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Enzymatic and chemoenzymatic synthesis of arene *trans*-dihydrodiols

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Abstract

Several potential approaches to the enzyme-catalysed synthesis of arene *trans*-diols have been examined including epoxidation/hydrolysis, bis-benzylic hydroxylation, *cis*-dihydroxylation/alcohol dehydrogenation/ketone reduction, *cis*-dihydroxylation/*cis–trans* isomerisation and multi-enzyme synthesis of *trans*-dihydrodiol secondary metabolites from primary metabolites. The lack of general applicability of these enzymatic methods has led to the development of several chemoenzymatic routes for the synthesis of a series of *trans*-dihydrodiols from the readily available *cis*-dihydrodiol precursors. Partial hydrogenation of *cis*-dihydrodiol metabolites to yield the corresponding *cis*-tetrahydrodiols followed by a regioselective Mitsunobu inversion process gave *trans*-tetrahydrodiols that were in turn converted to *trans*-dihydrodiols. The formation of anti-benzene dioxides or iron tricarbonyl complexes from the corresponding *cis*-dihydrodiol precursors provided shorter and more convenient chemoenzymatic routes to *trans*-dihydrodiols. The application of *cis*-dihydrodiol metabolites of polycyclic azaarenes in the synthesis of the corresponding arene oxides followed by chemical hydrolysis provides a convenient route to *trans*-dihydrodiols.

Keywords: Monooxygenase; Dioxygenase; *trans*-Dihydrodiols; Enzymatic synthesis; Chemoenzymatic synthesis

1. Introduction

The metabolism of aromatic rings in the environment occurs via two major pathways. In procaryotic systems (bacteria) the initial metabolites are *cis*-dihydrodiols and catechols formed via dioxygenase and *cis*-diol dehydrogenase biocatalysis, respectively [1–3]. Eucaryotic systems (plants, animal and fungi) utilise alternative enzymes in the initial steps, e.g. monooxygenase and epoxide hydrolase enzymes, yielding arene oxide and *trans*-dihydrodiol metabolites, respectively [4]. Both *cis*- and *trans*-dihydrodiols and arene oxide metabolites can readily form phenolic products as a result of aromatisation reactions (dehydration/dehydrogenation or isomerisation) and are generally considered to be transient intermediates [1–4]. The complementary roles played by procaryotic and eucaryotic metabolism of monosubstituted benzene substrates is shown in Scheme 1. With the availability of mutant and recombinant strains of bacteria, and large capacity fermenters, it is possible to intercept and isolate *cis*-dihydodiol intermediates on the multigram scale. These bioproducts, commercially available over the past decade, are being widely used as enantiopure synthetic precursors [1–3].

trans-Dihydrodiols are more stable than the corresponding *cis*-dihydrodiols. Thus, the 2,3-*cis*-dihydrodiols of chlorobenzene and bromobenzene were found

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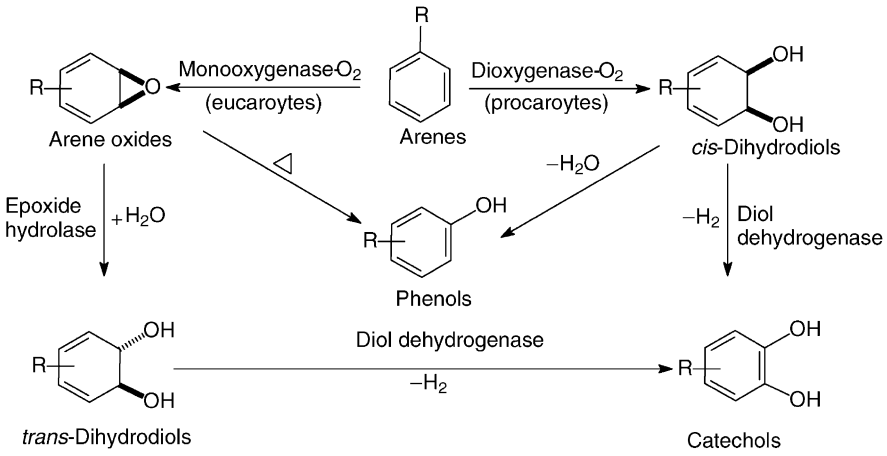


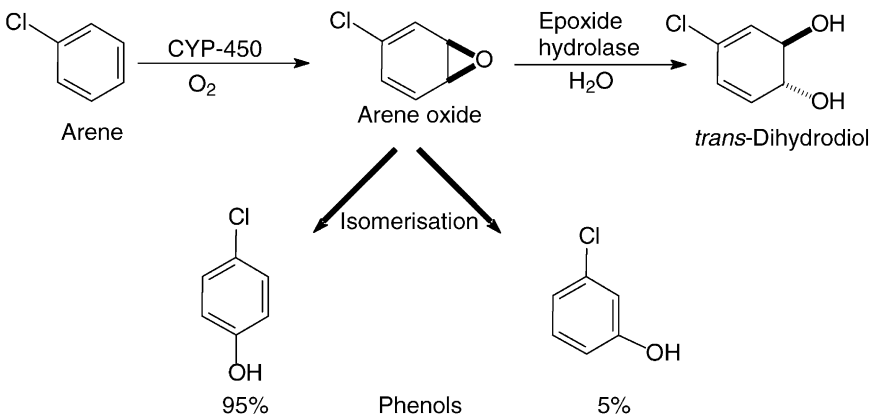
Scheme 1. Hydroxylation reactions of monosubstituted benzenes in eucaryotic and procaryotic systems.

