Chiral Arene Hydrates of Naphthalene: Enzymatic and Chemical Syntheses

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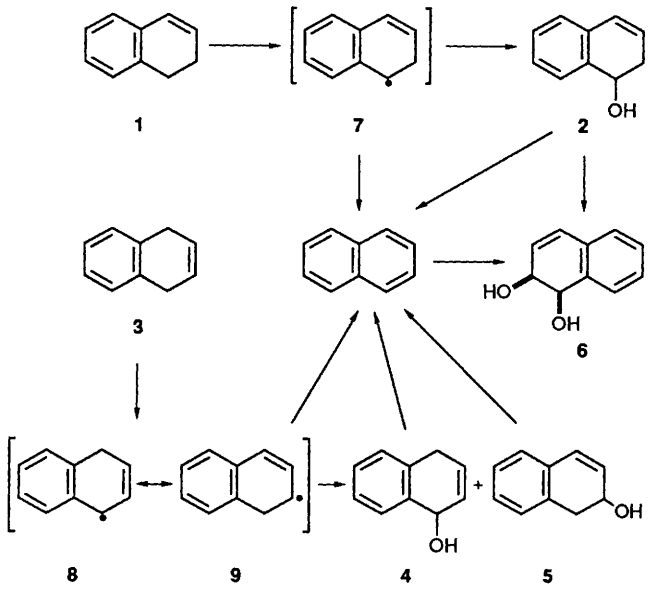
Bacterial metabolism of the dihydronaphthalenes **1** or **3** using cultures of *Pseudomonas putida* UV4 yielded the arene hydrates of naphthalene **2** or a mixture of **4** and **5** respectively; these arene hydrates have also been obtained in homochiral form either by synthesis from enantiomerically pure alcohols **10** and **12** and arene oxide **11** precursors or by chiral stationary phase HPLC separation of arene hydrate enantiomers.

The metabolism of polycyclic aromatic hydrocarbons (*e.g.* naphthalene,[1–5] anthracene,[3,6] phenanthrene,[3] quinoline[7,8]) and dihydroarenes (*e.g.* dihydronaphthalene[5] and dihydrobenzo[*e*]pyrene[9]) has been postulated to involve arene hydrates. Despite these early reports, due to their instability no arene hydrate had been isolated and structurally (or stereochemically) assigned until (+)-(*R*)-1-hydroxy-1,2-dihydronaphthalene **2** was obtained as a metabolite of 1,2-dihydronaphthalene **1** using a mutant strain of *Pseudomonas putida*.[10] A recent study with *P. putida* has shown an arene hydrate of acetophenone to be a metabolite of the parent arene.[11] As part of a systematic quest for enzymatically formed arene hydrates, 1,4-dihydronaphthalene **3** has also been used as a substrate for the bacterium *P. putida* UV4. Benzylic and allylic hydroxylation of compound **3** (catalysed by the endogenous benzene dioxygenase) has now been found to yield two further arene hydrates of naphthalene **4** and **5**.

The optimal proportions of arene hydrate metabolies **4** and **5** relative to *cis*-dihydrodiol metabolite **6** (81 : 9 : 10 respectively by ^{1}H NMR analysis) were obtained using alkene **3** as substrate for *P. putida* and a short incubation time (0.5 h). Metabolism of 1,2-dihydronaphthalene **1**[10] and 1,4-dihydronaphthalene **3** each yielded a homochiral sample of the *cis*-dihydrodiol metabolite **6**. As shown in Scheme 1, metabolite **6** could have arisen from naphthalene formed by (*a*) dehydration of arene hydrates **2**, **4** and **5**, and/or (*b*) loss of a



