan-fused naphthalene ring in preference to the alternative 10,11-fjord-region bond in the furan-fused phenyl ring.



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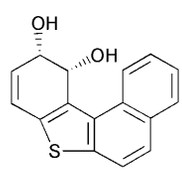
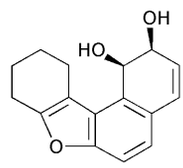


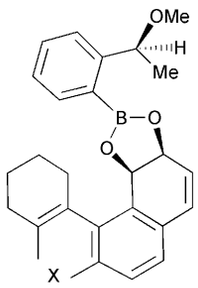
11

Dioxygenase-catalysed *cis*-dihydroxylation of benzo[*b*]furan (using TDO)[3] and dibenzofuran (NDO, BPDO)[2,3] was less regioselective (two *cis*-dihydrodiols were formed in each case) than found with benzo[*b*]naphtho[1,2-*d*]furan **8**. The regioselectivity for a modified type of fjord region (pseudo-fjord region) during dioxygenase-catalysed *cis*-dihydroxylation was examined using tetrahydrobenzo[*b*]naphtho[1,2-*d*]furan **12** and *S. yanoikuyae* B8/36, but no evidence of dihydroxylation was found. However with *P. putida* 9816/11, *cis*-dihydrodiol **14** was isolated as a single enantiomer (8% isolated yield, $[a]_D$ +70, $CHCl_3$); based upon ^{1}H-NMR spectral analysis of the MEBBA derivatives **20**$_S$ and **20**$_R$ (Table 1) an ee value of >98% and a (1*R*,2*S*) configuration was assigned.



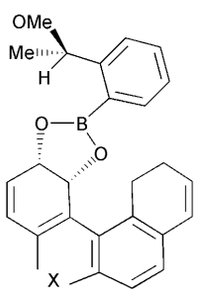
14

Benzo[*b*]naphtho[1,2-*d*]thiophene **9** proved to be an acceptable substrate for *Sphingomonas yanoikuyae* B8/36 again yielding a single fjord-region *cis*-dihydrodiol which was identified as compound **11** (2% isolated yield, $[a]_D$ −21, MeOH) from its NMR spectral analysis including coupling constants ($J_{10,11}$ 5.3 Hz) and NOE data (irradiation of H-1 and H-4 gave enhancements of 8% and 5% on signals H-11 and H-5 respectively). Formation of the MEBBA derivative **22**$_S$ and **22**$_R$ confirmed the



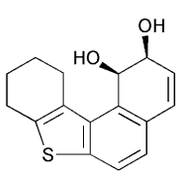
X= O (**20**$_S$), or S (**23**$_S$)

structure and showed that a single enantiomer (98% ee) of (11*R*,10*S*) configuration had been formed (Table 1). In contrast with the result obtained with benzo[*b*]naphtho[1,2-*d*]furan **8** as substrate, where exclusive *cis*-dihydroxylation occurred at the 1,2-fjord-region bond in the furan-fused naphthalene ring, the oxidation of the isosteric substrate **9** was found to occur only at the 10,11-fjord-region bond in the thiophene-fused benzene ring. It was surprising to find that substrates **8** and **9**, with similar structures, were oxidized regioselectively but at different fjord-region bonds. However, studies on isosteric benzo[*b*]-thiophene and benzo[*b*]furan substrates, using TDO, and on dibenzofuran, using NDO, had also found differences in regioselectivity during *cis*-dihydroxylation.[14–16] *cis*-Dihydrodiol **11** was formed in very low yield (<1%) when substrate **8** was oxidized in the presence of NDO using intact cells of *P. putida* 9816/11.