to aromatise to the corresponding phenols under aqueous conditions at a much faster rate (>1000-fold) compared with their *trans*-dihydrodiol isomers. The instability of some *cis*-dihydrodiols has placed a severe limitation upon their value in synthesis and thus the stable *trans*-dihydrodiols seem to be more useful as chiral precursors. However, *trans*-dihydrodiols have not been readily available, in sufficient quantities, either by enzyme catalysed or by chemoenzymatic methods. A short review of our earlier and current attempts to obtain enantiopure *trans*-dihydrodiols by enzymatic and chemoenzymatic methods, mainly using oxygenases as biocatalysts, and multi-enzyme approaches adopted by other groups, is presented in this article.

2. Enzyme-catalysed routes to *trans*-dihydrodiols

2.1. Epoxidation/hydrolysis

A possible mechanism to account for the formation of arene oxide, arene *trans*-dihydrodiol and phenol metabolites of monocyclic arenes in eucaryotic systems was postulated over 30 years ago by Jerina et al. at the National Institutes of Health, Bethesda, USA [5]. This is exemplified by the metabolism of chlorobenzene using animal liver enzymes (Scheme 2). The evidence for arene oxide intermediates, during the metabolism of monocyclic arenes, has relied mainly upon the observation of the migration of an atom or



Scheme 2. The proposed role of a 3,4-arene oxide in the eucaryotic metabolism of chlorobenzene.

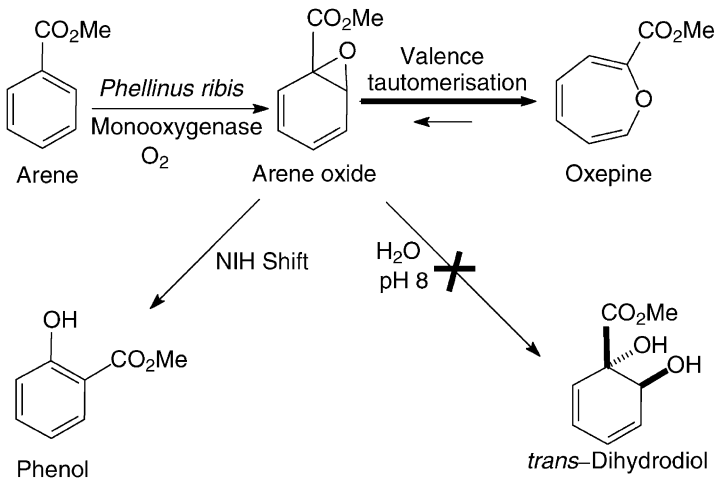
group to, and retention at, an adjacent carbon (NIH shift) [5,6]. It has, however, been shown that this process can occur without the intermediacy of an arene oxide and thus questions have arisen about its requirement to explain the NIH shift [6–8].

The quest for *direct* evidence of arene oxide formation during aromatic hydroxylation of benzene substrates has recently been renewed during the studies of secondary metabolites from wood-rotting fungi of the *Phellinus* family; methylbenzoate and methylsalicylate were among the identified metabolites of benzoic acid [9]. Evidence that methylsalicylate had been formed with the migration of a carbomethoxy group was found when deuterium-labelled samples of methylbenzoate were added to the cultures of *P. ribis*, *P. pomaceous* and *P. tremulae*; this provided an unprecedented example of the NIH shift of a carbomethoxy group in vivo [9]. The presence of the NIH shift was consistent with the formation of an arene oxide intermediate and indeed the oxepine valence tautomer of the 1,2-arene oxide of methylbenzoate was isolated and identified by comparison with a chemically synthesised sample (Scheme 3).

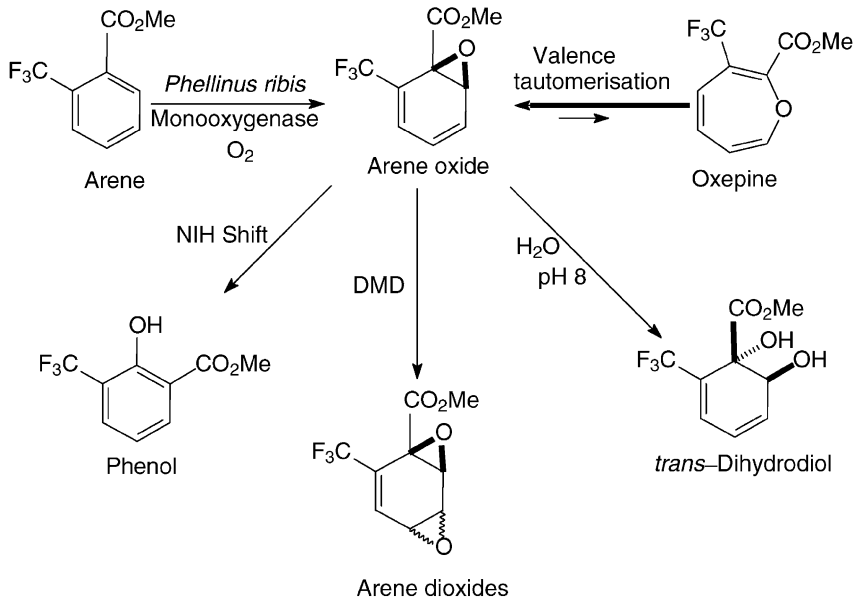
The stability of the oxepine derivative of methylbenzoate allowed it to be isolated and characterised. In common with all previous attempts to chemically hydrolyse benzene oxides/oxepines, it was again not possible to chemically hydrolyse this metabolite to the *trans*-dihydrodiol; the corresponding phenol, formed by dehydration, was obtained instead. Using other monosubstituted methylbenzoates as substrates for *P. ribis* cultures, the migration of a carbomethoxy group was again observed during methyl salicylate formation.

