PERKIN 1

Dioxygenase-catalysed oxidation of dihydronaphthalenes to yield arene hydrate and *cis*-dihydro naphthalenediols

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Biotransformation of 1,2- and 1,4-dihydronaphthalene substrates, using growing cultures of *Pseudomonas putida* UV4, resulted in dioxygenase-catalysed benzylic monohydroxylation, *cis*-tetrahydro diol and *cis*-dihydro diol formation, trihydroxylation and dehydrogenation. The arene hydrates, (*R*)-1,2-dihydronaphthalen-1-ol 5 and (*R*)-1,4-dihydronaphthalen-1-ol 7, were isolated as enantiopure metabolites while 1,2-dihydronaphthalen-2-ol 8 was found in almost racemic form. The structure, enantiopurity and absolute stereochemistry of these arene hydrates of naphthalene were confirmed by chemical synthesis. Deuterium labelling studies, and the use of enantiomerically pure arene hydrates 5 and 7 as substrates, were used to establish the metabolic pathways for the formation of (1*R*,2*S*)-1,2-dihydronaphthalene-1,2-diols 2, from both 1,2-dihydronaphthalene 3 and 1,4-dihydronaphthalene 6 substrates.

Introduction

Bacterial metabolism of arenes has been shown to occur *via* *cis*-dihydro diol metabolites using mutant strains which are deficient in *cis*-dihydro diol dehydrogenase, and hence allow the *cis*-dihydro diol intermediates to accumulate. Thus, mutant strains (119 [1] and UV4 [2]) of *Pseudomonas putida* yield (1*R*,2*S*)-*cis*-1,2-dihydronaphthalene-1,2-diol **2** in enantiopure form as a result of dioxygenase-catalysed asymmetric dihydroxylation of naphthalene **1**.[1,2]

Preliminary studies on the bacterial metabolism of 1,2-dihydronaphthalene **3** [3,4] and 1,4-dihydronaphthalene **6** [4] have shown that benzylic monohydroxylation can also occur as a result of dioxygenase-catalysed oxidation. Bacterial asymmetric *cis*-dihydroxylation was also found to yield both *cis*-tetrahydro diol **4** and *cis*-dihydro diol **2** metabolites. A similar type of benzylic hydroxylation was reported earlier by Gibson *et al.* during the dioxygenase-catalysed oxidation of indene.[5] A more comprehensive study of the mechanism and stereochemistry of biotransformation of dihydronaphthalene substrates **3** and **6** and of enzyme-catalysed/chemical synthesis routes to the enantiopure arene hydrates **5**, **7** and **8** is presented.

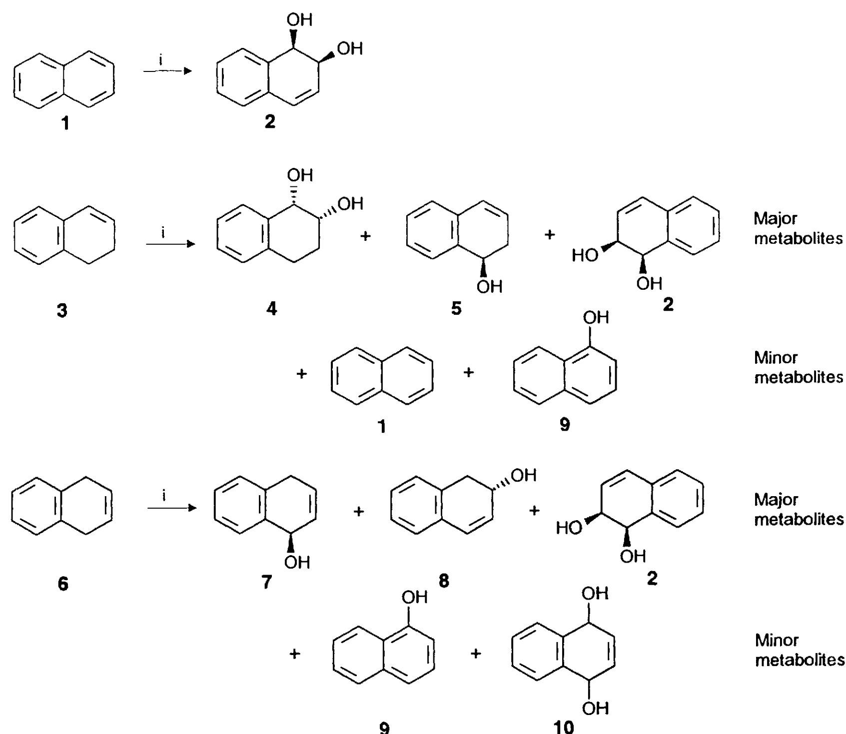
Results and discussion

(a) Biotransformations

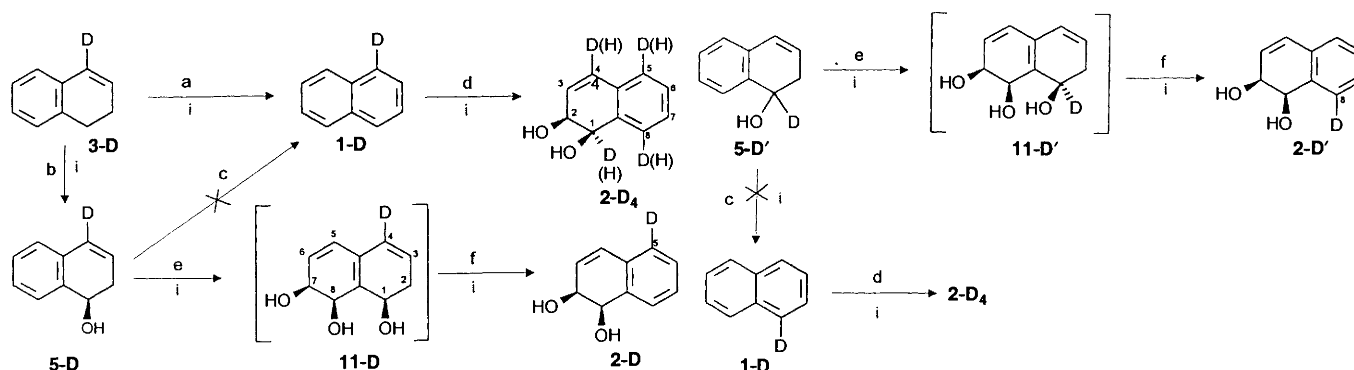
Metabolism of 1,2-dihydronaphthalene **3** in the presence of the soil bacterium *P. putida* UV4, followed by solvent extraction and product analysis using ^{1}H NMR spectroscopy and GC/MS (on the trimethylsilyl ether derivatives), showed the presence of three major metabolites. The NMR and mass spectral data of the mixture suggested the presence of one monohydroxylation **5** and two dihydroxylation (**2** and **4**) products whose relative proportions changed with time during the biotransformation process. Thus, a higher proportion of the monol **5** was initially observed but this decreased during the course of the biotransformation relative to the proportion of diol products **2** and **4**. Purification of the extract by flash column chromatography yielded the monol **5** as the least polar metabolite which crystallized from hexane ($[\alpha]_D$ +52, $CHCl_3$).† The structure, enantiopurity (>98% ee) and absolute configuration (1*R*) were established by chemical synthesis (see later). A more polar chromatographic fraction was found to be a mixture of the *cis*-diols **2** and **4**. Recrystallization of this fraction (dichloromethane–hexane) yielded pure (+)-(1*R*,2*S*)-*cis*-1,2-dihydronaphthalene-1,2-diol **2** ($[\alpha]_D$ +244, $CHCl_3$) which proved to be both structurally and stereochemically indistinguishable from the product isolated after biotransformation of naphthalene **1** in *P. putida* UV4. The mother liquors contained mainly tetrahydro diol **4** contaminated with minor amounts of the dihydro diol **2**. Since the latter mixture of diols could not readily be separated by chromatographic methods the small proportion of residual *cis*-dihydro diol **2** was converted into the more polar epoxide derivative by *m*-chloroperbenzoic acid (MCPBA) oxidation. The unchanged *cis*-tetrahydro diol **4** was then isolated in pure form by preparative TLC (PLC). The metabolite **4**, ($[\alpha]_D$ +39, $CHCl_3$) was found to be enantiomerically pure (>98% ee). The *cis*-tetrahydro diol metabolite **4** was assigned the *opposite* absolute configuration (1*S*,2*R*) to that obtained after catalytic hydrogenation of the *cis*-dihydro diol **2** (1*R*,2*S*). The GC/MS analysis of the crude mixture of metabolites also showed traces of naphthalene **1** and 1-naphthol **9**.

