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A Comparative Study of the Synthesis of 3-Substituted Catechols using an Enzymatic and a Chemoenzymatic Method

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Abstract: A series of *cis*-dihydrodiol metabolites, available from the bacterial dioxygenase-catalysed oxidation of monosubstituted benzene substrates using *Pseudomonas putida* UV4 , have been converted to the corresponding catechols using both a heterogeneous catalyst (Pd/C) and a naphthalene *cis*-diol dehydrogenase enzyme present in whole cells of the recombinant strain *Escherichia coli* DH5α(pUC129: *nar B*). A comparative study of the merits of both routes to 3-substituted catechols has been carried out and the two methods have been found to be complementary. A similarity in mechanism for catechol formation under both enzymatic and chemoenzymatic conditions, involving regioselective oxidation of the hydroxyl group at C-1, has been found using deuterium labelled toluene *cis*-dihydrodiols. The potential, of combining a biocatalytic step (dioxygenase-catalysed *cis*-dihydroxylation) with a chemocatalytic step (Pd/C-catalysed dehydrogenation), into a one-pot route to catechols, from the parent substituted benzene substrates, has been realised.

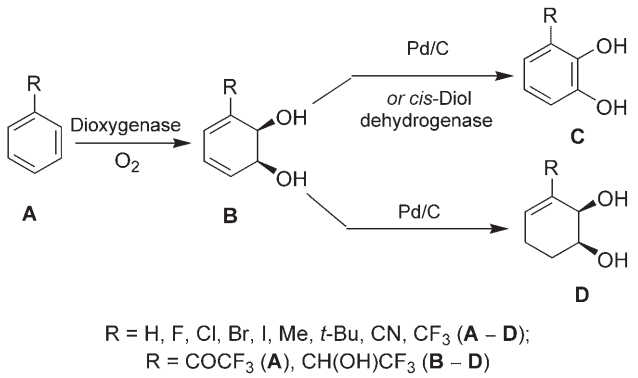
Keywords: chemoenzymatic synthesis; dehydrogenation; enzyme catalysis; heterogeneous catalysis

Introduction

Catechols (1,2-dihydroxybenzenes) are ubiquitous in nature and are extensively used in the chemical and pharmaceutical industries. For example, catechols and their substituted derivatives are employed in many processes, such as the manufacture of photographic developers, inks, insecticides, rubber, plastics, synthetic flavours, cosmetics, antiseptics, antioxidants and antihypertensive drugs.[1] In biology, compounds containing the catechol functionality play an important role in diverse metabolic pathways, since they are intermediates in the biodegradation of aromatic compounds[2–4] and are present in cells as endogenous substances such as catecholamine neurotransmitters including adrenaline, noradrenaline, dopamine and L-DOPA.[5] Catechols act as antioxidants in eukaryotic cells, preventing degenerative diseases caused by free radicals, such as cancer, heart disease and immune system decline.

The synthesis of substituted catechols by chemical methods is often complex and may involve severe reaction conditions, resulting in low yields and the formation of both isomeric mixtures of 3- and 4-catechols and by-products.[6] This has led to the continuing quest for more generally applicable chemical routes. Thus, several one-pot chemical syntheses of substituted catechols from the corresponding phenols, have recently been reported.[6,7]

The difficulties experienced in the chemical production of substituted catechols from more readily available precursors, e.g., the corresponding substituted benzenes, have prompted a search for alternative enzymatic or chemoenzymatic routes to catechols. These include the synthesis of the parent catechol from glucose using a multi-enzyme pathway,[8,9] and the production of substituted catechols from the corresponding phenols catalysed by a monooxygenase.[10,11] The use of dioxygenase enzymes to catalyse the *cis*-dihydroxylation of benzene and substituted benzene derivatives **A**, followed by *cis*-diol dehydrogenase-catalysed desaturation of the resulting *cis*-dihydrodiols **B**, can, in principle, provide a route to the corresponding 3-substituted catechols **C** based upon the major bacterial biodegradation pathway for aryl rings (Scheme 1).[2–4] This has been achieved using enzymes present in whole cell mutant or recombinant bacterial strains. Thus, the UV4 mutant strain of *Pseudomonas putida* containing toluene dioxygenase (TDO) can accumulate the *cis*-dihydrodiol derivatives **B** from a wide range of substituted benzene substrates since the corresponding toluene *cis*-diol dehydrogenase enzyme activity has been blocked.[12,13] The combination of a preformed *cis*-dihydrodiol metabolite using one bacte-



Scheme 1.

rial strain, e.g., *P. putida* UV4, as substrate for a *cis*-diol dehydrogenase enzyme, present in a different wild-type[14] or recombinant strain,[15] has been used to produce 3-substituted catechols **C** (Scheme 1). Thus, 3-fluorocatechol **C** (R = F) was produced using UV4 and ML2 strains of *P. putida*.[14] A series of enantiopure catechols **C** (R = stereogenic carbon or sulphur centre) has also been isolated from the corresponding *cis*-dihydrodiols **B** using a naphthalene *cis*-diol dehydrogenase.[15] Therein, the gene for this enzyme, which was originally present in a *Rhodococcus* species (NDD_R), was expressed and utilised in the recombinant strain *Escherichia coli* DH5α(pUC129:*nar B*). 3-Substituted catechols **C** have also been obtained directly from biotransformation of the corresponding substituted benzene substrates **A** using either a mutant, e.g., *P. putida* 6(12),[4] or recombinant strains, e.g., *E. coli* JM 109 (pDTG602) or *E. coli* JM 101 (pUC625B) containing *both* dioxygenase and *cis*-diol dehydrogenase enzymes.[15–18] Unfortunately the yields of catechols **C** obtained by use of the latter strains were very limited since they proved to be potent inhibitors of the first enzyme (dioxygenase) and thus exerted feed-back control of the two-step process.[4]

Heterogeneous catalysts have also been reported for the dehydrogenation of *cis*-dihydrodiols **B** to catechols **C**.[19,20] However, only a limited number of dihydrodiols **B** were studied and indicated that the chemocatalytic route could only produce the catechol **C** in relatively low yields due to disproportionation into the corresponding *cis*-tetrahydrodiols **D** (Scheme 1). Ryback and Schofield claimed that the relative yield of the catechols **C** could be increased by using elevated temperatures (up to 80 °C) and high proportions of oxygen and catalyst. However, the experimental details were only provided for two catechols **C** (R = F and CF_3) from the corresponding *cis*-dihydrodiols **B**.[21] The limited data available in these reports, showed that there is a potential route to catechols **C** from benzene and substituted benzene derivatives **A** using a chemoenzymatic route as shown in Scheme 1.