Methylbenzoate substituted with a trifluoromethyl group at the *ortho*-position was, however, found to yield a metabolite that was identified as a remarkably stable benzene oxide (Scheme 4) [9,10]. The stability of this monocyclic arene oxide allowed its unprecedented conversion to the dioxide (>90% yield) and the *trans*-dihydrodiol (30% yield) by chemical epoxidation and aqueous hydrolysis, respectively.

Despite the isolation of a stable benzene oxide metabolite, and its chemical hydrolysis, this approach to the synthesis of *trans*-dihydrodiols proved to be of limited applicability. To date the range of arene oxide metabolites, available in low yield (<5%), from *Phellinus* species has only been confined to alkylbenzoates. Attempts to obtain arene oxide or oxepine metabolites from methylbenzoates using a range of pure cytochrome P450 isozymes (>10) were unsuccessful. Furthermore, both the 1,2-arene oxide isolated from 2-trifluoromethyl methylbenzoate in *P. ribis* (Scheme 4) [9], and a series of 2,3-benzene oxides obtained by chemoenzymatic synthesis from the corresponding enantiopure 2,3-*cis*-dihydrodiols [11], were found to have racemised spontaneously via the corresponding oxepine tautomers.



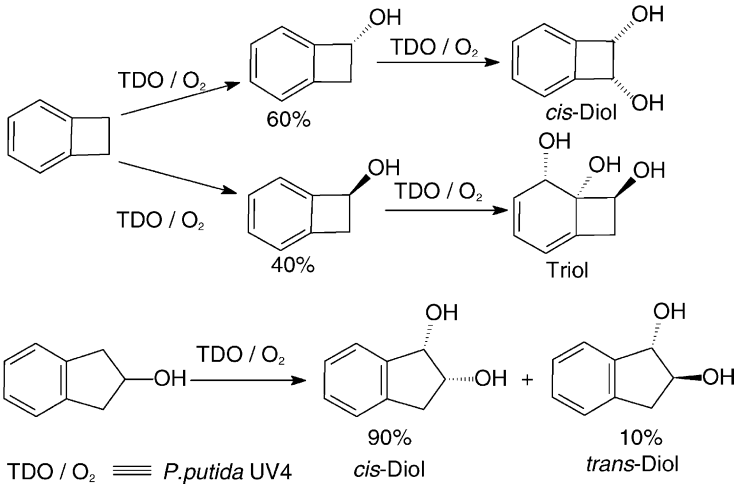
Scheme 3. The isolation of a 1,2-arene oxide/oxepine intermediate in the eucaryotic metabolism of methylbenzoate.



Scheme 4. The isolation of a 1,2-arene oxide/oxepine in the eucaryotic metabolism of *ortho*-trifluoromethyl methylbenzoate.

2.2. *bis*-Benzylic hydroxylation

The possibility that vicinal *trans*-diol metabolites could be obtained via a sequential dioxygenase-catalysed monohydroxylation process was briefly examined. The ability of toluene dioxygenase (TDO) enzymes, present in whole cell cultures of *Pseudomonas putida* UV4, to catalyse benzylic hydroxylations was observed with benzocyclobutene [12] and 2-hydroxyindan substrates [13]. The dioxygenase-catalysed bis-benzylic hydroxylation of benzocyclobutene substrate yielded mono-, di- and tri-hydroxylated products. Both the *cis*-1,2-diol of benzocyclobutene and the derived ketol were isolated as metabolites but no trace of the *trans*-1,2-diol was obtained (Scheme 5) [12].



Scheme 5. Dioxygenase-catalysed bis-benzylic hydroxylation of benzocycloalkanes.

The *trans*-1,2-dihydrodiol metabolite from 2-indanol was also found to be formed only as a minor metabolite (10%) in comparison to the major *cis*-isomer (90%, Scheme 5) [13]. On the basis of this limited study it appeared that the sequential monohydroxylation pathway to *trans*-diols using dioxygenase enzymes was not generally applicable.