The origin of the *cis*-dihydro diol **2** and naphthalene **1** formed during biotransformation of 1,2-dihydronaphthalene **3** by *P. putida* UV4 was investigated with the aid of specifically ^{2}H-labelled substrates. When 1,2-dihydro[4-$^{2}H_1$]naphthalene (**3-D**) is used as a substrate it could, in principle, yield [1-^{2}H]naphthalene (**1-D**) *via* dehydrogenation (step a) or *via* benzylic hydroxylation to yield 1,2-dihydro[4-$^{2}H_1$]naphthalen-1-ol (**5-D**; step b) followed by dehydration (step c; Scheme 1). The bioproduct [1-$^{2}H_1$]naphthalene (**1-D**) may, in turn, act as a substrate during dioxygenase-catalysed *cis*-dihydroxylation to yield the *cis*-dihydro diol (**2-D$_4$**) (step d). On the assumption that *cis*-dihydroxylation of [1-$^{2}H_1$]naphthalene (**1-D**) occurred with equal facility at the 1,2-, 3,4-, 5,6- and 7,8-bonds, an equal distribution of deuterium label (25% $^{2}H_1$) should occur at the C-1, C-4, C-5 and C-8 positions in the product (**2-D$_4$**). In practice, the deuterium content at positions C-1, C-4 and C-8 was equal (*ca.* 16%) while C-5 contained a significantly higher content (*ca.* 52% $^{2}H_1$) based upon ^{2}H NMR spectral analysis of the *cis*-dihydro diol (**2-D$_4$**). If the *cis*-dihydro diol metabolite **2** was formed *via* (i) benzylic hydroxylation of 1,2-dihydro[4-$^{2}H_1$]naphthalene **3-D** to yield the alcohol **5-D** (step b), (ii) *cis*-

† Throughout $[\alpha]_D$ values are recorded in 10^{-1} deg cm^3 g^{-1}.



Reagent: i, *P. putida* UV4



Scheme 1 *Reagent:* i, *P. putida* UV4

Scheme 2 *Reagent:* i, *P. putida* UV4

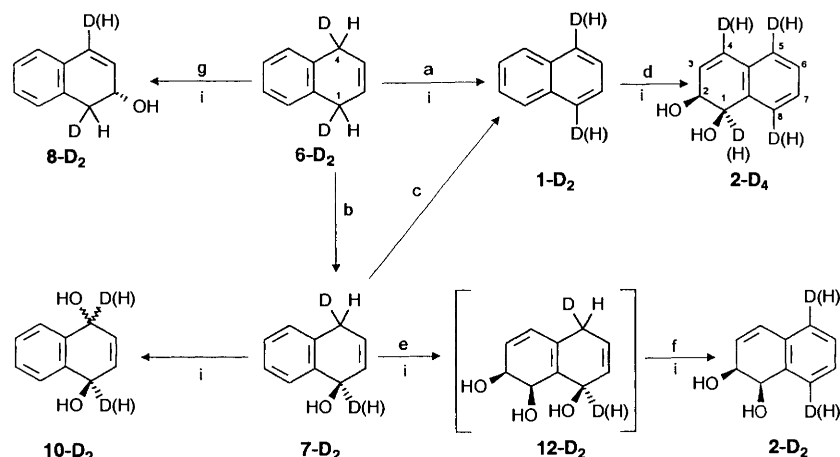
dihydroxylation to give the triol **11-D**, as a transient intermediate, (step e) and (iii) spontaneous dehydration (step f), then the deuterium label would be located exclusively at C-5 in the diol **2-D**.

The presence of deuterium label at C-1, C-4, C-5 and C-8 (with a preference for C-5) in the *cis*-dihydro diol metabolite **2** (Scheme 1) is consistent with the metabolic pathways involving the naphthalene intermediate **1-D** (steps a and d or steps b, c and d) occurring simultaneously with a further step involving a triol intermediate **11-D** (steps b, e and f).

In order to provide further evidence for the metabolic routes *via* naphthalene **1-D** from 1,2-dihydro[4-$^2H_1$]naphthalene **3-D**, *i.e.* sequence of steps a and d and/or steps b, c and d (Scheme 1), a labelled sample of racemic 1,2-dihydronaphthalen-1-ol **5-D'** was added as substrate to cultures of *P. putida* UV4 (Scheme 2). The major bioproduct (> 95% relative yield) was identified as *cis*-1,2-dihydro[8-$^2H_1$]naphthalene-1,2-diol **2-D'** with 1-naphthol being a minor product (< 5% relative yield). Unfortunately the total recovery of unchanged substrate and bioproducts from 1,2-dihydronaphthalen-1-ol **5** was rather poor (20–50%) partly due to some loss of the more volatile monohydroxylated products during work-up. Nevertheless, the location of deuterium label exclusively at the C-8 position of the *cis*-dihydro diol **2-D'** is consistent with a metabolic pathway involving *cis*-dihydroxylation of the monol **5-D'** to yield an unstable triol **11-D'** (step e) followed by a rapid dehydration to give the isolated product **2-D'** (step f). This experiment also precludes the alternative dehydration pathway (step c) for formation of naphthalene **1-D** from the arene hydrate **5-D'**.