The use of a TDO biocatalyst in the production of *cis*-dihydrodiols **B**, followed by a heterogeneous catalyst for the dehydrogenation step to yield catechols **C** by a chemoenzymatic route, should in principle avoid the problem of product inhibition and thus afford an attractive alternative to the two-step biocatalytic route. Prior to the current study, there have been no reports on the relative merits of the enzyme-catalysed (dioxygenase/*cis*-diol dehydrogenase) and chemoenzymatic methods (dioxygenase/Pd/C) in the context of general applicability, yield, or mechanism. Therefore, the major objectives of the current programme were (i) to carry out a comparison of the enzymatic and chemoenzymatic routes from *cis*-dihydrodiols **B** to catechols **C**, (ii) to optimise the chemoenzymatic approach for the synthesis of catechols **B** and to minimise the production of the other disproportionation products **D**, (iii) to examine the potential for combining the biocatalyst (TDO), the heterogeneous catalyst (Pd/C), and the aromatic substrate **A** to yield catechol **C** in a one-pot process, and (iv) to synthesise selected catechol and *cis*-tetrahydrodiol bioproducts of particular value as synthetic precursors.

Results and Discussion

Both the enzymatic and chemoenzymatic methods for the synthesis of catechol **C** (R = H) and nine functionalized 3-substituted catechols **C** from the corresponding monosubstituted benzene precursors **A** (R = F, Cl, Br, I, CN, CF_3, Me, *t*-Bu, $COCF_3$) required the initial formation of the corresponding *cis*-dihydrodiol precursors **B**. Samples of all the corresponding *cis*-dihydrodiols, with the exception of the compound **B** (R = *t*-Bu), were readily available from previous work in these laboratories using the enzyme TDO (present in whole cells of the UV4 constituent mutant strain of the bacterium *Pseudomonas putida*).[12,13] In the case of the ketone substrate **A** (R = $COCF_3$) it was found that *P. putida* UV4 cells contained a dehydrogenase enzyme as well as TDO resulting in a one-pot stereoselective ketone reduction/arene *cis*-dihydroxylation and thus the enantiopure *cis*-dihydrodiol product **B** had an exocyclic chiral (*S*)-substituent [R = $CH(OH)CF_3$].[15]

Dehydrogenation and Hydrogen Transfer Hydrogenation of *cis*-Dihydrodiols B to Yield Catechols C and *cis*-Tetrahydrodiols D, Respectively, Using Pd/C

Among the parameters examined during the current study of the dehydrogenation of *cis*-dihydrodiols **B** with Pd/C to yield the corresponding catechols **C**, were the effects of *cis*-dihydrodiol substituent type,

solvent, temperature, catalyst state and gas atmosphere. In the model experiments, the *cis*-dihydrodiols **B** used were mainly derived from fluorobenzene **A** (R = F) and also from toluene **A** (R = Me) in some cases. The *cis*-dihydrodiol from fluorobenzene **B** (R = F) was selected as a model compound due to it being readily available. It was also the only member of the *cis*-dihydrodiol series **B** [R = H, F, Cl, Br, I, CN, CF_3, Me, *t*-Bu, $CH(OH)CF_3$] formed as a mixture of two enantiomers from the TDO-catalysed reaction of fluorobenzene. This is important as in the NDD_R-catalysed conversion of *cis*-dihydrodiols **B** to catechol **C** (R = F) one enantiomer is oxidised faster whereas both are converted at the same rate using Pd/C.

The reaction of *cis*-dihydrodiols **B** (R = F or Me) was found to proceed at similar rates and give similar yields of catechols **C** (R = F or Me) in different solvents (including water, methanol and acetonitrile). As a result all other model experiments were carried out in methanol. When the reaction was conducted using several *cis*-dihydrodiols **B** (R = F, Cl, Me, CF_3, CN), over a range of temperatures (20–60 °C) it was found that thermal dehydration to yield the corresponding phenols was a serious problem at the higher temperatures. Thus use of higher temperatures recommended in the earlier patent[21] was not considered to be suitable for many of the *cis*-dihydrodiols **B** tested in the current study. All subsequent experiments were carried out at 30 °C.

The Pd/C catalyst was used either as received or following pre-reduction in hydrogen. For all *cis*-dihydrodiol substrates **B**, except bromobenzene (R = Br), only a small increase in rate and no significant advantage in terms of yield was obtained by pre-reduction of the catalyst. For the bromobenzene *cis*-dihydrodiol **B** (R = Br) the pre-reduced catalyst did increase the rate of reaction and, therefore, for this substrate only, the catalyst was pre-reduced; in all other cases the catalyst was used as received.

The composition of the gas atmosphere on the rate of dehydrogenation of the model *cis*-dihydrodiol **B** (R = F) showed a marked effect on both the rate of formation of catechol **C** (R = F) and the catechol selectivity. Figure 1 shows the effect of the gas composition of the atmosphere in methanol solvent at 30 °C and a stirring speed of 1000 rpm (Figure 1). The initial rates of formation for catechol **C** (R = F, ●) and *cis*-tetrahydrodiol **D** (R = F, □) were thus found to decrease until the proportion of O_2 reached 15 % when the reaction rate became too slow to measure.

The results in Table 1 showed a dependence on the oxygen partial pressure with the selectivity towards the formation of catechol **C** (R = F) increasing as the concentration of oxygen in the gas phase rises. Thus it was possible to obtain a complete conversion and 90 % yield of catechol **C** (R = F) after three days. However, the increase in selectivity was obtained at the expense of the rate with the reactions ranging from 100 % conversion in 1 min under inert atmosphere to only 65 % conversion under an atmosphere containing 30 % oxygen in seven days at ambient temperature and no observable reaction under 100 % oxygen. It is noteworthy that while a maximum yield of 67 % was reported earlier for catechol **C** (R = F),[21] this required a very high proportion of catalyst. Using 0.5 g 14 % Pd on asbestos (4.7 = Pd/substrate) and an elevated temperature (56 °C) only 0.015 g of catechol was formed.

This inverse relationship between the rate of oxidation of *cis*-dihydrodiol **B** (R = F) and selectivity to-

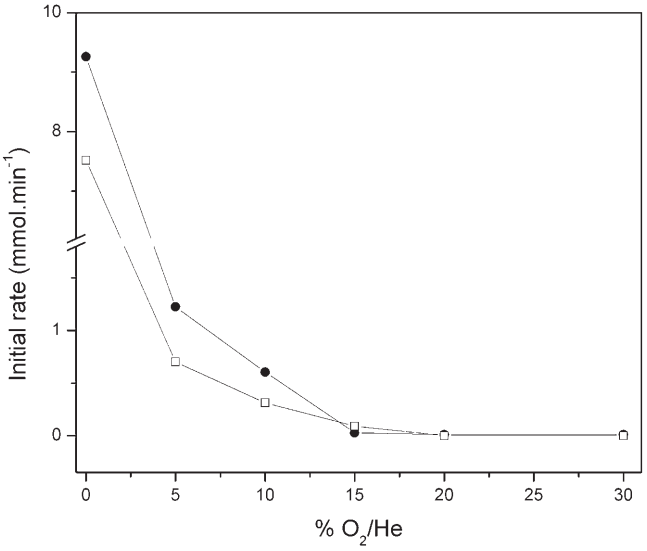


Figure 1. Variation in the initial rate of formation of catechol **C** (R = F, ●) and *cis*-tetrahydrodiol **D** (R = F, □) as a function of the percentage concentration of oxygen in helium with a total pressure of 1 atm during the reaction of 80 mM of *cis*-dihydrodiol **B** (R = F) using 10 % Pd/C (0.04 = Pd/substrate) in methanol at 30 °C.