2.3. *cis*-Dihydroxylation/*cis*-diol dehydrogenation/ketone reduction

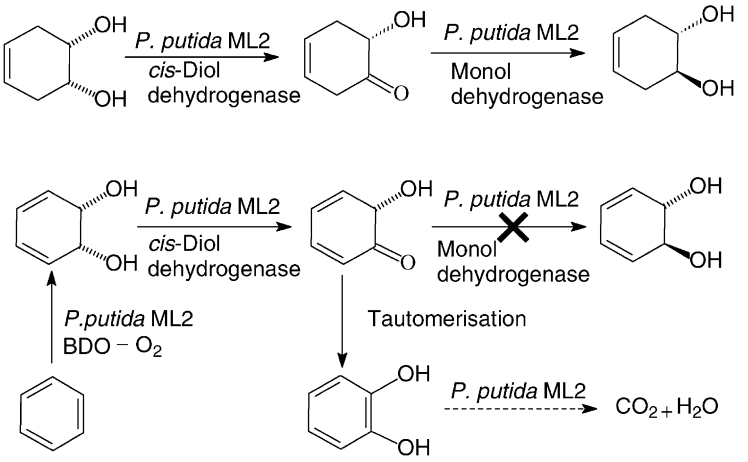
The synthesis of *cis*-dihydrodiol metabolites of arenes, using mutant or recombinant strains, provides a convenient route to produce multigram quantities [1–3]. If the synthesis and isomerisation of *cis*-dihydrodiols to *trans*-dihydrodiols could be achieved during the biotransformation conditions, this would be a very useful enzymatic method. Using a wild-type strain of *P. putida* (ML2), containing both benzene dioxygenase (BDO) and benzene *cis*-diol dehydrogenase (BDD), the *cis*-1,2-dihydroxycyclohex-4-ene substrate was dehydrogenated to form a ketol and reduced to yield an enantiopure sample of *trans*-1,2-dihydroxycyclohex-4-ene (74% yield, Scheme 6).

The synthesis of catechols from *cis*-dihydrodiols is catalysed by *cis*-diol dehydrogenase enzymes. The dehydrogenation of one allylic hydroxyl group in the *cis*-1,2-dihydrodiol of benzene could, in principle, be followed by: (a) reduction of the ketodiene functionality to yield the *trans*-dihydrodiol; or (b) tautomerisation to yield the catechol (Scheme 6). In practice no evidence of the *trans*-isomer was found and it was assumed that the benzene *cis*-dihydrodiol was mineralised exclusively via the catechol tautomer. While the enzyme-catalysed oxidation of dihydrobenzene *cis*-diols to ketols followed by their reduction to single enantiomer dihydrobenzene *trans*-diols has been proven, this approach does not appear to be generally applicable in the context of benzene *trans*-dihydrodiol synthesis (Scheme 6).

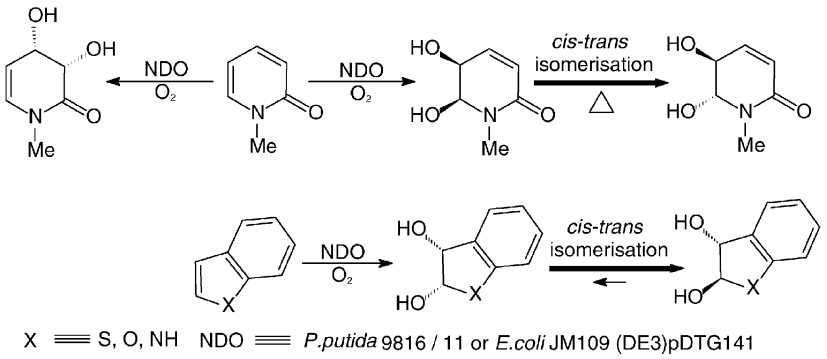
2.4. *cis*-Dihydroxylation/*cis*–*trans* isomerisation

The synthesis of *cis*-dihydrodiol intermediates, using dioxygenase biocatalysts, and their isomerisation to *trans*-dihydrodiols, has been achieved using heterocyclic arenes as substrates (Scheme 7) [14–17].

The electron-rich five-membered heterocyclic rings present in benzothiophene and benzofuran were found to yield *cis*-dihydrodiols as major metabolites in the presence of TDO and naphthalene dioxygenase (NDO) enzymes [14]. NMR analysis of the heterocyclic diols indicated that spontaneous equilibration was occurring with a strong preference (>90%) being observed for the *cis*-isomer in $CDCl_3$ and for the *trans*-isomer in D_2O (>80%) (Scheme 7). It is probable that diol metabolites formed by dioxygenase-catalysed oxidation of thiophene, furan and pyrrole rings will prefer to exist as the *trans*-isomers in aqueous solution. While the heterocyclic diol metabolite from indole has been



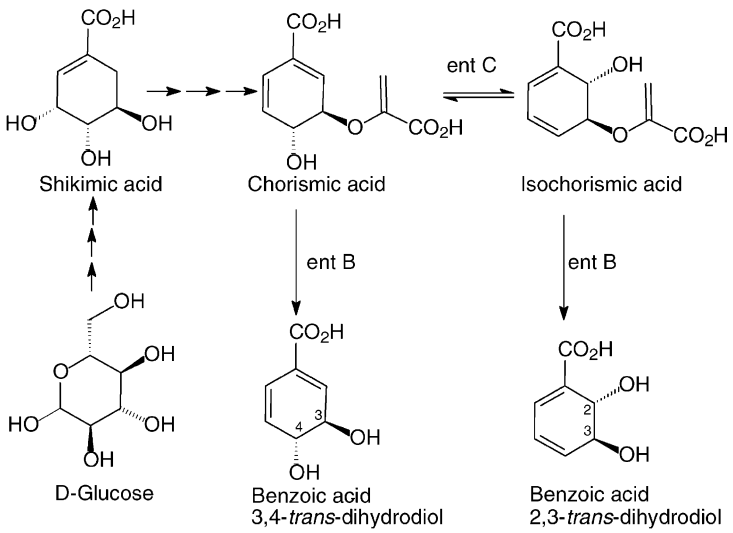
Scheme 6. Oxidation of *cis*-diols to form ketols and their reduction to yield *trans*-diols.