Addition of (+)-(*R*)-1,2-dihydronaphthalen-1-ol **5** ($[\alpha]_D$ +52, isolated metabolite) as a substrate to *P. putida* UV4 yielded the *cis*-dihydro diol **2** (14% relative yield, 1*R*,2*S*, > 98% ee) and 1-naphthol **9** (6% relative yield) along with recovered substrate **5** (80% relative yield). By contrast, biotransformation of (−)-(*S*)-1,2-dihydronaphthalen-1-ol **5**, ($[\alpha]_D$ −52, from chemical synthesis, see later) under similar conditions, gave 1-naphthol **9** (71% relative yield) in the presence of recovered substrate (29% relative yield) without evidence of *cis*-dihydro diol formation. The latter result is consistent with the involvement of a triol intermediate **11** having a *cis* relationship between the hydroxy groups at C-1, C-7 and C-8, *i.e.* a 1*R*,7*S*,8*R* configuration (as shown in **11-D**, Scheme 1). The formation of 1-naphthol **9**, particularly from metabolism of (−)-(*S*)-1,2-dihydronaphthalen-1-ol **5**, could be the result of (*S*)-monol dehydrogenase enzyme activity similar to that previously found to oxidise selectively (1*S*)-indanol to indan-1-one in *P. putida* UV4[6] and F39/D.[7] The results of metabolism of 1,2-dihydro[4-$^2H_1$]napthalene **3-D** and 1,2-dihydro[1-$^2H_1$]napthalen-1-ol **5-D'** thus provide further support for concurrent biotransformation pathways occurring (*i.e.* steps a and d *and* steps b, e and f, Scheme 1).

The formation of the *cis*-dihydro diol **2** of naphthalene as a major metabolite of 1,2-dihydronaphthalene **3** has thus been



Scheme 3 *Reagent:* i, *P. putida* UV4

shown to involve monohydroxylation (step b), dihydroxylation (step e) and a dehydration sequence (step f) by the labelling experiments outlined in Schemes 1 and 2. A similar 'monol→triol→diol' biotransformation sequence has also been observed when 2,3-dihydrobenzofuran,[8] 2,3-dihydrobenzothiophene[9] and benzocyclobutene[6] were used as substrates for *P. putida* UV4. The triol intermediates derived from 2,3-dihydrobenzofuran[8] and 2,3-dihydrobenzothiophene[9] were, however, in common with compound **11**, too unstable to be isolated. The involvement of the enantiopure triol intermediate **11** during biotransformation of 1,2-dihydronaphthalene **3** using *P. putida* UV4 contrasts with the recent observations of Gibson *et al.*[10] using *P. putida* strains 9816/11 and F39/D. In the latter study, 1,2-dihydronaphthalene **3** was biotransformed into the *cis*-dihydro diol **2** without involvement of the triol **11**. This could be due to a subtle difference in the dioxygenase enzyme activity between the individual *P. putida* mutant strains or a difference in experimental procedures.

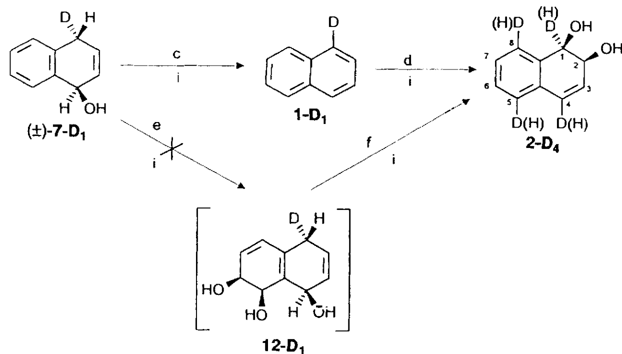
The biotransformation of 1,4-dihydronaphthalene **6**, using *P. putida* UV4, also gave *cis*-1,2-dihydronaphthalene-1,2-diol **2** as a major product along with other metabolites whose relative proportions varied during the course of the experiment. For example, after 0.5 h the biotransformation extracts showed a large proportion of unchanged substrate in the presence of three products which were identified by ^{1}H NMR spectroscopy and GC/MS analysis. Extraction and separation of the metabolites using PLC yielded (1*R*)-1,4-dihydronaphthalen-1-ol **7** ($[\alpha]_D$ −159, $CHCl_3$) as the major product with (2*S*)-1,2-dihydronaphthalen-2-ol **8** ($[\alpha]_D$ −7, $CHCl_3$) and (1*R*,2*S*)-*cis*-1,2-dihydronaphthalene-1,2-diol **2** ($[\alpha]_D$ +244, $CHCl_3$) as minor components of the mixture. The monol product **7** and *cis*-dihydro diol **2** proved to be enantiopure (>98% e.e.) while the monol **8** showed only a slight excess (*ca.* 3%) of the (2*S*)-enantiomer. The structures, absolute configurations and enantiomeric excess values of the monols **7** and **8** were determined by chiral stationary phase HPLC and synthesis (see later).

When the biotransformation of 1,4-dihydronaphthalene **6** by *P. putida* UV4 was allowed to proceed for a longer period (20 h), (1*R*,2*S*)-*cis*-1,2-dihydronaphthalene-1,2-diol **2** was the major metabolite (*ca.* 90% relative yield). Fractional crystallization of the latter product mixture yielded a pure sample of *cis*-dihydro diol **2** ($[\alpha]_D$ +244, $CHCl_3$) which was stereochemically indistinguishable from the samples isolated when either naphthalene **1** or 1,2-dihydronaphthalene **3** were used as substrates. The minor component (*ca.* 10%) was found to decompose into 1-naphthol **9** during attempts to separate it from the *cis*-dihydro diol **2**. The structure of this minor metabolite has not been unequivocally established but was tentatively identified as 1,4-dihydronaphthalene-1,4-diol **10** on the basis of the following observations: (i) the minor metabolite was inseparable from the *cis*-dihydro diol **2** by TLC or HPLC chromatographic methods, (ii) the ^{1}H NMR spectral characteristics recorded of the mixture of metabolites (singlets at δ 5.15 and 6.22) were consistent with structure **10** and (iii) the literature contains a report[11] of the facile dehydration of 1,4-dihydronaphthalene-1,4-diol **10** to yield 1-naphthol **9**. Metabolite **10** could, in principle, be formed by further benzylic hydroxylation of the monol **7**.

The metabolism of 1,4-dihydronaphthalene **6** was investigated using the labelled substrate **6-D$_2$**, which contained a single deuterium atom at each of positions C-1 and C-4 (Scheme 3). The formation of *cis*-1,2-dihydronaphthalene-1,2-diol **2** from 1,4-dihydro[1,4-$^2H_2$]naphthalene **6-D$_2$** could proceed *via* a naphthalene intermediate **1-D$_2$** as a result of direct dehydrogenation (step a) or by benzylic hydroxylation (step b) and dehydration of the monol (**7-D$_2$**, step c). The *cis*-dihydro diol metabolite **2-D$_4$** formed *via* the naphthalene **1-D$_2$** would contain an equal distribution of deuterium label at positions C-1, C-4, C-5 and C-8 (Scheme 3). Owing to the presence of primary kinetic isotope effects during the formation of the naphthalene intermediate (**1-D$_2$**, step a and/or steps b and c), it is difficult to predict the deuterium content of the derived *cis*-dihydro diol **2-D$_4$**. However, the observation of an equal distribution of deuterium (*ca.* 28%) at positions C-1, C-4, C-5 and C-8 in the *cis*-diol **2-D$_4$** is consistent with naphthalene **1-D$_2$** being a precursor (Scheme 3, step a or steps b and c) and having a deuterium content of *ca.* 56% at positions C-1 and C-4 as a result of a primary kinetic isotope effect. The equal distribution of deuterium label at positions C-1 and C-4 (or C-5 and C-8) is incompatible with any significant involvement of the transient triol **12-D$_2$** to yield the *cis*-diol **2-D$_2$** (Scheme 3, steps b, e and f). A concentration of deuterium label only at positions C-5 and C-8 would have been expected if the triol **12-D$_2$** had been the sole precursor of the *cis*-dihydro diol **2-D$_4$**.