X= S (**22**$_S$)

Addition of the tetrahydrobenzo[*b*]naphtho[1,2-*d*]thiophene **13** as substrate to *S. yaniokuyae* B8/36 proved to be exceptional in yielding two *cis*-dihydrodiols. Since the thiophene-fused benzene ring was not present in substrate **13**, it was anticipated that *cis*-dihydroxylation would occur exclusively at the available pseudo-fjord-region bond (1,2-) in the thiophene-fused naphthalene ring. The more polar bioproduct (R_f 0.25, 10% MeOH in $CHCl_3$ as eluant) was found to be the expected *cis*-dihydrodiol **15** (3% isolated yield, $[\alpha]_D$ +110, MeOH). It was enantiopure (98% ee) and had a (1*R*,2*S*) configuration (MEBBA derivative formation, **23**$_S$ and **23**$_R$, Table 1). The second bioproduct (R_f 0.27) was identified as (1*R*,2*S*)-*cis*-1,2-dihydroxy-1,2-dihydrobenzo[*b*]naphtho[1,2-*d*]thiophene **17** (2% isolated yield, $[\alpha]_D$ +18, MeOH, >98% ee, MEBBA derivative formation, **21**$_S$ and **21**$_R$, Table 1). The absolute configurations assigned to each of the new fjord- (**7**, **10**, **11**, and **17**) and pseudo-fjord-region *cis*-dihydrodiols (**14** and **15**) were found to be similar to the PAH *cis*-dihydrodiols previously isolated from dioxygenase-catalysed dihydroxylation (benzylic *R*, allylic *S*).[3]



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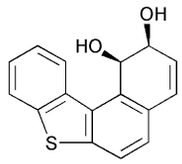
The regiocomplementary formation of the fjord-region *cis*-dihydrodiols **11** and **17** is consistent with BPDO-catalysed direct *cis*-dihydroxylation of benzo[*b*]naphtho[1,2-*d*]thiophene **9** (to yield diol **11**) and 8,9,10,11-tetrahydrobenzo[*b*]naphtho[1,2-*d*]-thiophene **13** (to yield diol **15**) followed by an unprecedented



17

bis-desaturation (assumed to be BPDO-catalysed to yield diol **17**, Scheme 2). Dioxygenase-catalysed mono-desaturation reactions have been observed earlier with indane (using NDO to yield indene)[17] and 1,2-dihydronaphthalene as substrates (using TDO to yield naphthalene).[18] The sequence of desaturation (dehydrogenation) steps involved in the dioxygenase-catalysed synthesis of *cis*-dihydrodiol **17** has not been determined. However, since the *cis*-hexahydrodiol metabolite **15** was isolated, it is probable that the metabolic sequence first involves the formation of diol **15**. This step appears to be followed by an initial desaturation step to yield a transient, 8,9-dihydrobenzo[*b*]naphtho[1,2-*d*]thiophene *cis*-tetrahydrodiol **16** (or the isomeric 10,11-dihydrobenzo[*b*]naphtho[1,2-*d*]thiophene) and a second desaturation step to give the dihydrodiol **17**. Recent studies have shown that some of the initially formed *cis*-dihydrodiol metabolites using BPDO undergo a further type of sequential or tandem dioxygenase-catalysed oxidation (bis-*cis*-dihydroxylation).[19,20]

Conclusion

The earlier reported preference shown for bay region bonds during biphenyl dioxygenase-catalysed *cis*-dihydroxylation of PAHs or heteroPAHs has been extended to the exclusive oxidation of the more congested fjord-region and pseudo-fjord-region bonds of tetracyclic-arenes and tetracyclic-tetrahydroarenes respectively. *cis*-Dihydroxylation at a pseudo-fjord-region bond of tetrahydrobenzo[*b*]naphtho[1,2-*d*]thiophene **13** was followed by an unusual bis-desaturation process to yield the second *cis*-dihydrodiol regioisomer **17** from benzo[*b*]-naphtho[1,2-*d*]thiophene **9**.