Scheme 7. Dioxygenase-catalysed *cis*-dihydroxylation of heterocyclic rings and isomerisation to yield *trans*-diols.

postulated to exist as a *cis*-isomer, it is more likely to prefer the *trans*-geometry prior to dehydration and subsequent dimerisation to yield indigo.

The electron-poor pyridine ring in 2-substituted quinolines has recently been metabolised via dioxygenase-catalysed dihydroxylation to yield *cis*-dihydrodiols [16,17]. To date a *trans*-dihydrodiol metabolite of a pyridine ring has not been isolated. 1-Methyl-2-pyridone is, however, known to have significant resonance energy and a structure similar to a 1,2-disubstituted pyridine. NDO-catalysed dihydroxylation of 1-methyl-2-pyridone has been found to yield two rather unstable diol metabolites in ca. 70% yield (Scheme 7) [15]. The major 5,6-diol was found to exist exclusively as the initially formed *cis*-isomer which readily isomerised to the more stable *trans*-isomer upon gentle heating (ca. 50 °C) [15]. Both the 5,6- and 3,4-diols have recently been identified as single enantiomers having the same (*S*,*S*) absolute configurations as shown in Scheme 7 [16,17]. The configurations of these heterocyclic *cis*-diols are in accord with expectations based on an earlier stereochemical predictive model for dioxygenase-catalysed dihydroxylation of 1,2-disubstituted benzenes [1] assuming that 1-methyl-2-pyridone was behaving as a benzene substrate of similar size and shape. While the



Scheme 8. Enzyme-catalysed synthesis of *trans*-dihydrodiols of benzoic acid [18,19].

formation of *cis*-dihydrodiol metabolites, and their isomerisation to the corresponding *trans*-isomers, has been clearly established for heterocyclic ring systems, this has not yet been observed for carbocyclic rings. Since this approach also appeared to be of limited applicability, alternative methods were explored.

2.5. Multi-enzyme synthesis of trans-dihydrodiol secondary metabolites from primary metabolites

Chorismic acid and isochorismic acid are naturally occurring derivatives of the 3,4- and 2,3-*trans*-dihydrodiols of benzoic acid associated with the multi-enzyme shikimate biosynthetic pathway (Scheme 8). Both *trans*-(2*S*,3*S*)-dihydroxy-2,3-dihydrobenzoic acid [18,19], and *trans*-(3*R*,4*R*)-dihydroxy-2,3-dihydrobenzoic acid [18] have been obtained in good yields (e.g. 73% yield of the *trans*-2,3-dihydrodiol) from D-glucose by metabolic deregulation and construction of recombinant bacterial strains. While this approach provides a valuable enzyme-catalysed route to the *trans*-dihydrodiols of benzoic acid and carboxylic acid derivatives, the range of *cis*-dihydrodiols currently available by this method is rather limited.

3. Chemoenzymatic routes to *trans*-dihydrodiols

The chemoenzymatic synthesis of the parent *trans*-dihydrodiol metabolite of benzene was achieved using dehydrogenase enzymes expressed in the whole cell cultures of *P. putida* ML2 which were able to catalyse the oxidation of *cis*-1,2-dihydroxycyclohex-4-ene to a ketol and its reduction to an enantiopure *trans*-1,2-dihydroxycyclohex-4-ene (Scheme 9). The latter *trans*-tetrahydrodiol was finally converted to the corresponding *trans*-dihydrodiol using known chemical steps (60% yield) [20]. The enzyme-catalysed *cis–trans* isomerisation process used in the chemoenzymatic synthesis of benzene *trans*-dihydrodiol (Scheme 9) has not yet been applied to the synthesis of other *trans*-dihydrodiols.

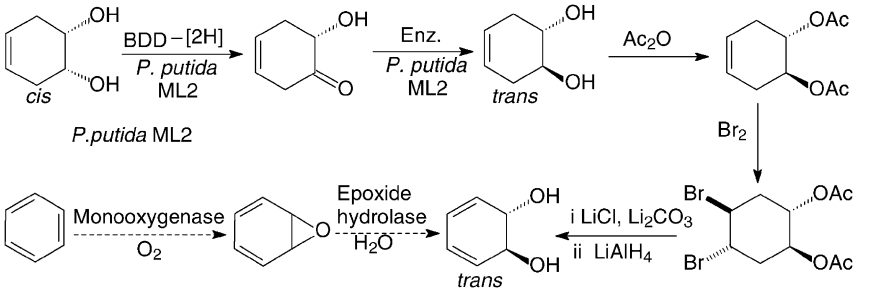
An alternative chemoenzymatic route to *trans*-dihydrodiols from *cis*-dihydrodiols [21] is shown in Scheme 10 (R = Cl, Br, I). The *cis–trans* isomerisation step was carried out on the *cis*-tetrahydrodiol using the Mitsunobu method (Ph_3P, diethyl azodicarboxylate, *p*-nitrobenzoate) where inversion at the allylic hydroxyl stereogenic centre occurred exclusively.

The 2,3-*trans*-dihydrodiols were obtained from the corresponding *cis*-dihydrodiols in seven steps with an overall yield of 35–50% [21]. Further examples of 2,3-*trans*-dihydrodiols (e.g. R = –CH=CH_2) have been obtained by substitution of the iodine atom in the 2,3-*trans*-dihydrodiol derivative of iodobenzene.