When racemic *trans*-1,4-dihydro[4-$^2H_1$]naphthalen-1-ol **7-D$_1$** was added as substrate to *P. putida* UV4, the *cis*-dihydro diol **2-D$_4$** was isolated as the major metabolite and again showed an equal distribution of deuterium label at positions C-1, C-4, C-5 and C-8 (*ca.* 28%, Scheme 4). This observation can be rationalized by the rapid dehydration of the substrate **7-D$_1$** *via* a conjugate *syn*-1,4-elimination[12] to yield the naphthalene **1-D$_1$** followed by *cis*-dihydroxylation (Scheme 4, steps c and d) and is incompatible with involvement of the triol intermediate **12-D$_1$** (Scheme 4, steps e and f).

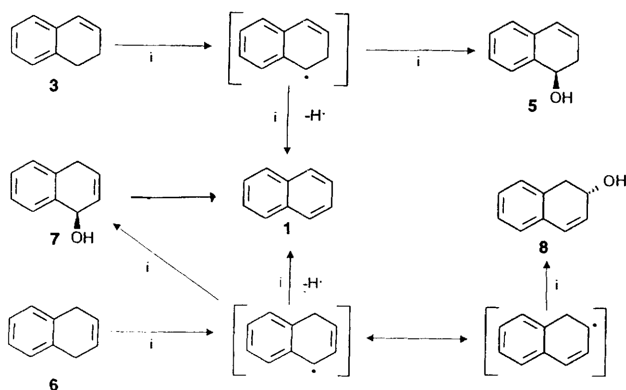
The *cis*-dihydro diol metabolite **2-D$_4$** obtained from the substrate **7-D$_1$** (Scheme 4) was accompanied by a minor metabolite which decomposed to 1-naphthol **9** during attempted isolation and is again tentatively assumed to be 1,4-dihydronaphthalene-1,4-diol **10-D$_2$**. When (*R*)-1,4-dihydronaphthalen-1-ol **7** ($[\alpha]_D$ −159, >98% ee, isolated metabolite) was added as a substrate to *P. putida* UV4, the



Scheme 4 *Reagent:* i, *P. putida* UV4

cis-dihydro diol **2** ([α]$_D$ +244, >98% ee) was isolated as the sole metabolite (36% yield). Addition of (*S*)-1,4-dihydronaphthalen-1-ol **7** ([α]$_D$ +156, >98% ee, isolated by preparative HPLC, see later) to *P. putida* UV4 yielded only 1-naphthol **9** (40% yield). These results suggest that only the (*R*)-enantiomer of the monol **7** is formed from 1,4-dihydronaphthalene **6** by the organism and that it is dehydrated rapidly to yield naphthalene **1**. The formation of 1-naphthol **9** from the (1*S*)-enantiomer of arene hydrate **7** may again be rationalized in terms of selective benzylic alcohol oxidation under the influence of an (*S*)-monol dehydrogenase to yield the corresponding ketone (which exists as the phenol **9**).

The formation of 1,2-dihydronaphthalen-2-ol **8** as a minor metabolite of 1,4-dihydronaphthalene **6** can be explained in terms of a dioxygenase-catalysed oxidation *via* a benzylic/allylic radical (Scheme 5). This type of mechanism has previously been



Scheme 5 *Reagent:* i *P. putida* UV4

proposed by Gibson *et al.* for the benzylic hydroxylation of indene using *P. putida* 39D.[5] The proposal that this mechanism[3,4] may also be extended to the benzylic hydroxylations of 1,2-dihydronaphthalene **3** and 1,4-dihydronaphthalene **6** has received support from the recent studies of Gibson *et al.* using purified dioxygenase enzymes.[10] Scheme 5 shows the formation of naphthalene **1**, and the monohydroxylation products **5**, **7** and **8** from both 1,2-dihydronaphthalene **3** and 1,4-dihydronaphthalene **6**, *via* benzylic and allylic radicals.

The presence of the monol **8** as a metabolite of 1,4-dihydronaphthalene **6** and the absence of compound **8** as a metabolite of 1,2-dihydronaphthalene **3** suggests that allylic hydroxylation is not a preferred oxidation pathway in *P. putida* UV4.

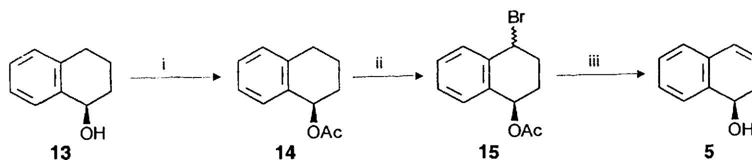
In conclusion, the use of deuterium labelled substrates (**3-D**, **5-D′**, **6-D$_2$** and **7-D$_1$**) with *P. putida* UV4 provides evidence for (i) dehydrogenation (Scheme 1, step a and possibly Scheme 3, step a), (ii) benzylic hydroxylation (Scheme 1, step b; Scheme 3, step b), (iii) allylic alcohol formation (Scheme 3, step g), (iv) triol formation (Scheme 1, step e; Scheme 2, step e) and (v) dehydration (Scheme 1, step f; Scheme 2, step f; Scheme 3, step c). The greater propensity of 1,4-dihydronaphthalen-1-ol **7** to dehydrate compared with 1,2-dihydronaphthalen-1-ol **5** or 1,2-dihydronaphthalen-2-ol **8**[13] may be an important factor in the rapid formation of naphthalene **1** from 1,4-dihydronaphthalene **6**. The absence of the triol intermediate **12-D$_1$** (Scheme 4) may also be linked to the relative instability of the benzylic alcohol **7-D$_1$** which may dehydrate (step c) before *cis*-dihydroxylation (step e) can occur.

The benzylic/allylic alcohol metabolites **5**, **7** and **8**, isolated from *P. putida* UV4 biotransformations of substrates **3** and **6**, have previously been described as arene hydrates[3,4] since they are formally equivalent to adducts of water and an arene. Although arene hydrates have been postulated as liver metabolites in early studies (*e.g.* arene hydrates of naphthalene[14]) unequivocal direct evidence for their formation from the parent arenes has been unavailable. The isolation of compounds **5**, **7** and **8** as biotransformation products from dioxygenase-catalysed oxidation of substrates **3** and **6** provides the first unequivocal evidence for the structure and absolute stereochemistry of arene hydrate metabolites. Liver microsomal metabolism studies of 1,2-dihydronaphthalene,[15] 1,2-dihydroanthrancene,[15] 5,6-dihydroquinoline,[16] 7,8-dihydroquinoline[16] and 9,10-dihydrobenzo[*e*]pyrene[17] have provided evidence of monooxygenase-catalysed oxidation to yield arene hydrates of similar structure to compounds **5** and **8**.