Table 1. Variation of conversion and selectivity for the dehydrogenation of *cis*-dihydrodiol **B** to yield catechol **C** with selected oxygen concentrations (R = F).[a]

% O_2 in He	Time	Conversion [%]	Catechol Selectivity [%]
0	1 min	100	60
5	< 5 min	100	64
10	5 min	100	66
15	2 h	100	76
20	3 d	100	90
30	7 d	65	100

[a] The only by-product for all experiments was the result of the concomitant hydrogenation to give *cis*-tetrahydrodiol **D**. *Reaction conditions:* 80 mM of *cis*-dihydrodiol, 10 wt % Pd/C (0.04 = Pd/substrate), 20 cm^3 methanol, 1 atm total pressure, 30 °C, stirring rate 1000 rpm.

wards the catechol formed, i.e., that an increase in the rate results in a decrease in the selectivity, has also been shown in the case of dehydrogenation of α,β-unsaturated alcohols.[22] High selectivity for catechol formation is found in the presence of oxygen due to the surface hydrogen atoms formed during the dissociative adsorption of the *cis*-dihydrodiol substrate **B** being oxidised to yield water in preference to reduction of another substrate molecule to form a *cis*-tetrahydrodiol **D**. Interestingly, the initial rates of reaction to form catechol **C** (R = F) and *cis*-tetrahydrodiol **D** (R = F), as shown in Figure 1, are approximately negative first order, with respect to p_{O_2} up to 0.15 bar thereafter the rate is approximately zero order. This behaviour is expected for a catalyst poison whereby above a certain concentration the surface coverage has saturated. Similar behaviour has been found with other platinum group metal-catalysed oxidations of alcohols which have shown that oxygen can also cause overoxidation of the catalyst surface which poisons the catalyst and reduces the rate of oxidation.[23–25] This is particularly problematic at the relatively low temperatures used in the present experiments due to the strong metal-oxygen bond formed and this results in the low rates of reaction observed herein.

In order to attempt to reduce the proportion of *cis*-tetrahydrodiol **D** (R = F) being formed as a by-product whilst maintaining the high reaction rate found in the absence of oxygen, a range of hydrogen accepting alkene solvents/additives were employed, e.g., cyclohexene, *cis*-2-butene-1,4-diol, 3,4-dihydroxy-1-butene, α-methyl styrene and 1,3-cyclohexadiene. However, these alkenes had little effect and only the presence of an oxygen atmosphere led to a significant increase in the proportion of catechol **C** observed.

Figure 2 [*top* (R = F) and *bottom* (R = Me)] shows the kinetic profiles for the formation of catechol **C** and *cis*-tetrahydrodiol **D** (R = F or Me) from the corresponding *cis*-dihydrodiol substrates **B** (R = F or Me). The nature of the substituent R in the *cis*-dihydrodiols **B** has a significant effect on the rate of reaction observed, with the initial consumption rate for *cis*-dihydrodiol **B** (R = F) being (5.4 mM min^{-1}) and catechol **C** (R = F) formation (3.3 mM min^{-1}). This contrasts with the rates observed when the toluene *cis*-dihydrodiol **B** (R = Me) was used where both its consumption rate (11.9 mM min^{-1}) and its catechol formation rate (8.1 mM min^{-1}) were significantly higher. In comparison it was observed that the substituent R has only a limited effect on the overall selectivity towards catechol formation **C**.

The formation of the *cis*-tetrahydrodiol **D** (R = F) under relatively mild conditions (Pd/C, methanol solvent, room temperature, atmospheric pressure in the absence of H_2 gas) could in principle offer a valuable alternative route to other arene *cis*-tetrahydrodiols, particularly where the parent *cis*-dihydrodiol **B** was

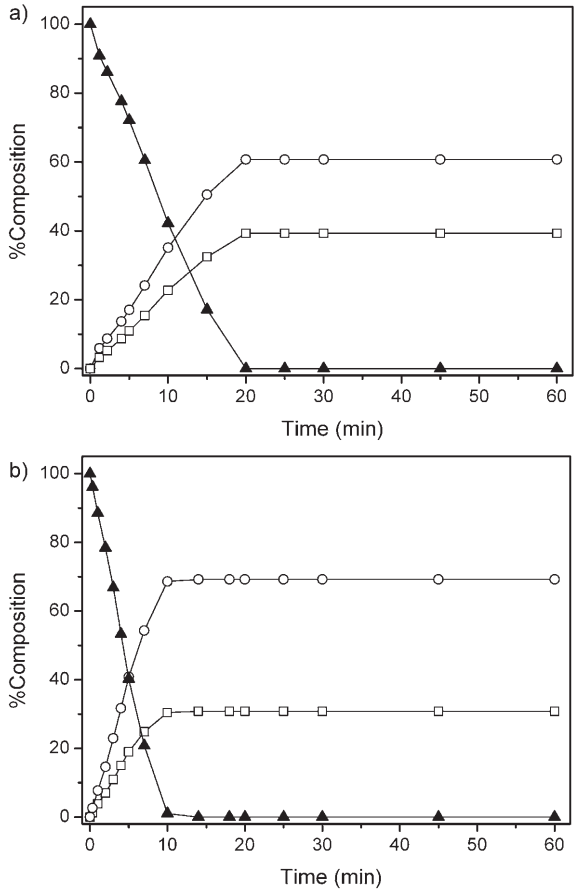


Figure 2. Kinetic profile for *cis*-dihydrodiol **B** consumption (R = F or Me, ▲), catechol **C** (R = F or Me, ○) and *cis*-tetrahydrodiol **D** (R = F or Me, □] formation from (*top*) fluorobenzene *cis*-dihydrodiol **B** (R = F) and (*bottom*) toluene *cis*-dihydrodiol **B** (R = Me) in methanol (20 cm^3). *Reaction conditions:* 80 mM of *cis*-dihydrodiol, 10 wt % Pd/C (0.005 = Pd/substrate), atmosphere of N_2, 30 °C, and stirring rate of 1000 rpm.

less stable or more likely to undergo a second hydrogenation to yield the corresponding arene *cis*-hexahydrodiol. In order to evaluate the general applicability of the chemoenzymatic route to catechols **C** and *cis*-tetrahydrodiols **D**, a series of *cis*-dihydrodiol substrates **B** were examined under similar conditions (Table 2). Except for benzene *cis*-dihydrodiol **B** (R = H), where both alkene bonds were found to be hydrogenated to yield *cis*-1,2-dihydroxycyclohexane, the hydrogen transfer process was confined to the 5,6-bond of other *cis*-dihydrodiols **B** [R = F, Cl, Br, I, Me, *t*-Bu, CN, CF_3, $CH(OH)CF_3$] to yield the corresponding *cis*-tetrahydrodiols **D**. No evidence was found for hydrogenation of the alternative 3,4-bond to yield isomeric *cis*-tetrahydrodiols. Due to the very low reaction rates found using an oxygen-containing atmosphere, coupled with the fact that the *cis*-tetrahydrodiol **D** was a valuable precursor in the synthesis of stable cyclohexane *cis*-diols and other derivatives,[13,26,27] all of the re-

Table 2. Product distribution and time required for total conversion of *cis*-dihydrodiols **B** to catechols **C**, *cis*-tetrahydrodiols **D** and phenols using Pd/C in methanol at 100% conversion.[a]

R	Time [min]	Relative products distribution [%]		
		C	**D**	Phenols
H	10	66	4[b]	4
F	20	60	40	0
Cl	20	60	0	40
Br[c]	30	39	0	61
I	$\gg$120	0	0	0
CN	60	49	44	7
Me	10	69	31	0
$C(Me)_3$	7	53	45	2
CF_3	120	56	35	9
$CH(OH)CF_3$	4	62	38	0

[a] *Reaction conditions:* 80 mM of *cis*-dihydrodiol, 10wt% Pd/C (0.005 = Pd/substrate), 20 cm^3 methanol, atmosphere of N_2, 30 °C, and stirring rate of 1000 rpm.