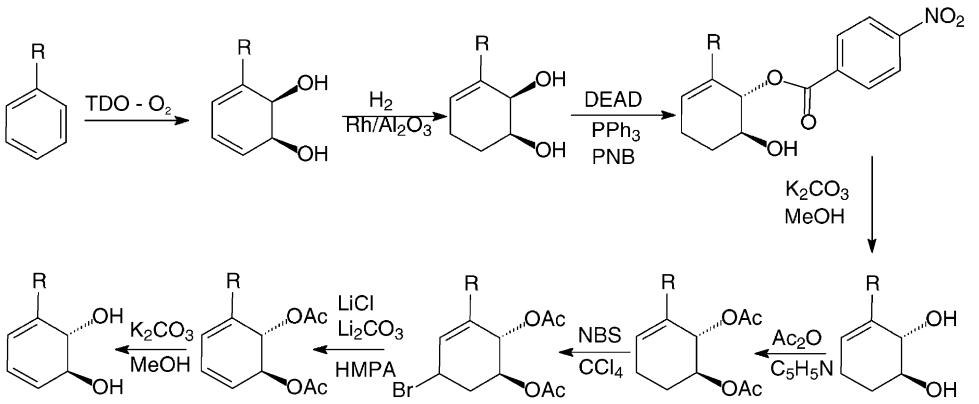
A modification of the chemoenzymatic route shown in Scheme 10 has also been used to obtain the 3,4-*trans*-dihydrodiol of bromobenzene from the *cis*-dihydrodiol metabolite available by biotransformation of *ortho*-bromoiodobenzene with *P. putida* UV4 (Scheme 11) [21]. This approach involved an eight-step synthesis from the *cis*-dihydrodiol metabolite but the overall yield was low (5%).

A new chemoenzymatic approach to the synthesis of 3,4-*trans*-dihydrodiols from a 2,3-*cis*-dihydrodiol precursor was based on the synthesis of tetraol and benzene dioxide intermediates (Scheme 12, R = Cl, Br, I) [10].

A major advantage of the routes shown in Schemes 10 and 12–14 was that the 2,3-*cis*-dihydrodiol



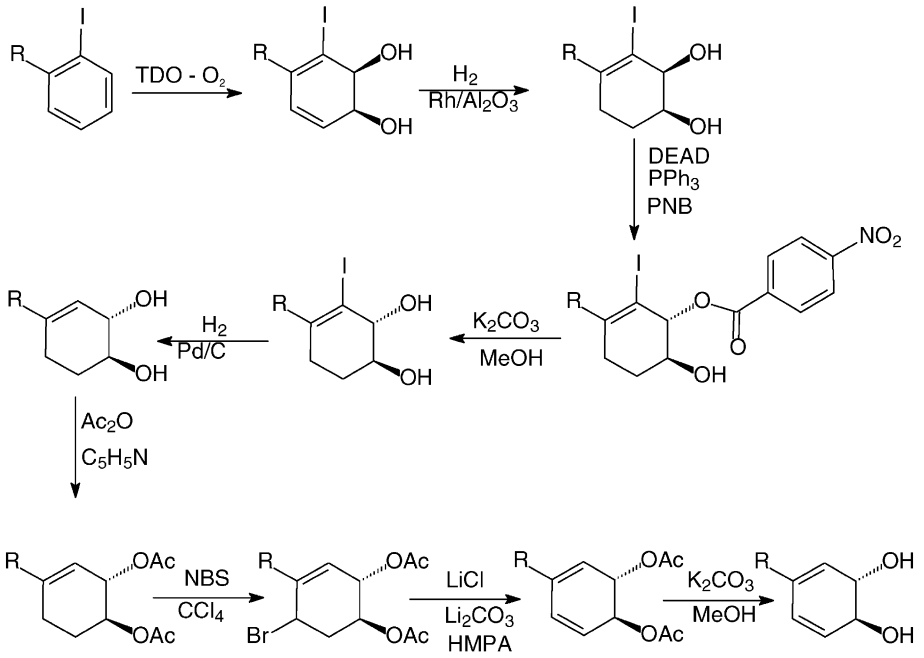
Scheme 9. Chemoenzymatic synthesis of *trans*-(1*S*,2*S*)-1,2-dihydroxy-1,2-dihydrobenzene.



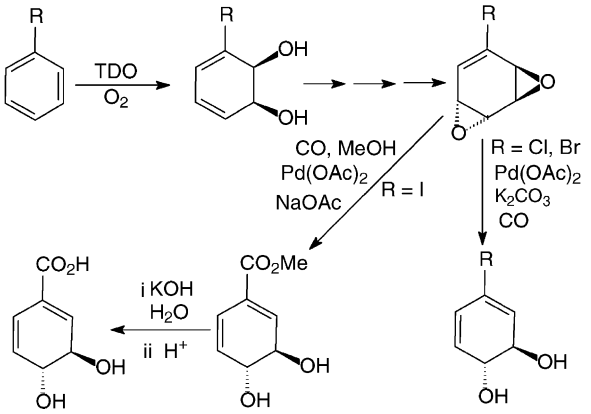
Scheme 10. Chemoenzymatic synthesis of *trans*-2,3-dihydroxy-1,2-dihydrobenzenes from the corresponding 2,3-*cis*-dihydrodiols.

precursors were available as metabolites in excellent yields from the corresponding benzene substrates. Furthermore, the 3,4-*trans*-dihydrodiol derivatives were obtained in fewer steps from the corresponding 2,3-*cis*-dihydrodiol (20–25% yield) (Schemes 12 and 13) [10]. Iodobenzene 2,3-*cis*-dihydrodiol could be converted into the 3,4-*trans*-dihydrodiol of benzoic acid [10], a metabolite of glucose (Scheme 8) [18,19]. The general applicability of the chemoenzymatic route involving benzene dioxide intermediates is shown in Scheme 13 where both 3,4- and 1,2-*trans*-dihydrodiols were synthesised from 2,3-*cis*-dihydrodiols directly or via the acetonide.