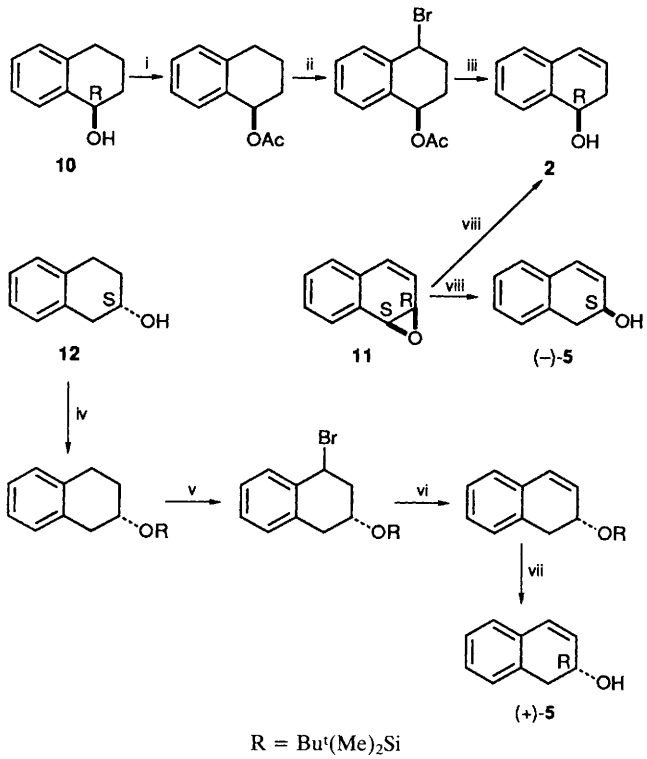
hydrogen atom from the radical intermediates **7**, **8** and **9**, in each case followed by the normal oxidation pathway to yield the *cis*-dihydrodiol product. A novel alternative mechanism for the formation of metabolite **6** involving oxidation of the arene hydrates to yield unstable triol intermediates, followed by dehydration (as previously suggested for arene hydrate **2**, on the basis of [^{2}H]-labelling experiments[10]) may also be applicable to arene hydrates **4** and **5**.

Arene hydrate **4** was found to dehydrate much more readily than arene hydrates **2** and **5** under acidic conditions.[12] Thus, preparative TLC separation was carried out rapidly on silica gel using diethyl ether–pentane (4 : 1) containing a trace of triethylamine as eluent to yield 1-hydroxy-1,4-dihydronaphthalene (**4**, high R_f, $[\alpha]_D$ − 152°), 2-hydroxy-1,2-dihydronaphthalene (**5**, low R_f, $[\alpha]_D$ −7°) and *cis*-1,2-dihydroxy-1,2-dihydronaphthalene **6**.†

Using chiral stationary phase (CSP) HPLC analysis [Chiralcel OB, 250 × 4.6 mm, propan-2-ol–hexane (1 : 9) as eluent] a baseline resolution of racemic arene hydrates **2** (α 1.32) and **4** (α 3.2) and a partial resolution of enantiomers of arene hydrate **5** (α 1.11) were obtained. When the arene hydrate metabolites **2** and **4**, resulting from benzylic hydroxylation of the dihydroarenes **1** and **3** in *P. putida*, were analysed by this CSP–HPLC method they were found to be essentially homochiral [96–98% enantiomeric excess (e.e.)], whereas the allylic hydroxylation product **5** was of low optical purity (*ca.* 3% e.e) (Table 1).

The relative roles of asymmetric synthesis and kinetic resolution in the production of the chiral arene hydrate metabolites **2**, **4** and **5** by *P. putida* are currently under investigation. Preliminary results show that when a racemic sample of arene hydrate **2** (produced by chemical synthesis) was used as substrate for *P. putida* the residual arene hydrate (isolated after incubation for 20 h) was found to be essentially optically pure (>98% e.e.) and of the same absolute configuration (1*R*) to that obtained using alkene **1** as substrate, *i.e.* a kinetic resolution process had occurred. The *cis*-dihydrodiol **6** was also isolated as a metabolite of arene hydrate **2** (Table 1). Regardless of the origin of the chirality in arene hydrates **2**, **4** and **5**, their formation is consistent with the initial involvement of a benzyl radical **7** and a benzyl-allyl radical **8**⟷**9**.

† All $[\alpha]_D$ measurements were recorded at ambient temperature in chloroform solution.