Experimental

^{1}H NMR spectra were recorded at 300 MHz (Bruker Avance DPX-300) and 500 MHz (Bruker Avance DRX-500) in $CDCl_3$ solvent unless stated otherwise. Chemical shifts (δ) are reported in ppm relative to $SiMe_4$ and coupling constants (J) are given in Hz. Electron impact mass spectra were recorded at 70 eV on a VG Autospec Mass Spectrometer, using a heated inlet system. Accurate molecular weights were determined by the peak matching method with perfluorokerosene as standard. Elemental microanalyses were obtained on a Perkin-Elmer 2400 CHN microanalyser. Optical rotations are given in 10^{-1} deg cm^2 g^{-1} and were measured on a Perkin-Elmer 421 polarimeter at ambient temperature. Circular dichroism spectra were recorded on a Jasco J-720 instrument in acetonitrile solvent. The syntheses of (−)-(*S*)- and (+)-(*R*)-2-(1-methoxyethyl)benzeneboronic acid (MEBBA) and ^{1}H-NMR analysis of the corresponding diastereomeric boronate esters were carried by the reported methods.[7,12]

The substrates benzo[*c*]phenanthrene **2**,[21] benzo[*b*]naphtho-[1,2-*d*]furan **8**,[22] benzo[*b*]naphtho[1,2-*d*]thiophene **9**,[23] 8,9,10,11-tetrahydrobenzo[*b*]naphtho[1,2-*d*]furan **12**,[22] and 8,9,10,11-tetrahydrobenzo[*b*]naphtho[1,2-*d*]thiophene **13**[23] were synthesised by the literature procedures.

Biotransformations of substrates **2**, **8**, **9**, **12**, and **13** were carried out using *Sphingomonas yanoikuyae* B8/36 and/or

Pseudomonas putida 9816/11 in the manner described.[7] The growing cultures containing the substrates were incubated (18 h) in the dark at 30 °C with shaking (220 rpm). The cells were subsequently removed by centrifugation and the supernatant extracted thoroughly with ethyl acetate after saturation with sodium chloride. The extracts were dried ($MgSO_4$), concentrated, and the products purified by PLC on glass plates (20 × 20 cm) coated with Merck Kieselgel 60 PF_{254}.

Biotransformations using *S. yanoikuyae* B8/36

(a) Benzo[*c*]phenanthrene 2. Substrate **2** (0.1 g, 0.44 mmol) yielded (−)-(1*S*,2*R*)-*cis*-1,2-dihydroxy-1,2-dihydrobenzo[*c*]-phenanthrene **7** (0.14 g, 12%); R_f 0.5 ($CHCl_3$–MeOH, 95 : 5), mp 97 °C (from pentane–CH_2Cl_2); $[\alpha]_D$ −222 (*c* 0.68, $CHCl_3$) (Found: M^+, 262.0997. $C_{18}H_{14}O_2$ requires *M*, 262.0994); δ_H (500 MHz, $CDCl_3$) 4.54 (1 H, m, H-2), 5.42 (1 H, dd, $J_{1,2}$ 6.2, $J_{1,3}$ 1.7, H-1), 6.00 (1 H, ddd, $J_{3,1}$ 1.7, $J_{3,2}$ 1.7, $J_{3,4}$ 9.6, H-3), 6.67 (1 H, dd, $J_{4,2}$ 2.6, $J_{4,3}$ 9.6, H-4), 7.37 (1 H, d, *J* 8.0, Ar-H), 7.62–7.70 (4 H, m, Ar-H), 7.83 (1 H, d, *J* 8.0, Ar-H), 7.90 (1 H, dd, $J_{9,10}$ 7.0, $J_{9,11}$ 1.6, Ar-H, H-9), 9.12 (1 H, dd, $J_{12,11}$ 7.2, $J_{12,1}$ 1.6, H-12); δ_C (125 MHz, $CDCl_3$) 67.68, 71.26, 104.58, 118.47, 125.81, 126.28, 126.92, 127.07, 127.59, 128.05, 128.27, 128.52, 129.66, 129.83, 130.68, 131.11, 146.20; *m*/*z* 262 (15), 244 (100), 215 (42), 189 (35); Electronic CD data: λ 367 nm ($\Delta\varepsilon$ −2.590), 349 ($\Delta\varepsilon$ −2.959), 329 ($\Delta\varepsilon$ −19.69), 317 ($\Delta\varepsilon$ −22.68), 289 ($\Delta\varepsilon$ −4.0), 273 ($\Delta\varepsilon$ 11.12), 265 ($\Delta\varepsilon$ −19.24), 242 ($\Delta\varepsilon$ 71.66), 209 ($\Delta\varepsilon$ −44.42); >98% ee by the MBBA method.