An alternative chemoenzymatic route from 2,3-*cis*-dihydrodiol bacterial metabolites to the corresponding 2,3-*trans*-dihydrodiols has recently been found based on the reported formation of iron tricarbonyl complexes (Scheme 14) [22,23].



Scheme 11. Chemoenzymatic synthesis of *trans*-3,4-dihydroxy-1,2-dihydrobenzenes.

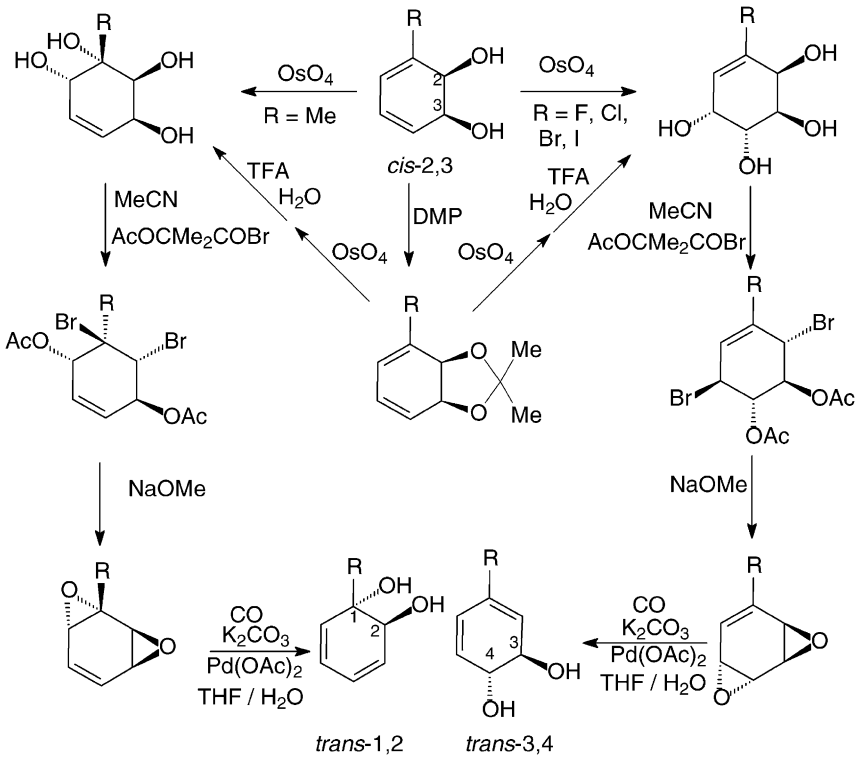


Scheme 12. Chemoenzymatic synthesis of *trans*-3,4-dihydroxy-1,2-dihydrobenzenes from the corresponding 2,3-*cis*-dihydrodiols.

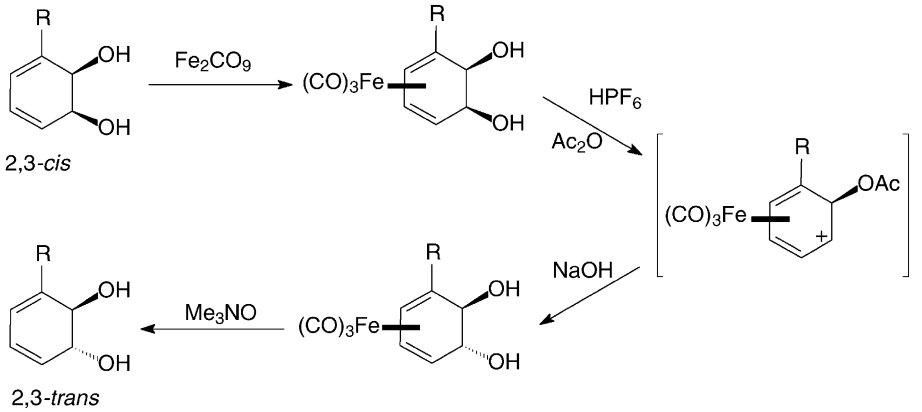
The regioselective inversion of configuration was observed for the iron tricarbonyl complexes of 2,3-*cis*-dihydrodiols (e.g. R = Br, CF_3) where carbocation intermediates were involved. Preliminary studies of the four-step synthesis of 2,3-*trans*-dihydrodiols from the corresponding 2,3-*cis*-dihydrodiols (Scheme 14) showed that this isomerisation process occurred with an overall yield of ca. 20%. Iron carbonyl complexes of a series of *cis*- and *trans*-dihydrodiols had previously been synthesised but not decomplexed [22].