[b] 26% of the totally hydrogenated *cis*-diol was detected by GC.

[c] The catalyst was pre-reduced under H_2 atmosphere before the reaction.

actions shown in Table 2 were carried out under a nitrogen atmosphere. In addition to the catechol products **C** which were formed in all cases (39–69% yield), the *cis*-tetrahydrodiols **D** were also found to be present in significant quantities (31–45% yield) in six of the ten examples shown in Table 2.

Dehydration to form the corresponding phenols as major products (40–61%) without any of the corresponding *cis*-tetrahydrodiols **D** was mainly observed when the *cis*-dihydrodiol metabolites **B** from chlorobenzene (R = Cl) and bromobenzene (R = Br) were used. This is consistent with the fact that the partial reduction of the chloro- and bromo-*cis*-dihydrodiols **B** to yield *cis*-tetrahydrodiols **D** usually requires high pressures of hydrogen using $Rh\text{-}Al_2O_3$.[26,27] In addition, the reaction of the bromobenzene *cis*-dihydrodiol **B** (R = Br) only took place when the catalyst was pre-activated with hydrogen, whilst in the case of the iodobenzene *cis*-dihydrodiol **B** (R = I) no significant reaction was observed, i.e., compound **B** was recovered intact. For *cis*-dihydrodiol metabolites **B** from chlorobenzene (R = Cl), and bromobenzene (R = Br) dehalogenation is a potential reaction. The subsequent strongly adsorbed halogen can poison the surface reducing the rate of dehydrogenation as observed for R = Br. In the case of R = Br, the higher rate of reaction observed following pre-reduction of the catalyst is likely to be due to the increased surface hydrogen concentration which reacts with bromine forming HBr and thus freeing active sites. This reaction will also occur with R = Cl to form HCl but due to the stronger C–Cl bond compared with C–Br, the poisoning effect is much smaller. The halogenated substrates **B** are also easily decomposed to phenols under acidic conditions and the presence of the catalyst exacerbates the problem by producing both HCl and HBr. Thus both dehalogenated and halogenated phenols were formed during the reaction of *cis*-dihydrodiols **B** (R = Cl and Br).

The *cis*-tetrahydrodiols **D** [R = H, F, CN, Me, *t*-Bu, CF_3, $CH(OH)CF_3$] were easily separated from the corresponding catechols **C** using flash column chromatography with silica gel. Both compounds were eluted with a mixture of ethyl acetate/hexane. The use of 10% of ethyl acetate/hexane recovered the *cis*-tetrahydrodiols **D**, while a proportion of 25% recovered the catechol **C**. However, it is important to ensure the complete conversion of the *cis*-dihydrodiol **B** due to the difficulty of separating the substrate from the corresponding *cis*-tetrahydrodiol **D**. It is noteworthy that from the ten *cis*-dihydrodiols **B** used in this study only the iodobenzene derivative failed to give the required catechol **C** (R = I).

Authentic samples of the monosubstituted benzene *cis* tetrahydrodiols **D** [R = F, Cl, Br, I, CF_3 and $CH(OH)CF_3$] were available from previous studies.[13,26,27] As in the present study, partial hydrogenation of *cis*-dihydrodiol derivatives **B** occurred exclusively at the 5,6-bond using dihydrogen (1 atm) and Pd/C (R = H, CF_3) or Rh/Al_2O_3 [R = $CH(OH)CF_3$] or 1–3 atm dihydrogen and Rh/Al_2O_3 (R = F, Cl, Br, I).[26,27] The regioselectivity for the 5,6-bond may be due to the adsorption geometry of the substrate with the more substituted double bond being forced away from the surface by the steric effects. The fact that substrate with both electron donating, e.g., *t*-Bu, and electron withdrawing substituents, e.g., CF_3, both show this selectivity is consistent with a steric influence rather than an electronic effect (Table 2).

The possibility of recycling the catalyst was explored, since its recovery and reuse is important to scale up the reactions. Figure 3 shows the percentage of conversion and catechol **C** (R = F) selectivity during the reaction of *cis*-dihydrodiol **B** (R = F) observed after recycling three times using the catalyst recovered from the previous step. At each stage the solution containing the reaction products was isolated by filtration, and the catalyst that remained in the filter was washed with methanol and dried. No significant changes in reaction rate or selectivity were observed with each reaction. Furthermore, analysis of the mother liquor after reaction by inductively coupled plasma-atomic emission spectroscopy (ICP-AES) showed that the amount of palladium present was <1 ppm.

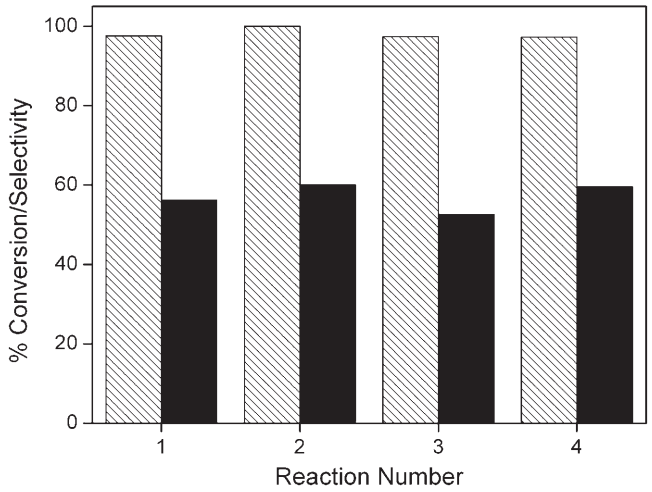


Figure 3. Comparison of the percentage of conversion (hatched) and catechol **C** selectivity (solid) for the Pd/C catalysed reaction of *cis*-dihydrodiol **B** (R = F), in methanol, as a function of the number of reactions performed. *Reaction conditions:* 20 cm^3 of solvent, 80 mM of *cis*-dihydrodiol, 10 wt% Pd/C (0.005 = Pd/substrate), atmosphere of N_2, 30 °C, and stirring rate of 1000 rpm.