(b) Benzo[*b*]naphtho[1,2-*d*]furan 8. Substrate **8** (0.1 g, 0.5 mmol) yielded (+)-(1*R*,2*S*)-*cis*-1,2-dihydroxy-1,2-dihydrobenzo[*b*]naphtho[1,2-*d*]furan **10** (0.16 g, 14%); R_f 0.2 ($CHCl_3$–MeOH, 95 : 5); mp 115 °C (from CH_2Cl_2); $[\alpha]_D$ +42 (*c* 0.9, $CHCl_3$) (Found: M^+, 252.0796. $C_{16}H_{12}O_3$ requires *M* 252.0786); δ_H (500 MHz, $CDCl_3$) 4.74 (1 H, ddd, $J_{2,1}$ 5.0, $J_{2,3}$ 2.5, $J_{2,4}$ 2.5, 2-H), 5.41 (1 H, d, $J_{1,2}$ 5.4, 1-H), 5.91 (1 H, ddd, $J_{3,4}$ 9.8, $J_{3,2}$ 1.7, $J_{3,1}$ 1.7, 3-H), 6.56 (1 H, dd, $J_{4,3}$ 9.8, $J_{4,2}$ 2.6, 4-H), 7.22 (1 H, d, $J_{5,6}$ 8.3, 5-H), 7.39 (1 H, dd, $J_{9,8}$ 6.8, $J_{9,10}$ 6.8, 10-H), 7.45 (1 H, d, $J_{6,5}$ 8.3, 6-H), 7.50 (1 H, dd, $J_{8,7}$ 8.4, $J_{8,9}$ 7.3, 9-H), 7.58 (1 H, d, $J_{7,8}$ 8.2, 8-H), 8.19 (1H, d, $J_{10,9}$ 7.6, 11-H); δ_C (125 MHz, $CDCl_3$) 66.65, 69.80, 111.88, 112.19, 123.04, 123.25, 126.51, 127.04, 127.16, 127.74, 128.55, 129.28, 155.99, 156.90; *m*/*z* 252 (30), 234 (100), 205 (85), 149 (55); electronic CD data: λ 294 nm ($\Delta\varepsilon$ 1.213), 267 ($\Delta\varepsilon$ −1.872), 224 ($\Delta\varepsilon$ 5.549), 205 ($\Delta\varepsilon$ −99.7); >98% ee by the MEBBA method.