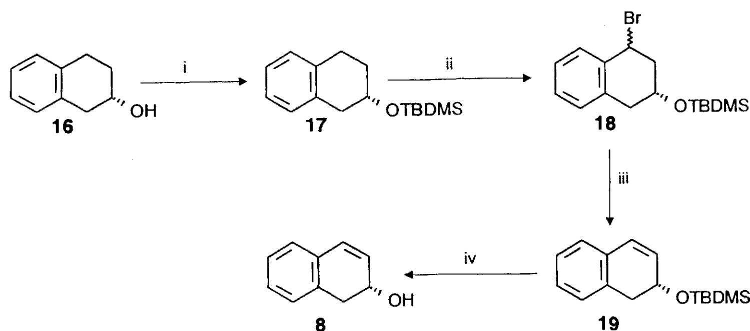
(b) Synthesis of enantiopure arene hydrates of naphthalene

Chemical synthetic routes for the arene hydrates **5**, **8** and **7** of naphthalene have previously been reported.[12,18,19] Unfortunately, none of these compounds had been obtained in optically active form and the literature methods used did not appear to be the most appropriate for the isolation of enantiopure compounds. Hence, alternative synthetic routes were used based upon alcohol precursors which were readily available as single enantiomers of known absolute configuration. (+)-(1*R*)-1,2-Dihydronaphthalen-1-ol **5** ([α]$_D$ +52, $CHCl_3$) was obtained in a total yield of *ca.* 30% from the commercially available (−)-(*R*)-1,2,3,4-tetrahydronaphthalen-1-ol **13** ([α]$_D$ −32, $CHCl_3$), *via* the acetate **14** ([α]$_D$ +98, $CHCl_3$) and the bromoacetate **15** ([α]$_D$ +49, $CHCl_3$) intermediates (Scheme 6).

Similarly, a sample of (−)-(*S*)-1,2,3,4-tetrahydronaphthalen-2-ol **16** ([α]$_D$ −61, available from the hydrogenolysis of the tetrahydro diacetate of the *cis*-diol metabolite **2**) was converted into the *tert*-butyl dimethylsilyl ether (TBDMS) derivative **17** ([α]$_D$ −50, $CHCl_3$) prior to benzylic bromination to yield the bromo TBDMS derivative **18**. Dehydrobromination gave the unstable TBDMS derivative **19** which was immediately deprotected using Bu_4NF–THF to yield the (+)-(*R*)-enantiomer of arene hydrate **8** ([α]$_D$ +267, $CHCl_3$) in a total yield of *ca.* 15% (Scheme 7).



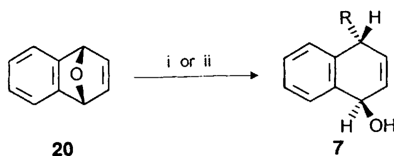
Scheme 6 *Reagents:* i, Ac_2O/pyridine; ii, NBS/CCl_4; iii, NaOMe/THF



Scheme 7 *Reagents*: i, $Bu^tSiMe_2OSO_2CF_3$–CH_2Cl_2–Et_3N; ii, NBS–CCl_4; iii, NaOMe–THF; iv, Bu_4NF–THF

1,2-Dihydro[1-$^2H_1$]napththalen-1-ol **5-D′** was prepared from 1,2,3,4-tetrahydro[1-$^2H_1$]naphthalen-1-ol **13** (from $LiAlD_4$ reduction of 1-tetralone) as precursor using the method outlined in Scheme 6.

Using the literature method,[12] a racemic sample of *trans*-1,4-dihydro[4-$^2H_1$]naphthalen-1-ol **7-D$_1$** was obtained from $Li(Bu^tO)_3AlD$ reduction of 1,4-epoxy-1,4-dihydronaphthalene **20**. The deuterium atom was inserted *trans* to the hydroxy group and thus the naphthalene formed by dehydration contained a deuterium atom at C-1 by a *syn* elimination mechanism (Scheme 8).



Scheme 8 *Reagents*: i, $Li(Bu^tO)_3AlH$ (R = H); ii, $Li(Bu^tO)_3AlD$ (R = D)

The individual enantiomers of racemic samples of the arene hydrates **5**, **7** and **8** were found to be separable by chiral stationary phase (CSP) HPLC (Chiralcel OB, hexane–propan-2-ol). The enantiopurity of the metabolites **5** ($>98\%$ ee, α 1.32), **7** ($>98\%$ ee, α 3.30) and (**8**, *ca.* 3% ee, α 1.10) was determined both by $[\alpha]_D$ measurement and by CSP HPLC. A semi-preparative version of the Chiralcel OB column provided a direct separation method for the individual enantiomers of arene hydrate **7** ($[\alpha]_D$ ± 159, $CHCl_3$).

Synthetic routes to the enantiopure arene hydrates **5** and **7** outlined in Schemes 6 and 7 have proved to be generally applicable to the synthesis of heterocyclic analogues[16] and to larger members of the PAH series, *e.g.* anthracene and phenanthrene. Application of the latter arene hydrates as reference compounds will facilitate the quest for arene hydrates as metabolites of the larger PAHs.

Experimental

Samples of (−)-(*R*)-1,2,3,4-tetrahydronaphthalen-1-ol **13** and 1,4-epoxy-1,4-dihydronaphthalene **20** were obtained from the Aldrich Chemical Company. ^1H NMR spectra were recorded at 300 and 500 MHz using General Electric QE300 and GN500 instruments, respectively. Coupling constants are reported in Hz. ^2H NMR spectra were recorded at 400 MHz using a Bruker WP400 instrument. Mass spectral data was recorded at 70 eV using an AEI-MS902 instrument updated by VG Autospec Instruments and accurate molecular weights were determined by the peak-matching method. CSP HPLC analysis was carried out using a Perkin-Elmer Series 3B model HPLC instrument and Chiralcel OB or OJ columns. Optical rotations were measured using a Perkin-Elmer Model 241 polarimeter at 589 nm.

Biotransformations were carried out using *P. putida* UV4 and the shake flask or fermentor method. The conditions for the biotransformations were identical with those reported previously.[20–22]

Biotransformation of 1,2-dihydronaphthalene 3

A fermentor scale (8 dm^3) biotransformation of 1,2-dihydronaphthalene **3** (8.0 g) over a 12 h period followed by dichloromethane extraction yielded a crystalline product mixture (4.83 g) which appeared to contain three major components by ^1H NMR analysis. Separation of these components was achieved by flash chromatography on silica gel. Elution with diethyl ether–hexane (1:1) yielded (+)-(*R*)-1,2-dihydronaphthalen-1-ol **5** (0.8 g). Elution with MeOH–CH_2Cl_2 (5:95) yielded a mixture of the diols **2** and **4**. The major diol **2** (2.8 g) was separated by crystallization from the mixture (CH_2Cl_2). The residual minor portion of the *cis*-dihydro diol **2** present in the mother liquor was oxidized to the 3,4-epoxy derivative of 1,2-dihydronaphthalene **3** upon being stirred with *meta*-chloroperoxybenzoic acid in CH_2Cl_2 at ambient temperature overnight. Separation of the more polar epoxide by PLC on silica gel using MeOH–$CHCl_3$ (96:4) yielded a pure sample of the *cis*-tetrahydro diol **4** (1.0 g).