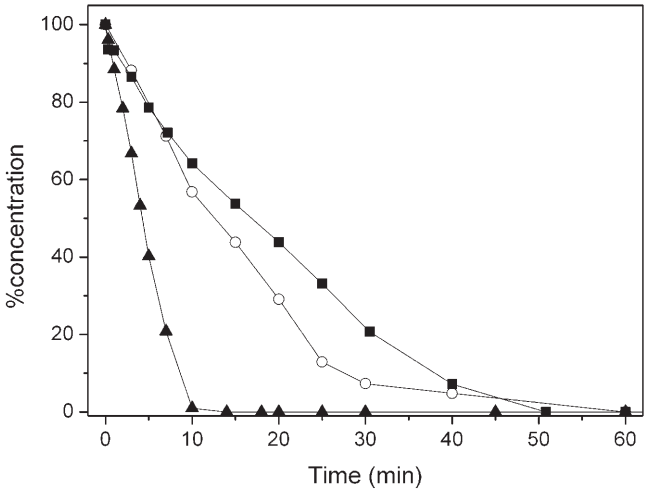
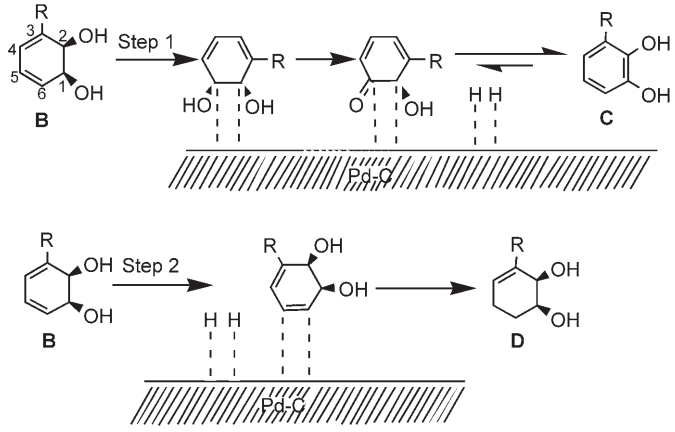


Figure 4. Comparison of the kinetic profile for the consumption of non-deuteriated toluene-*cis*-dihydrodiol **B** (R = Me; ▲], 1,5-D_2-toluene-*cis*-1,2-dihydrodiol **B** (R = Me; ○) and D_8-toluene-*cis*-1,2-dihydrodiol **B** (R = CD_3; ■] in methanol. *Reaction conditions:* 80 mM of *cis*-dihydrodiol, 10 wt% Pd/C (0.005 = Pd/substrate), 20 cm^3 of methanol, atmosphere of N_2, 30 °C, and stirring rate of 1000 rpm.

Possible Mechanism for the Dehydrogenation and Hydrogen Transfer Hydrogenation of *cis*-Dihydrodiols B to Yield Catechols C and *cis*-Tetrahydrodiols D, Respectively, Using Pd/C

Scheme 2 shows one possible reaction pathway for the dehydrogenation reaction of a typical *cis*-dihydrodiol **B** using a Pd/C catalyst. Therein, two hydrogen atoms are transferred from the C-1 position (and the attached OH group) of the *cis*-dihydrodiol **B** to the catalyst surface leading to a ketodiene intermediate which prefers to exist as the tautomeric catechol **C**. At the same time, some of the hydrogen atoms that remained adsorbed on the catalyst surface can reduce a further molecule of unreacted substrate **B** at the more accessible 5,6-bond, leading to the *cis*-tetrahydrodiol **D**.

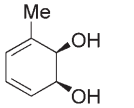
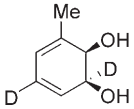
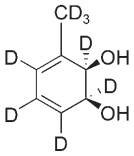


Scheme 2.

In order to distinguish between which hydrogen atom (from C-1 or C-2) in *cis*-dihydrodiol **B** contributes more to the rate-determining step during the dehydrogenation reaction, 3,5-D_2-toluene and perdeuteriated D_8-toluene substrates were synthesised. The corresponding time variation and rate data for the protiated (▲), 1,2,4,5,6,1′,1′,1′-D_8- (■), and 1,5-D_2-selectively deuteriated (○) toluene-*cis*-dihydrodiol **B** (R = Me or CD_3) are shown in Figure 4 and Table 3. The kinetic isotopic effect (KIE) was calculated using the initial rates for the protiated and deuteriated *cis*-dihydrodiols **B** (R = Me or CD_3). As expected, a primary KIE is observed for the D_8-*cis*-dihydrodiol **B** (R = CD_3, ■) relative to the non-deuteriated *cis*-dihydrodiol **B** (R = Me, ▲], indicating that a C–H bond dissociation is the kinetically significant step (Figure 4). Furthermore, the similarity in the reaction kinetics (Figure 4) of the D_8-(■) and 1,5-D_2-selectively deuteriated species (○) shows that the most likely mechanism involves formation of the carbonyl at position C-1 of the ketodiene intermediate preferentially over position C-2, i.e., the dissociation of the C-1–H bond is favoured (Scheme 2). In addition, the ratio of catechol **C** to *cis*-tetrahydrodiol **D** did not change significantly with deuteriation indicating that bond forming reactions and activation of the surface hydrogen do not influence the rate of reaction.

^1H and ^2H NMR spectroscopy coupled with GC-MS analysis of the reaction products of 1,5-deuteriated *cis*-dihydrodiol **B** (R = Me) in MeOH solvent showed the formation of mono-deuteriated (5-D) 3-methyl-

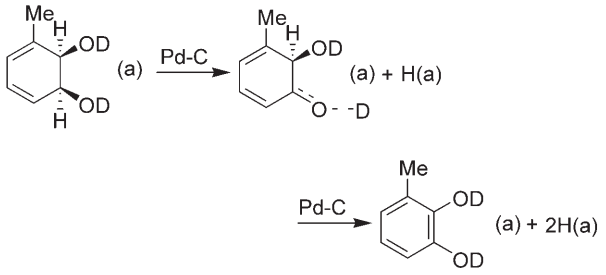
Table 3. Comparison of the initial rates of *cis*-dihydrodiol **B** (R = Me or CD_3) consumption and 3-methylcatechol **C** (R = Me or CD_3) formation and catechol selectivity at 100% conversion as a function of the deuterium incorporation into *cis*-dihydrodiol **B** (R = Me) and ratio (k_H/k_D) of the conversion rate of the protiated to the deuteriated *cis*-dihydrodiols **B** (R = Me).[a]

Substrate **B**	Initial rates ($mM\,min^{-1}$) Substrate **B** consumption	Catechol **C** formation	% Catechol selectivity	k_H/k_D
	11.9	8.1	69	–
	4.3	3.0	71	2.8
	3.5	2.5	72	3.4

[a] *Reaction conditions:* 80 mM of *cis*-dihydrodiol **B** (R = Me or CD_3), 10 wt% Pd/C (0.005 = Pd/substrate), 20 cm^3 methanol, atmosphere of N_2, room temperature, 1000 rpm.