(c) Benzo[*b*]naphtho[1,2-*d*]thiophene 9. Substrate **9** (0.1 g, 0.42 mmol) yielded (+)-(10*S*,11*R*)-*cis*-10,11-dihydroxy-10,11-dihydrobenzo[*b*]naphtho[1,2-*d*]thiophene **11** (0.006 g, 2%), R_f 0.2 ($CHCl_3$–MeOH, 95 : 5), mp 100–103 °C decomp. (from CH_2Cl_2), $[\alpha]_D$ +21 (*c* 0.3, MeOH) (Found: M^+, 268.0561. $C_{16}H_{12}SO_2$ requires *M* 268.0558); δ_H (500 MHz, $CDCl_3$) 4.71 (1 H, m, H-10), 5.58 (1 H, d, $J_{11,10}$ 5.3, H-11), 5.96 (1 H, dd, $J_{8,9}$ 9.8, $J_{9,10}$ 1.5, H-9), 6.55 (1 H, dd, $J_{9,8}$ 9.8, $J_{8,10}$ 2.7, H-8), 7.22 (1 H, d, $J_{5,6}$ 8.1, H-5), 7.47–7.52 (2 H, m, H-2 and H-3), 7.79 (1 H, d, $J_{5,6}$ 8.1, H-6), 7.87 (1 H, m, H-4), 8.52 (1 H, d, $J_{1,2}$ 7.8, H-1); δ_C (12 MHz, $CDCl_3$) 65.9, 70.5, 123.4, 123.9, 125.3, 126.1, 126.2, 127.2, 127.5, 129.4, 129.8, 130.6, 134.6, 134.9; *m*/*z* 268 (M^+, 80%), 250 (100), 221 (88), 196 (23), 86 (17); electronic CD data: λ 303 nm ($\Delta\varepsilon$ −2.816), 259 ($\Delta\varepsilon$ −9.45), 241 ($\Delta\varepsilon$ 2.583), 227 ($\Delta\varepsilon$ −9.124), 197 ($\Delta\varepsilon$ 1.578); >98% ee by the MEBBA method.

(d) 8,9,10,11-Tetrahydrobenzo[*b*]naphtho[1,2-*d*]thiophene 13. Substrate **13** (0.35 g, 1.46 mmol) yielded (+)-(1*R*,2*S*)-*cis*-1,2-dihydroxy-1,2,8,9,10,11-hexahydrobenzo[*b*]naphtho[1,2-*d*]thiophene **15** (0.01 g, 3%), R_f 0.25 ($CHCl_3$–MeOH, 90 : 10); mp 98–100 °C decomp. ($CHCl_3$); R_f 0.25 (10% MeOH–$CHCl_3$); $[\alpha]_D$ +110 (*c* 0.4 in MeOH) (Found: M^+, 272.0878. $C_{16}H_{16}SO_2$ requires *M* 272.087102); δ_H (500 MHz, $CDCl_3$) 1.70 (2 H, m, H-9), 1.88 (2 H, m, H-10), 3.17–3.28 (2 H, m, H-8), 3.41 (2 H, m, H-11), 4.52 (1 H, m, H-2), 5.11 (1 H, dd, $J_{1,2}$ 5.0, $J_{1,3}$ 1.3, H-1), 5.72 (1 H, m, H-3), 6.38 (1 H, dd, $J_{4,3}$ 9.8, $J_{4,2}$ 2.7, H-4), 6.87 (1 H, d, $J_{5,6}$ 8.0, H-5), 7.54 (1 H, d, $J_{6,5}$ 7.9, H-6); δ_C (125 MHz, $CDCl_3$) 22.9, 23.0, 26.5, 26.9, 65.9, 70.7, 123.1, 123.2, 123.9, 126.9, 128.2, 128.6, 129.5, 129.7, 138.3, 138.9; *m*/*z* 272 (M^+, 15%), 254 (100), 226 (56), 86 (27); electronic CD data: λ 259 nm ($\Delta\varepsilon$ 5.875), 226 ($\Delta\varepsilon$ −6.38), 194 ($\Delta\varepsilon$ 3.116); ee >98% by the MEBBA method.