The relative yields of metabolites obtained after the 12 h biotransformation were determined by ^1H NMR analysis as **5** (18%), **2** (61%) and **4** (21%). A time course experiment on the biotransformation of 1,2-dihydronaphthalene **2** showed that the relative proportion of arene hydrate **5** was optimal (*ca.* 60%) during the early phase (*ca.* 3 h) while the *cis*-diol metabolites **2** and **4** were present as the major bioproducts in the latter stages (12 h).

(+)-(R)-1-*Hydroxy*-1,2-*dihydronaphthalene* **5**.—Mp 52–53 °C (bp 101–103 °C/0.5 mmHg[18]), $[\alpha]_D$ +52 (*c* 0.5, $CHCl_3$); δ_H(300 MHz, $CDCl_3$) 1.72 (1 H, d, $J_{OH,1}$ 6.5, OH), 2.59 (2 H, m, 2-H, 2′-H), 4.77 (1 H, m, 1-H), 5.98 (1 H, m, 3-H), 6.54 (1 H, d, $J_{4,3}$ 9.8, 4-H), 7.10 (1 H, m, ArH) and 7.20–7.30 (2 H, m, ArH) and 7.35 (1 H, m, ArH).

(+)-(1R,2S)-cis-1,2-*Dihydronaphthalene*-1,2-*diol* **2**.—Mp 114–115 °C (CH_2Cl_2–hexane) (lit.,[1] 115–116 °C); $[\alpha]_D$ +218 (*c* 0.6, MeOH) (lit.,[1] $[\alpha]_D$ +220, MeOH); δ_H[300 MHz, $(CD_3)_2CO$] 4.28 (1 H, m, 2-H), 4.58 (1 H, d, $J_{1,2}$ 5.7, 1-H), 6.25 (1 H, dd, $J_{3,2}$ 4.2, $J_{3,4}$ 9.3, 3-H), 6.51 (1 H, d, $J_{4,3}$ 9.3, 4-H) and 7.1–7.5 (4 H, m, ArH).

(+)-(1S,2R)-cis-1,2,3,4-*Tetrahydronaphthalene*-1,2-*diol* **4**.—Mp 129–130 °C (CH_2Cl_2–hexane) (lit.,[1] 129–130 °C), $[\alpha]_D$ +39 (*c* 3.1, $CHCl_3$); δ_H(300 MHz, $CDCl_3$) 2.00 (2 H, m, 3-H, 3′-H), 2.81 (1 H, m, 4-H), 2.96 (1 H, m, 4′-H), 4.03 (1 H, m, 2-H), 4.71 (1 H, d, $J_{1,2}$ 3.7, 1-H) and 7.11–7.45 (4 H, m, ArH).

GC/MS analysis of the crude product mixture after trimethylsilylation using bis(trimethylsilyl)trifluoroacetamide (25m BP 1 column, 154 °C) showed that in addition to the major metabolites **2**, **4** and **5**, naphthalene **1** was present as a minor component ($<5\%$).

1,2-Dihydro[4-$^2H_1$]naphthalene **3-D** was prepared in 83% overall yield from 1,2,3,4-tetrahydronaphthalen-1-one by reduction with $LiAlD_4$ and acid-catalysed dehydration (toluene-*p*-sulfonic acid in benzene). Distillation under reduced

pressure yielded 1,2-dihydro[4-$^2H_1$]naphthalene **3-D**, bp 42–44 °C/1 mmHg (lit.,[23] 84–85 °C/12 mmHg), >98% incorporation of ^2H at C-4. Biotransformation of 1,2-dihydro[4-$^2H_1$]naphthalene **3-D** under similar conditions to those used for the unlabelled dihydronaphthalene **3** yielded the major metabolites **4** and **5** with the deuterium label (>98% $^2H_1$) located at positions C-1 and C-4, respectively (^1H NMR analysis). The *cis*-dihydro diol metabolite **2-D**/**2-D$_4$** was found to contain a major portion of the deuterium label at C-5 (52 ± 3% $^2H_1$) and an equal proportion (16 ± 3% $^2H_1$) of label at positions C-1, C-4 and C-8 (^2H NMR analysis).

Biotransformation of 1,2-dihydro[1-$^2H_1$]naphthalen-1-ol 5-D′, (+)-(1*R*)- and (−)-(1*S*)-1,2-dihydronaphthalen-1-ol 5

A small scale (0.1 g) biotransformation of the racemic arene hydrate **5-D′** was carried out over a 24 h period. Extraction with ethyl acetate of the NaCl-saturated aqueous culture medium and concentration of the dried organic extracts yielded a crude mixture of metabolites. ^1H NMR analysis of the latter showed it to be a mixture of the residual arene hydrate **5-D′** (67% relative yield), *cis*-dihydro diol **2-D′** (28% relative yield) and 1-naphthol **9** (5% relative yield). The two major components were separated by preparative TLC on silica gel using ethyl acetate–hexane (2:3). The recovered arene hydrate **5-D′** was found to have an excess of the (1*R*)-enantiomer (30% ee). The ^1H NMR spectrum of *cis*-dihydro diol **2-D′** was similar to that of the *cis*-dihydro diol **2** except for the absence of one aromatic proton in the range δ 7.1–7.4.

When a similar biotransformation was carried out using the (+)-(1*R*)-enantiomer of the arene hydrate **5** (0.05 g) for 20 h, the product (0.03 g) showed the presence of recovered substrate **5** (85% relative yield), *cis*-dihydro diol **2** (14% relative yield) and 1-naphthol **9** (6% relative yield) by ^1H NMR and GC-MS analysis. Using the (−)-(1*S*)-enantiomer of the arene hydrate **5** as substrate (0.05 g) under identical conditions the product (0.03 g) showed the presence of recovered substrate (29% relative yield) and 1-naphthol **9** (71% relative yield) only.

Biotransformation of 1,4-dihydronaphthalene 6

Biotransformation of 1,4-dihydronaphthalene **6** (3 g) was carried out in a fermentor (5 dm^3) over a 23 h period. Extraction of the culture medium by EtOAc and ^1H NMR analysis showed the major product (*ca.* 90%) to be (+)-(1*R*,2*S*)-*cis*-1,2-dihydronaphthalene-1,2-diol **2**. A minor component (*ca.* 10%) was detected in the crude ^1H NMR spectrum which showed characteristic singlets at δ 5.15 and 6.22. This minor component showed chromatographic characteristics identical with those of the major metabolite **2** but was found to readily decompose to yield 1-naphthol **9**. The residual *cis*-dihydro diol metabolite **2** was indistinguishable from that obtained from metabolism of substrate **3**. When the biotransformation was repeated and terminated after a much shorter period (0.5 h), a major proportion of the substrate remained and the *cis*-dihydro diol metabolite **2** was found to be present as a relatively minor component (10%), in the presence of two monohydroxylation products **7** (81%) and **8** (9%). Preparative TLC was employed to separate the monols **7** and **8** (diethyl ether–hexane, 20:80) and the *cis*-dihydro diol **2** (MeOH–$CHCl_3$, 4:96).