catechol **C** (R = Me) as well as di- (1,5-D_2, 52%), tri- (1,5,5-D_3 or 1,5,6-D_3, 38%) and tetra- (1,5,5,6-D_4 or 1,5,6,6-D_4, 10%) deuteriated species of *cis*-tetrahydrodiol **D** (R = Me). The fact that there is a distribution of deuterium incorporation into *cis*-tetrahydrodiol **D** (R = Me), i.e., that the trideuteriated species is not the only product, indicates that the reaction is not concerted, as expected. Interestingly, the proportion of catechol formed is always greater than the *cis*-tetrahydrodiol, irrespective of R, indicating that surface hydrogen coupling to form dihydrogen, which then desorbs, also takes place and thus reducing the formation of *cis*-tetrahydrodiol **D.**

The role of the solvent was also probed using MeOD. No significant changes in the initial rate of *cis*-diol consumption ($-11.9\,mM\,min^{-1}$) or catechol formation ($7.9\,mM\,min^{-1}$), were observed. Furthermore, with the exception of facile hydrogen/deuterium exchange at the hydroxy positions, no additional incorporation of deuterium was observed in the *cis*-tetrahydrodiol **D** (R = Me) product. Although formally the dehydrogenation mechanism may be considered as the initial formation of a carbonyl at C-1 coupled with a keto-enol tautomerisation (Scheme 2), this is a valid but simplified view. The fact that no isotopic exchange occurs between the C–H and O–D bonds indicates that the O–D bond does not fully dissociate during reaction and this deuterium cannot be used for the subsequent hydrogenation to form the *cis*-tetrahydrodiol **D**. The proposed surface reaction is shown in Scheme 3.



Scheme 3.

The conclusion that catechol **C** (R = Me) is formed mainly *via* oxidation of the less sterically hindered C-1 hydroxy group of *cis*-dihydrodiol **B** (R = Me) using Pd/C as chemocatalyst, is of interest in the context of the *cis*-diol dehydrogenase-catalysed formation of catechols **C** from the corresponding *cis*-dihydrodiols **B**. Thus, a similar conclusion was reached by Ribbons et al.[28] when using 1-D-toluene-*cis*-1,2-dihydrodiol **B** (R = Me) as substrate for toluene-*cis*-2,3-dihydrodiol dehydrogenase (TDD) as biocatalyst from *P. putida* F1. TDD-catalysed the exclusive transfer of the deuterium atom from position C-1 to NAD during formation of 3-methylcatechol **C** (R = Me). Similarly, when the conversion of 2-D-naphthalene-*cis*-1,2-dihydrodiol to its relatively unstable catechol (1,2-dihydroxynaphthalene) was catalysed by naphthalene diol dehydrogenase (NDD_P, from *P. putida* NCIB 9816), oxidation was again found to occur exclusively at the less hindered position bearing an OH group (C-2).[29] A similar mechanism has also been proposed for the conversion of biphenyls using a biphenyl diol dehydrogenase enzyme.[30] A primary KIE (k_H/k_D) of *ca.* 1.9 was found using the biocatalyst NDD_P and 2-D-naphthalene-*cis*-1,2-dihydrodiol substrate[29] while a value of *ca* 2.8 was found using the chemocatalyst Pd/C and 1,5-D_2-toluene-*cis*-1,2-dihydrodiol **B** (R = Me) as precursor of the corresponding catechol **C** (R = Me) during the current study.

A Comparison of Relative Yields of Catechols C Obtained from the Corresponding *cis*-Dihydrodiols B Using the Chemocatalytic (Pd/C) and Biocatalytic (NDD_R) Approaches

The results presented in Table 2 showed that the relative rates of formation of catechols **C** from the corresponding *cis*-dihydrodiols **B** using a chemocatalyst (Pd/C) appeared to be higher when the substituent R was $CH(OH)CF_3$ or Me and lower for substituents CF_3 and I. Unfortunately 3-iodocatechol **C** (R = I), arguably the most important member of this series in

terms of its synthetic versatility, could not be formed using the Pd/C method. In order to compare the chemocatalytic route with the biocatalytic route, all ten *cis*-dihydrodiols **B** [R = H, F, Cl, Br, I, CN, CF_3, Me, *t*-Bu, $CH(HO)CF_3$] were used as substrates for naphthalene *cis*-diol dehydrogenase (NDD_R) enzyme present in whole cells of the recombinant strain *Escherichia coli* DH5α(pUC129:*nar B*) constructed using the NDD gene originally expressed by *Rhodococcus* sp. NCIMB 12038.[31,32] Enzyme activity of the NDD_R enzyme was determined using an established assay in the *E. coli nar B* whole cells. The NDD_R enzymatic activity for all ten *cis*-dihydrodiols was determined spectrophotometrically at 340 nm by measuring the initial rate of reduction of NAD^+ and accumulation of NADH.[33]

The NDD_R specific enzymatic activity of the crude cell-free extracts was first determined for the natural substrate, naphthalene *cis*-1,2-dihydrodiol, and found to be typically in the range 0.017–0.030 μmol $min^{-1} mg^{-1}$. This substrate was assigned a relative activity value of 100% against which the relative values for the other ten *cis*-dihydrodiols **B** [R = H, F, Cl, Br, I, CN, CF_3, Me, *t*-Bu, $CH(HO)CF_3$] were measured (Table 4).

Previous studies using crude cell-free extracts from a *P. putida* strain containing NDD_P[3] where the relative activities of catechol formation from the halobenzene *cis*-dihydrodiols **B** (R = F, Cl, Br and I) were compared, indicated that NDD_P-catalysed formation of 3-fluorocatechol **C** (R = F) was generally slower compared with the other three 3-halocatechols **C** (R = Cl, Br, I), these three being produced at approximately similar rates. More recent studies from these laboratories using pure NDD_P enzyme, isolated from a *P. putida* strain (NCIMB 8859), also showed a similar pattern of activity.[34] Thus, the *cis*-dihydrodiol of naphthalene was again found to the best substrate for NDD_P and the relative activities for the other *cis*-dihydrodiols **B** (R = H, F, Cl, Br, I) were found to be in the sequence I = Br = Cl > Me > F > H.

It should be noted that the purified NDD_R enzyme present in cell-free extracts from *E. coli nar B* showed only 40% amino acid homology with the analogous pure NDD_P gene (nahB) isolated from a *Pseudomonas* strain.[32] However, it is noteworthy that, while NDD_R and NDD_P may be considered as different enzymes, clearly the binding and catalytic sites must be similar. This is evident from the observation that in each case the *cis*-dihydrodiol of naphthalene was the best substrate and the relative activities of the other *cis*-dihydrodiol substrates **B** (R = H, F, Cl, Br, Me) showed some similarity. Significantly, the *cis*-dihydrodiol from iodobenzene **B** (R = I), proved to be a better substrate than the other halobenzene *cis* -dihydrodiols **B** using NDD_R (Table 4).