The second diol isolated and identified was (+)-(1*R*,2*S*)-*cis*-1,2-dihydroxy-1,2-dihydrobenzo[*b*]naphtho[1,2-*d*]thiophene **17** (0.005 g, 1.5%), R_f 0.26 ($CHCl_3$–MeOH, 95 : 5); $[\alpha]_D$ +18 (*c* 0.4 in MeOH) (Found: M^+, 272.0872. $C_{16}H_{12}SO_2$ requires *M* 272.0871); δ_H (500 MHz, $CDCl_3$) 4.67 (1 H, m, H-2), 5.28 (1 H, d, $J_{1,2}$ 5.2, H-1), 5.97 (1 H, dd, $J_{3,4}$ 9.7, $J_{3,2}$ 1.3, H-3), 6.48 (1 H, dd, $J_{4,3}$ 9.7, $J_{4,2}$ 2.7, H-4), 7.20 (1 H, d, $J_{5,6}$ 9.6, H-5), 7.41 (1 H, m, H-9), 7.51 (1 H, m, H-10), 7.74–7.77 (2 H, m, H-6 and H-8), 8.12 (1 H, d, $J_{11,10}$ 8.5, H-11); δ_C (125 MHz, $CDCl_3$) 65.7, 70.9, 122.8, 125.5, 125.9, 127.3, 128.7, 128.9, 129.6, 129.7, 131.5, 131.9, 133.5; *m*/*z* 268 (37), 250 (100), 222 (67), 194 (86), 166 (94), 86 (86); electronic CD data: λ 312 nm ($\Delta\varepsilon$ −3.447), 261 ($\Delta\varepsilon$ 11.65), 253 ($\Delta\varepsilon$ 9.112), 229 ($\Delta\varepsilon$ −2.297), 209 ($\Delta\varepsilon$ 6.922); ee >98% by the MEBBA method.

Biotransformations using *P. putida* 9816/11

(a) Benzo[*b*]naphtho[1,2-*d*]furan 8. Substrate **8** (0.1 g, 0.45 mmol) yielded a single product (0.013 g, 11%), which was indistinguishable from the sample of (+)-(1*R*,2*S*)-1,2-dihydrobenzo[*b*]naphtho[1,2-*d*]furan-1,2-diol **10** obtained earlier from *S. yanoikuyae* B8/36.

(b) 8,9,10,11-Tetrahydrobenzo[*b*]naphtho[1,2-*d*]furan 12. Substrate **12** did not yield any identifiable bioproducts from *Sphingomonas yanoikuyae* B8/36. However, using *P. putida* 9816/11 substrate **12** (0.10 g, 0.45 mmol) yielded (+)-(1*R*,2*S*)-1,2,8,9,10,11-hexahydrobenzo[*b*]naphtho[1,2-*d*]furan-1,2-diol **14** (0.008 g, 8%), R_f 0.3 ($CHCl_3$–MeOH, 95 : 5), mp 117 °C (from hexane–CH_2Cl_2), $[\alpha]_D$ +70 (*c* 0.7, $CHCl_3$) (Found: M^+, 256.110031. $C_{16}H_{16}O_3$ requires *M* 256.109945); δ_H (500 MHz, $CDCl_3$) 1.82–1.98 (4 H, m, 8-H and 9-H), 2.74–2.91 (4 H, m, 10-H and 11-H), 4.66 (1 H, dd, $J_{2,1}$ 4.8, $J_{2,4}$ 2.5, 2-H), 5.00 (1 H, dd, $J_{1,2}$ 5.2, $J_{1,3}$ 1.4, 1-H), 5.79 (1H, ddd, $J_{3,4}$ 9.8, $J_{3,2}$ 1.8, $J_{3,1}$ 1.8, 3-H), 6.48 (1 H, dd, $J_{4,3}$ 9.9, $J_{4,2}$ 2.8, 4-H), 6.96 (1 H, d, $J_{6,5}$ 8.2, 5-H), 7.30 (1 H, d, $J_{5,6}$ 8.2, 6-H); δ_C (125 MHz, $CDCl_3$) 21.59, 21.90, 22.26, 23.18, 66.67, 70.05, 109.98, 111.47, 112.21, 122.62, 123.64, 127.18, 128.77, 130.67, 138.82, 156.92; *m*/*z* 256 (M^+, 45) 238 (100), 210 (85); electronic CD data: λ 326 nm ($\Delta\varepsilon$ 1.019), 312 ($\Delta\varepsilon$ −0.8294), 279 ($\Delta\varepsilon$ −3.557), 245 ($\Delta\varepsilon$ 6.044), 205 ($\Delta\varepsilon$ 0.6787); >98% ee by the MEBBA method.

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