(−)-(R)-1,4-*Dihydronaphthalen*-1-*ol* **7**.—Mp 60–61 °C (pentane) (lit.,[12] 48 °C, racemic), $[\alpha]_D$ −159 (*c* 0.3, $CHCl_3$); δ_H(300 MHz, $CDCl_3$) 1.87 (1 H, d, $J_{HO,1}$ 9.2, OH), 3.40 (2 H, m, 4-H, 4′-H), 5.15 (1 H, m, 1-H), 6.09 (2 H, m, 2-H, 3-H), 7.14–7.32 (3 H, m, ArH) and 7.6 (1 H, m, ArH).

(−)-(S)-1,2-*Dihydronaphthalen*-2-*ol* **8**.—Mp 26–28 °C (lit.,[24] 20–25 °C, racemic), $[\alpha]_D$ −7.1 (*c* 0.14, $CHCl_3$); δ_H(300 MHz, $CDCl_3$) 1.50 (1 H, s, OH), 3.05 (2 H, d, $J_{1,2}$ 6.0, 1-H, 1′-H), 4.47 (1 H, m, 2-H), 6.12 (1 H, dd, $J_{3,2}$ 4.3, $J_{3,4}$ 9.5, 3-H), 6.56 (1 H, d, $J_{4,3}$ 9.5, 4-H) and 7.09–7.25 (4 H, m, ArH).

The *cis*-dihydro diol metabolite **2** ($[\alpha]_D$ +244, $CHCl_3$) was indistinguishable from the sample obtained from biotransformation of 1,2-dihydronaphthalene **3**.

1,4-Dihydro-[1,4-$^2H_2$]naphthalene **6-D$_2$** was obtained from naphthalene **1** by treatment with sodium and EtOD according to the literature procedure.[25] The deuterium incorporation at C-1 and C-4 was found to be >98% by NMR analysis. A small scale (0.25 g) biotransformation of 1,4-dihydro[1,4-$^2H_2$]naphthalene **6-D$_2$** was carried out over a 16 h period. Extraction (EtOAc) and preparative TLC purification gave *cis*-dihydro diol **2-D$_4$** as the major metabolite (63%). ^2H NMR analysis of the *cis*-dihydro diol **2-D$_4$** showed an equal distribution of deuterium (28 ± 3%) at positions C-1, C-4, C-5 and C-8. Minor metabolites were found to include 1,2-dihydronaphthalen-2-ol **8** and a compound which readily rearranged to 1-naphthol **9**.

Biotransformation of *trans*-1,4-dihydro[4-$^2H_1$]naphthalen-1-ol 7-D$_1$, (−)-(1*R*)- and (+)-(1*S*)-1,4-dihydronaphthalen-1-ol 7

A small-scale biotransformation of racemic *trans*-1,4-dihydro[4-$^2H_1$]naphthalen-1-ol **7-D$_1$** (0.05 g, >98% $^2H_1$) was carried out over an 18 h period by the shake-flask method. The crude extract (EtOAc) was analysed by ^1H NMR spectroscopy and found to contain (1*R*,2*S*)-*cis*-1,2-dihydronaphthalene-1,2-diol **2-D$_4$**, (67% relative yield), 1-naphthol **9** (33% relative yield). Preparative TLC purification yielded the pure *cis*-dihydro diol **2-D$_4$** whose ^1H and ^2H NMR spectra showed an equivalent proportion of deuterium (*ca.* 28 ± 3% $^2H_1$) at positions C-1, C-4, C-5 and C-8.

When the biotransformation was repeated using (−)-(1*R*)-1,4-dihydronaphthalen-1-ol **7** (0.05 g, $[\alpha]_D$ −159) the only isolated product was (+)-(1*R*,2*S*)-*cis*-1,2-dihydronaphthalene-1,2-diol **2** (0.018 g), $[\alpha]_D$ +244 (36% yield). Using the (+)-(1*S*)-enantiomer of arene hydrate **7** (0.05 g), $[\alpha]_D$ +156 under identical biotransformation conditions yielded only 1-naphthol **9** (0.02 g, 40%).

The *trans*-1,4-dihydro[4-$^2H_1$]naphthalen-1-ol substrate **7-D$_1$** was synthesised from 1,4-epoxy-1,4-dihydronaphthalene **20** using lithium tri-*tert*-butoxyaluminium deuteride according to the literature procedure.[12]

Chemical synthesis of enantiopure arene hydrates (+)-(*R*)-5 and (+)-(*S*)-8

(+)-(*R*)-1-Acetoxy-1,2,3,4-tetrahydronaphthalene 14. An excess of acetic anhydride (1.5 cm^3) was added to a solution of (*R*)-1,2,3,4-tetrahydronaphthalen-1-ol **13** (0.5 g, 3.37 mmol; $[\alpha]_D$ −32) in pyridine (2 cm^3). The reaction mixture was stirred overnight at room temperature and then worked up to give the crude acetate (0.630 g, 98%). Distillation under reduced pressure yielded (+)-(*R*)-1-acetoxy-1,2,3,4-tetrahydronaphthalene **14**, bp 84 °C/0.4 mmHg, $[\alpha]_D$ +98 (*c* 4.4, $CHCl_3$) (lit.,[26] bp 105–110 °C/2 mmHg, racemic); δ_H(300 MHz, $CDCl_3$) 1.78–2.05 (4 H, m, 3-H, 3′-H, 2-H, 2′-H), 2.07 (3 H, s, $OCOCH_3$), 2.70–2.91 (2 H, m, 4-H, 4′-H), 5.99 (1 H, d, $J_{1,2}$ 3.8, 1-H) and 7.11–7.28 (4 H, m, ArH).

(*R*)-1-Acetoxy-4-bromo-1,2,3,4-tetrahydronaphthalene 15. A solution of (*R*)-1-acetoxy-1,2,3,4-tetrahydronaphthalene **14** (0.5 g, 2.63 mmol; $[\alpha]_D$ +98) in tetrachloromethane (20 cm^3) was heated to reflux temperature using an oil bath. *N*-Bromosuccinimide (0.5 g, 2.9 mmol) and a catalytic amount of azoisobutyronitrile (AIBN; *ca.* 0.005 g) were added to the refluxing solution which was then heated for a further 0.25 h using a heat lamp until the succinimide product was totally formed. The solution was cooled, filtered and concentrated to yield a mixture of *cis*- and *trans*-(*R*)-1-acetoxy-4-bromo-1,2,3,4-tetrahydronaphthalene **15** as a colourless oil (0.6 g, 86%), $[\alpha]_D$ +49 (*c* 4, $CHCl_3$); δ_H(300 MHz, $CDCl_3$) 2.03 (3 H, s, $OCOMe_A$), 2.17 (3 H, s, $OCOMe_B$), 2.03–2.50 (8 H, m, 2-H_A, 2-H_B, 2′-H_A,

2′-H_B, 3-H_A, 3-H_B, 3′-H_A, 3′-H_B), 5.50 (1 H, m, 4-H_B), 5.60 (1 H, m, 4-H_A), 6.05 (2 H, m, 1-H_A, 1-H_B) and 7.22–7.43 (8 H, m, ArH).