R = $Bu^t(Me)_2Si$

Scheme 1 *Reagents:* i, Ac_2O; ii, *N*-bromosuccinimide (NBS); iii, NaOMe; iv, $Bu^t(Me)_2SiCl$; v, NBS; vi, NaOMe; vii, Bu_4NF, viii, $LiAlH_4$

Parallel studies of the metabolism of the dihydronaphthalene **1** by rat liver microsomal enzymes[13] have again shown that arene hydrates **2** and **5** are formed as isolable metabolites, but the allylic hydroxylation product **5** was formed in a higher yield and optical purity than the benzylic hydroxylation product **2**.

The detection of arene hydrates **2**, **4** and **5** in optically active form from both microbial and animal enzyme sources prompted us to devise new and generally applicable synthetic routes to both racemic and homochiral forms (Scheme 1).

The availability of either enantiomer of 1-tetralol (Aldrich Chemical Co. Ltd.) and 2-tetralol [by acetylation, hydrogenolysis and hydrolysis of (1*S*,2*R*)-*cis*-1,2-dihydroxy-1,2,3,4-tetrahydronaphthalene and (1*R*,2*S*)-*cis*-dihydrodiol **6**, obtained using alkene **1** as substrate for *P. putida*] provided suitable precursors for the synthesis of arene hydrates **2** and **5**. Thus (−)-(*R*)-1-tetralol (**10**, $[\alpha]_D$ − 32°) was converted in three steps to the (1*R*)-arene hydrate (**2**, $[\alpha]_D$ + 49°) *via* the acetate (step i) and bromoacetate (step ii) intermediates in a total yield of 20% (steps i–iii). The general nature of this route has further been verified by application to arene hydrates analogous to **2** in the anthracene, phenanthrene and benzo[*e*]pyrene series.[14]

Although two benzylic carbon atoms are available in (−)-(*S*)-2-tetralol (**12**, $[\alpha]_D$ −61°), use of the bulky t-butyldimethylsilyl ether (TBDMS) group largely prevented bromination at the adjacent benzylic position. Thus, (2*R*)-arene hydrate **5** ($[\alpha]_D$ + 267°) was obtained by a four-step synthesis (steps iv–vii) *via* the TBDMS ether (step iv), the bromo TBDMS ether (step v), and the TBDMS ether of arene hydrate **5** (step vi) in a total yield of *ca.* 15%. Instability of the arene hydrate products **2** and **5** and the brominated intermediates led to a decrease in the overall yields.

Reduction of a small sample (0.01 g) of homochiral

Table 1 Metabolite products

Substrate	Metabolism time/h	Arene hydrate			*cis*-Dihydrodiol		
		Yield (%)[a]	E.e. (%)[b]	Absolute configuration	Yield (%)	E.e. (%)[b]	Absolute configuration
(**1**)	3	**2** 60	≥98	1*R*	**6** 15[c,d]	≥98	1*R*,2*S*
(±)-**2**	20	**2** 57	≥98	1*R*	**6** 43[d]	≥98	1*R*,2*S*
(**3**)	0.5	**4** 81	≥96	1*R*	**6** 10[d]	≥98	1*R*,2*S*
		5 9	*ca.* 3	2*S*			

[a] All substrates and arene hydrates were found to be relatively volatile. The total isolated yields of metabolites were generally in the range 10–15% based on the weight of the substrate added. The tabulated relative yields are from ^{1}H NMR analysis of crude extract. [b] Based upon $[\alpha]_D$ comparison with synthetic samples, CSP–HPLC analysis of the metabolites and ^{1}H NMR analysis of their methoxy(trifluoromethyl)-phenylacetyl derivatives. [c] Accompanied by (+)-(1*S*,2*R*)-*cis*-tetrahydrodiol in 20% relative yield. [d] Accompanied by other minor metabolites and substrate.