The chemoenzymatic synthesis of *trans*-dihydrodiols from the *cis*-dihydrodiol isomers has also been applied to polycyclic aromatic systems. Arene oxides have been synthesised from the corresponding *cis*-dihydrodiol metabolites, e.g. phenanthrene 3,4-oxide was obtained from the 3,4-*cis*-dihydrodiol metabolite of phenanthrene (Scheme 15) [24]. This 3,4-oxide was found to racemise spontaneously, via an unstable oxepine, and its hydrolysis to the corresponding 3,4-*trans*-dihydrodiol could not be to effected by chemical methods.

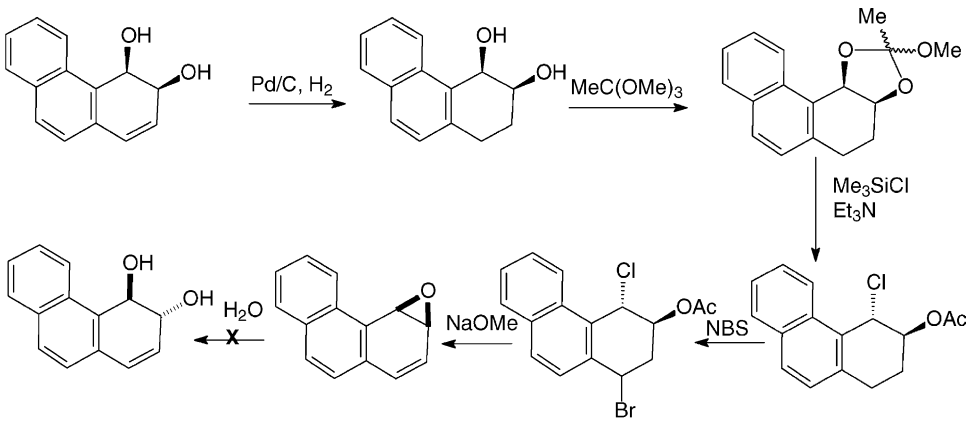
The chemoenzymatic synthesis of arene oxide and *trans*-dihydrodiol mammalian metabolites was, however, achieved from the corresponding *cis*-dihydrodiol bacterial metabolites in the polycyclic azaarene series,



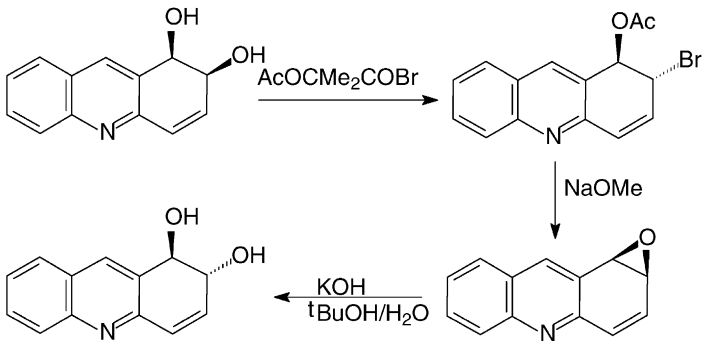
Scheme 13. Chemoenzymatic synthesis of *trans*-3,4- and 1,2-dihydroxy-1,2-dihydrobenzenes from 2,3-*cis*-dihydrodiols.



Scheme 14. Chemoenzymatic synthesis of 2,3-*trans*-dihydrodiols from 2,3-*cis*-dihydrodiols.



Scheme 15. Chemoenzymatic synthesis of phenanthrene 3,4-oxide from the *cis*-3,4-dihydrodiol metabolite of phenanthrene.



Scheme 16. Chemoenzymatic synthesis of acridine 1,2-oxide and the corresponding *trans*-dihydrodiol from the *cis*-1,2-dihydrodiol metabolite of acridine.

e.g. quinoline, acridine and azaphenanthrenes. This is exemplified by the synthesis of acridine 1,2-oxide in two steps from acridine *cis*-1,2-dihydrodol (Scheme 16) [25], instead of the earlier six-step sequence [26]. Acridine 1,2-oxide, in contrast with phenanthrene 3,4-oxide, was found to be configurationally stable and of sufficient chemical stability to undergo hydrolysis under aqueous alkaline conditions yielding enantiopure *trans*-1,2-dihydrodiol (65% yield) [25].

4. Conclusions

The enzyme-catalysed synthesis of *trans*-dihydrodiols in low yields from the corresponding arenes (via arene oxide intermediates) has not yet been sufficiently developed to allow their use in chemical synthesis. The application of metabolic pathway engineering has, however, been successfully used in the production of significant quantities of the 2,3- and 3,4-*trans*-dihydrodiols of benzoic acid. The disadvantages of low yields or limited substrate choice have, however, prompted the *search* for more generally applicable chemoenzymatic methods to make *trans*-dihydrodiols. New approaches to the synthesis of a wide range of mono- and polycyclic *trans*-dihydrodiols have now been developed based upon the readily available arene *cis*-dihydrodiol metabolites. These methods include: (a) selective inversion of configuration of the *cis*-tetrahydrodiols to *trans*-tetrahydrodiols (Mitsunobu inversion); (b) formation of mono- or diarene oxides followed by regioselective ring opening with inversion of configuration; and (c) stereo-controlled inversion of configuration at the other chiral centre in the iron tricarbonyl complexes of monocyclic arene *cis*-diols (via carbocation formation) and decomplexation to yield the *trans*-dihydrodiols. The availability of enantiopure *trans*-dihydrodiols by enzymatic and chemoenzymatic routes has facilitated their use as new chiral synthons [27].

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