A small sample of the *trans*-isomer $\mathbf{15}_{trans}$ was recrystallized from hexane to give pure material mp 86–88 °C, $[\alpha]_D$ +72 (*c* 0.58, $CHCl_3$) but the bulk of the material was used in the next stage without separation of *cis*- and *trans*-isomers.

(+)-(*R*)-1,2-Dihydronaphthalen-1-ol 5. Sodium methoxide (1.5 g, 26 mmol) was added to a stirred solution of *cis*/*trans*-1-acetoxy-4-bromo-1,2,3,4-tetrahydronaphthalene **15** (0.7 g, 2.6 mmol; $[\alpha]_D$ +49) in dry THF (50 cm^3) under nitrogen at 0 °C and these conditions were maintained for 6 h. Water (30 cm^3) was added to the mixture from which THF was then removed under reduced pressure. The aqueous solution was extracted (Et_2O) and the extract was dried and concentrated to yield the crude arene hydrate **5**. Purification by preparative TLC (silica gel; diethyl ether–hexane containing 0.1% triethylamine) gave the title compound **5**, (0.13 g, 34%) as a colourless oil which was purified by distillation (bp 53–55 °C/0.1 mmHg, $[\alpha]_D$ +52, $CHCl_3$). The (+)-(*R*)- arene hydrate **5** was found to be spectrally identical with the metabolite. The (−)-(1*S*)-form was also chemically synthesised starting from (*S*)-1,2,3,4-tetrahydronaphthalen-1-ol **13**.

(−)-(*S*)-2-*tert*-Butyldimethylsilyloxy-1,2,3,4-tetrahydronaphthalene 17. To a cooled solution (0 °C) of (−)-(*S*)-1,2,3,4-tetrahydronaphthalen-2-ol **16** (0.320 g, 2.16 mmol; $[\alpha]_D$ −61) in dry dichloromethane(15 cm^3), containing triethylamine (0.4 cm^3) and dimethylaminopyridine (0.135 g), was added *tert*-butyldimethylsilyl chloride (0.410 g, 2.71 mmol). The reaction mixture was stirred (12 h) at ambient temperature and then diluted with dichloromethane (20 cm^3), washed successively with 5% hydrochloric acid, water and 5% aqueous sodium hydrogencarbonate, dried (Na_2SO_4) and concentrated under reduced pressure to yield the crude TBDMS ether **17** (0.550 g, 97% yield) as a pale yellow oil. Distillation of this *in vacuo* gave the TBDMS ether **17** as a colourless oil, bp 60 °C at 0.01 mmHg, $[\alpha]_D$ −50 ($CHCl_3$) (Found: C, 73.0; H, 9.7. $C_{16}H_{26}SiO$ requires C, 73.3; H, 9.9%); δ_H(300 MHz, $CDCl_3$) 0.90 [15 H, s, $Si(CH_3)_2C(CH_3)_3$], 1.76 (1 H, m, 3′-H), 1.95 (1 H, m, 3-H), 2.75 (2 H, m, 1-H), 2.96 (2 H, m, 4-H), 4.05 (1 H, m, 2-H) and 7.08 (4 H, m, ArH).

(2*R*)-4-Bromo-2-*tert*-butyldimethylsilyloxy-1,2,3,4-tetrahydronaphthalene 18. To a solution of (−)-(*S*)-2-*tert*-butyldimethylsilyloxy-1,2,3,4-tetrahydronaphthalene **17** (0.260 g, 0.99 mmol; $[\alpha]_D$ −50, $CHCl_3$), in carbon tetrachloride (10 cm^3), under nitrogen atmosphere, were added *N*-bromosuccinimide (0.194 g, 1.09 mmol) and azoisobutyronitrile (0.005 g). The stirred reaction mixture was heated (60 °C) for 0.5 h using a heat lamp. After cooling, the reaction mixture was filtered to remove the precipitated succinimide and the filtrate was evaporated to yield the crude bromosilyloxy mixture of isomers **18** as a semisolid (0.33 g, 97%) (Found M − 1, 341.075 766. $C_{16}H_{24}BrOSi$ requires 341.079 34); δ_H(300 MHz, $CDCl_3$) 0.10 [6 H, s, $Si(CH_3)_2$], 0.15 [6 H, s, $Si(CH_3)_2$], 0.93 [9 H, s, $Si(CH_3)_3$], 0.94 [9 H, s, $Si(CH_3)_3$], 2.1–3.2 (8 H, m, 2 × 1-H, 2 × 1′-H, 2 × 3-H, 2 × 3′-H), 3.90 (1 H, m, 2-H), 4.12 (1 H, m, 2-H), 5.48 (1 H, m, 4-H), 5.65 (1 H, m, 4-H) and 7.00–7.40 (8 H, m, ArH). The crude bromo TBDMS ether mixture **18** was used without further purification in the next step.

(+)-(*R*)-1,2-Dihydronaphthalen-2-ol 8. A solution of compound **18** (0.350 g, 1.03 mmol) in dry THF (15 cm^3) was cooled to 0 °C under nitrogen and treated with sodium methoxide (0.480 g, 8.9 mmol). The reaction mixture was stirred at 0 °C for 3 h after which most of the THF was removed under reduced pressure. The residue was treated with diethyl ether (40 cm^3) and the ether solution was then washed with cold water. Evaporation, of the dried (Na_2SO_4) ether extract, under reduced pressure gave the crude (2*R*)-*tert*-butyldimethylsilyloxy-1,2-dihydronaphthalene **19** as a colourless oil (0.220 g, 82%); δ_H(300 MHz, $CDCl_3$) 0.09 [6 H, s, $Si(CH_3)_2$], 0.91 [9 H, s, $SiC(CH_3)_3$], 2.92 (2 H, m, 1-H), 4.67 (1 H, m, 2-H), 5.92 (1 H, dd, $J_{3,2}$ 2.2 $J_{3,4}$ 9.7, 3-H), 6.39 (1 H, d, $J_{4,3}$ 9.7, 4-H) and 7.02–7.20 (4 H, m, ArH).

To a cooled solution (0 °C) of the above crude silyloxydihydronaphthalene **19** (0.200 g, 0.77 mmol) in THF (5 cm^3) was added, dropwise, under nitrogen, a solution of tetrabutylammonium fluoride in THF (1 mol dm^{-3}; 1 cm^3). After being stirred for 2 h at 0 °C the reaction mixture was quenched with cold water (1 cm^3) and then concentrated by removal of most of the THF under reduced pressure. The residue was extracted into ether (25 cm^3) and the extract dried (Na_2SO_4) and evaporated to yield the crude title compound **8** as a light-yellow semisolid. Purification by PLC (silica gel, ether–hexane, 1:2 containing 0.1% triethylamine) and subsequent crystallization from hexane yielded pure arene hydrate **8** as colourless crystals (0.030 g, 27%), mp 26–28 °C (lit.,[24] 20–25 °C), $[\alpha]_D$ +267 ($CHCl_3$). The arene hydrate **8** was spectrally identical with the metabolite.

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