(−)-(1*S*,2*R*)-naphthalene 1,2-oxide [**11**, available from (*a*) earlier synthetic work[15] (*b*) by synthesis[14] from (1*S*,2*R*)*cis*-1,2-dihydroxy-1,2,3,4-tetrahydronaphthalene, a metabolite of dihydroarene **1** with *P. putida*[10]] using $LiAlH_4$ in diethyl ether (step viii) yielded (+)-(*R*)-1-hydroxy-1,2-dihydronaphthalene (**2**, 90%) and (−)-(*S*)-2-hydroxy-1,2-dihydronaphthalene (**5**, 10%). The separation of arene hydrates **2** and **5** by HPLC (microsorb SIL, 0.3% propan-2-ol in hexane as eluent) followed by enantiomer analysis using CSP–HPLC showed each arene hydrate to be optically pure.

Using a semi-preparative version of the Chiralcel OB CSP–HPLC column (250 × 10 mm) a racemic sample of arene hydrate **4**, obtained by the literature method,[16] was resolved into enantiomers ($[\alpha]_D$ ± 159°). Catalytic hydrogenation (Pd–C, H_2 in MeOH) of arene hydrate metabolite **4** ($[\alpha]_D$ −152°) yielded (−)-(*R*)-1-tetralol ($[\alpha]_D$ −30°) confirming the (1*R*)-configuration of arene hydrate metabolite **4**.

The development of these new general synthetic routes to arene hydrates **2** and **5** shown in Scheme 1 should facilitate the continuing search for arene hydrates as metabolites of both arenes and dihydroarenes in the polycyclic aromatic hydrocarbon series.

We acknowledge financial support from the biotechnology directorate of SERC (to N. D. S. and D. J. G.), DENI (to R. A. S. McM. and G. A. O'K.) and the Queen's University of Belfast (to R. A. and P. P.). We thank Dr S. C. Taylor (ICI, Billingham) for the gift of the *P. putida* strain.

Received, 1st August 1990; Com. 0/03540H

References

1 M. C. Bourne and L. Young, *Chem. Ind. (London)*, 1933, **52**, 271.
2 M. C. Bourne and L. Young, *Biochem, J.*, 1934, **28**, 803.
3 L. H. Chang and L. Young, *Proc. Soc. Exp. Biol. Med.*, 1943, **53**, 126.
4 L. Young, *Biochem. J.*, 1947, **41**, 417.
5 E. Boyland and J. B. Solomon, *Biochem. J.*, 1955, **59**, 518.
6 E. Boyland and A. A. Levi, *Biochem. J.*, 1936, **30**, 1225.
7 S. Tamura, *Acta Sch. Med. Univ. Kioto.*, 1924, **6**, 449.
8 J. N. Smith and R. T. Williams, *Biochem. J.*, 1955, **59**, 284.
9 A. W. Wood, W. Levin, D. R. Thakker, H. Yagi, R. L. Chang, D. E. Ryan, P. E. Thomas, P. M. Dansette, N. Whittaker, S. Turujman, R. E. Lehr, S. Kumar, D. M. Jerina and A. H. Conney, *J. Biol. Chem.*, 1979, **254**, 4408.
10 D. R. Boyd, R. A. S. McMordie, N. D. Sharma, H. Dalton, P. Williams and R. O. Jenkins, *J. Chem. Soc., Chem. Commun.*, 1989, 339.
11 P. W. Howard, G. R. Stephenson and S. C. Taylor, *J. Chem. Soc., Chem. Commun.*, 1990, 1182.
12 D. R. Boyd, R. A. S. McMordie, N. D. Sharma, R. A. More O'Ferrall and S. C. Kelly, *J. Am. Chem. Soc.*, 1990, **112**, 7822.
13 D. R. Boyd, R. Agarwal, R. A. S. McMordie, N. D. Sharma, J. Bessens, B. van Ommen and P. J. van Bladeren, *Biochem. Biophys. Res. Commun.*, submitted for publication.
14 D. R. Boyd, H. Dalton, D. J. Gray, R. A. S. McMordie and N. D. Sharma, manuscript in preparation.
15 S. K. Balani, D. R. Boyd, E. S. Cassidy, G. I. Devine, J. F. Malone, K. McCombe, N. D. Sharma and W. B. Jennings, *J. Chem. Soc., Perkin Trans. I*, 1983, 2751.
16 H. C. Brown and J. V. N. Vara Prasad, *J. Org. Chem.*, 1985, **50**, 3002.