Based on the results shown in Table 2 and Table 4 for the NDD_R-catalysed formation of ten 3-substituted catechols **C** [R = H, F, Cl, Br, I, CN, CF_3, Me, *t*-Bu, $CH(HO)CF_3$] from the corresponding *cis*-dihydrodiol precursors **B**, it is now possible a make an approximate comparison between the relative rates of formation of each catechol **C** using either the chemocatalytic (Pd/C) or biocatalytic (NDD_R) approaches. Thus, using the Pd/C catalytic system the reactivity sequence in decreasing order was: $CH(OH)CF_3$ > Me > H > *t*-Bu > F > Cl > Br > CN > CF_3 > I while the corresponding NDD catalytic sequence was: I > *t*-Bu > Br > Cl > Me > CF_3 > CN > F > $CH(OH)CF_3$. On this basis the two catalytic approaches could be used in a complementary manner, e.g., the best method for the formation of 3-iodocatechol **C** (R = I) would involve using NDD_R while the chiral catechol **C** [R = $CH(OH)CF_3$] would be more readily obtained using Pd/C.

A Tandem (One-Pot) Biocatalytic/Chemocatalytic Approach to the Synthesis of Catechols C from the Corresponding Monosubstituted Benzene Substrates A

The final objective of this study was to determine if the synthetic pathway **A**→**B**→**C** involving two biocatalysts (TDO and NDD) illustrated in Scheme 1 could also be performed as a one-pot procedure involving a biocatalyst (TDO) and chemocatalyst (Pd/C). If successful this procedure could avoid the isolation of the intermediate, *cis*-dihydrodiol **B**, during the formation of the catechol **C**. This would significantly reduce the down-steam processing involved with two separate biotransformations using whole cell systems. It could also overcome the problem of product inhibition associated the production of catechol **C** from an arene precursor **A** using both dioxygenase and *cis*-diol dehydrogenase enzymes in a single whole cell system. Figure 5 shows a schematic diagram to illustrate the differences between the sequential biocatalytic/che-

Table 4. Comparison of relative activities of NDD_R during formation of catechols **C** with respect to the activity of NDD_R relative to that shown by the naphthalene *cis*-1,2-dihydrodiol (100%) using UV absorption at 340 nm.[a]

R	H	F	Cl	Br	I	Me	*t*-Bu	CN	CF_3	$CH(OH)CF_3$
Relative activity (%)	5	23	55	56	67	50	60	26	50	13

[a] The standard deviation for all assays was < 3%.

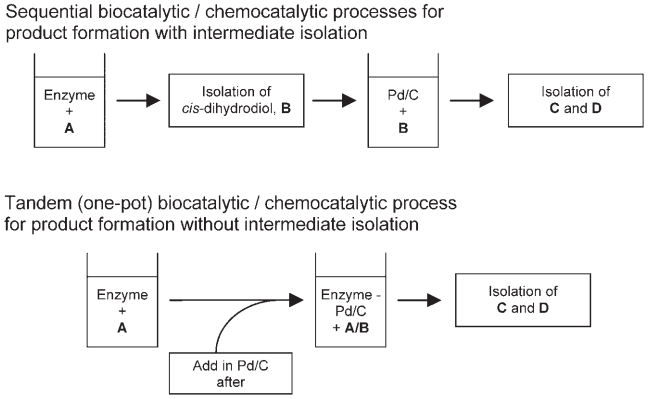


Figure 5. Schematic comparison of the sequential biocatalytic/chemocatalytic and the tandem (one-pot) biocatalytic/chemocatalytic processes.

mocatalytic process and the tandem (one-pot) biocatalytic/chemocatalytic process.

In order to achieve this objective it was important to know the maximum concentration of substrate that the bacterial cultures could support before the cells were significantly poisoned by the reactants **A** or products (*cis*-dihydrodiols **B**, *cis*-tetrahydrodiols **D** or catechols **C**) formed. Table 5 summarises the results obtained from a series of reactions where the Pd/C catalyst and the substrate **A** (R = F), were directly added either simultaneously to the biotransformation medium or with a delay of three or six hours after addition of the substrate. In all the cases, after 24 h the bacterial cell debris was removed by centrifugation and the products extracted from the aqueous supernatant. The model substrate used was fluorobenzene **A** (R = F) and the product distribution was analysed by ^{1}H NMR and GC-MS methods.

When, the Pd/C catalyst was added either 3 or 6 h after the addition of substrate **A** (R = F), complete conversion to catechol **C** (R = F) and *cis*-tetrahydrodiol **D** (R = F) in approximately equal amounts was observed after 24 h reaction time. However, when catalyst and substrate were added simultaneously, 17% of the fluorobenzene substrate remained, even after 24 h reaction. In the absence of the chemocatalyst (Pd/C), the biotransformation from the fluorobenzene substrate into the corresponding *cis*-dihydrodiol **B** as sole bioproduct is complete between 6–8 h depending on the particular cell colonies used. Thus when the Pd/C was added after 6 h, the biotransformation was expected to be almost complete and there was little observable effect on the TDO enzyme activity of the catechol **C** or *cis*-tetrahydrodiol **D** formed in the second step. In contrast, when substrate **A** and heterogeneous catalyst were added simultaneously, the *cis*-dihydrodiol **B** formed is then rapidly converted into catechol **C** and *cis*-tetrahydrodiol **D**. The catechol **C**, as expected, was then found to act as an inhibitor of the TDO enzyme thus reducing the biotransformation rate with the result that a significant proportion of the residual arene substrate **A** (17%) remained after 24 h.

The one-pot biocatalytic/chemocatalytic synthesis was performed in the presence of air and, therefore, it is surprising, given the low rates of reaction using an oxygen-containing environment (Table 1), that the reaction is so efficiently catalysed. In order to determine whether the solvent, culture medium or cells increased the rate of reaction, the dehydrogenation reactions were performed in water, in the culture medium and in the culture medium after the cells were removed (Table 6).

From Table 6, it is clear that the presence of the *P. putida* UV4 cells is crucial for the reaction to proceed effectively. Interestingly, even when the cells were re-

Table 5. Effect of time of addition of the Pd/C catalyst to the biocatalytic reaction of fluorobenzene **A** to the catechol **C** and *cis*-tetrahydrodiol **D** product distribution observed after 24 h overall reaction time (R = F).[a]

Time at which Pd/C catalyst was added [h]	Product distribution [%]		
	A	**C**	**D**
0[b,c]	17.0	41.0	40.0
3	0.0	44.0	56.0
6	0.0	52.0	48.0

[a] Reaction conditions: 10 mM of **A** (R = F), 10 wt% Pd/C (0.04 = Pd/substrate), 200 cm^3 culture medium, air, 30°C for 24 h on a rotary shaker (110 rpm).
[b] Fluorobenzene substrate also added at 0 h;
[c] Among the products 2% of catechol C (R = H) was detected by GC analysis.

Table 6. Comparison of the product distribution with medium type for the reaction of *cis*-dihydrodiol **B** (R = F) with Pd/C following 24 h reaction in air.[a]

Reaction medium	Reactant (**B**) and product (**C** and **D**) distribution (%)		
	B	**C**	**D**
P. putida UV4 cells and culture medium[b]	0	52	46
Residual culture medium[c]	52	27	20
Culture medium	99	1	0
Water	95	5	0

[a] *Reaction conditions:* 8 mM of *cis*-dihydrodiol **B** (R = F), 10 wt% Pd/C (0.04 = Pd/substrate), 200 mL culture medium, 30°C for 24 h on a rotary shaker (110 rpm).
[b] Among the products 2% of catechol **C** (R = H) was detected by GC;
[c] Cells were largely removed by centrifugation.

moved, although the reaction rate was decreased, a significant rate enhancement over pure water or culture medium was found. This rate increase is thought to be due to the combined process of cell growth and of TDO enzyme activity, both of which reduce the dissolved oxygen in the aqueous culture medium and effectively limit the surface oxygen concentration on the Pd/C catalyst. As reported earlier for methanol solvent, this increases the rate of reaction but decreases the selectivity towards the catechol. The significant concentration (46%) of *cis*-tetrahydrodiol **D** (R=F) formed in the presence of the *P. putida* UV4 cells supports this hypothesis. Due to the micellar nature of the cells in solution, it was difficult to fully remove the cells even with filtration and, therefore, it is likely that the rapid reaction found once the cells had been removed, is due to residual cell colonies present in the medium which had not been separated.

A meaningful comparison of the two step process, i.e., formation and isolation of the intermediate *cis*-dihydrodiol followed by the heterogeneously catalysed reaction, with the one-step process (concomitant biotransformation and Pd/C-catalysed dehydrogenation) needs to include the work-up steps as well as the reaction steps. Although the isolation and purification of *cis*-dihydrodiol intermediate **B** requires extensive work-up and waste production, it offers the advantage that the heterogeneous route can be performed at high concentrations of *cis*-dihydrodiol without any problems of enzyme inhibition. In the one-pot process, even if the catalyst is added after the biotransformation has been completed, the maximum substrate concentration for the initial TDO catalysed *cis*-dihydroxylation reaction is 10 mM, in the case of fluorobenzene **A** (R=F), before the cells become inactive. The most efficient procedure appears to be a hybrid approach where the biotransformation is performed using a large volume of culture medium and the reaction mixture is then concentrated but without isolation and purification of the intermediate. The concentrate, including bacterial cells and bioproduct **B**, is then reacted in the presence of the heterogeneous catalyst after which the product is extracted and purified. This has the advantage that only a fraction of the solvent waste is produced and only a small reactor volume is required for the second stage process whilst maintaining the high reaction rates. Using this approach from 4 g of fluorobenzene **A** (R=F) in 250 cm^3 water 2.4 g of pure 3-fluorocatechol **C** (R=F) were formed. This process has also been used to form 3-methylcatechol **C** (R=Me) with similar efficiencies.

Conclusions

A comparative study of the conversion of a series of monosubstituted benzene *cis*-dihydrodiols **B** into the corresponding catechols **C** has been carried out using both biocatalytic (NDD_R) and chemocatalytic (Pd/C) methods. This has allowed the best approach to be adopted for specific catechols **C** having particular synthetic value. The factors that determine the relative proportions of dehydrogenation (catechols **C**) and hydrogenation products (*cis*-tetrahydrodiols **D**) obtained when *cis*-dihydrodiols **B** react with Pd/C, have been evaluated. The mechanism of the Pd/C-catalysed disproportionation of *cis*-dihydrodiols has been determined using deuterium labelled precursors and appears to be very similar to that found during the NDD catalysed process. It is now possible to combine the biocatalytic (TDO) and chemocatalytic (Pd/C) steps into a one-pot synthetic route for catechol production from the corresponding monosubstituted benzene substrate.

Experimental Section

Materials

The catalyst 10wt% Pd/C (supplied by Aldrich) had a BET surface area of $682.3 \pm 7.7\ m^2 g^{-1}$ and 16% metal dispersion (measured by CO chemisorption). Methanol (Prolabo 99.8%, HPLC grade) and 18.2 MΩ distilled water were used as solvents in all the reactions, except for the kinetic experiments which were performed in methanol-d_4 (99.8% D, Euriso-top). Research grade helium, hydrogen, nitrogen and oxygen were supplied by BOC as pure gases and were mixed as required.

Experimental procedure

The reaction was monitored by regular sample analysis using a GC on a Hewlett Packard 6890 instrument fitted with a ZB-5 column (30 m, 0.25 μm diameter) and GC-MS using a Perkin–Elmer Clarus 500 instrument with an PE-5MS column (30 m, 0.25 μm diameter). The retention time of the peaks was compared against authentic samples. In each case the sample was removed from the reaction medium, filtered to extract the catalyst and to stop further reaction, and subsequently analysed. The reaction mixture, at the end of each experiment, was analysed by ^{1}H NMR spectroscopy using either 300 MHz (Bruker Avance DPX-300) or 500 MHz (Bruker Avance DRX-500) instruments 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.

The aromatic substrates **A** (R=H, F, Cl, Br, I, CN, CF_3, Me, *t*-Bu, $COCF_3$) and authentic samples of catechols **C** (R=H, F), were purchased (Aldrich) and used as received. Toluene-1,5-D_2 (99.2atom% D), used for the synthesis of isotopic toluene-*cis*-1,2-dihydrodiol **B** (R=Me), was obtained from C/D/N Isotopes Inc., and toluene-D_8 (99+

atom % D) from Aldrich. *cis*-Dihydrodiols **B** [R = H, F, Cl, Br, I, Me, CN, CF_3, $CF_3CH(OH)$][12,27] and *cis*-tetrahydrodiols **D** [R = H, F, Cl, Br, I, CF_3, $CF_3CH(OH)$], which had been fully characterised,[13,27,35-38] were available from earlier studies. Catechols **C** [R = Cl, Br, I, Me, $CF_3CH(OH)$], isolated during this study, showed identical physical and spectral characteristics to those reported.[2,3,7,40]

(−)-*cis*-(1*S*,2*R*)-3-Methyl-3-cyclohexene-1,2-diol D (R = Me)

White solid; mp 83–85 °C; $[\alpha]_D$: −155 (*c* 1.00, $CHCl_3$); MS: *m/z* = 128.0863 [M^+], calcd. for $C_7H_{12}O_2$: 128.0837; ^{1}H NMR (300 MHz): δ = 1.70 (2H, m, 6-H_A,6-H_B), 1.82 (3H, d, $J_{Me,4}$ = 1.8 Hz, Me), 2.04–2.12 (2H, m, 5-H_A, 5-H_B), 2.72 (2H, br s, OH), 3.75 (1H, m, 1-H), 3.92 (1H, d, $J_{2,1}$ = 3.3 Hz, 2-H), 5.56 (1H, m, 4-H); ^{13}C NMR (125 MHz): δ = 21.17, 24.24, 25.85, 70.02, 70.58, 125.95, 133.99; MS (EI): *m/z* = 128 (M^+, 1 %), 70 (100).

(+)-*cis*-(1*S*,2*R*)-3-*t*-butyl-3,5-cycloxadiene-1,2-diol B (R = *t*-Bu)

White solid; mp 52–54 °C; $[\alpha]_D$: +9 (*c* 1.10, MeOH); MS: *m/z* = 168.1137 [M^+], calcd. for $C_{10}H_{16}O_2$: 168.1150; ^{1}H NMR (300 MHz): δ = 1.14 (9H, s, *t*-Bu), 2.10 (1H, br s, OH), 3.18 (1H, br s, OH), 4.02 (1H, d, $J_{2,1}$ = 4.8 Hz, 2-H), 4.40 (1H, m, 1-H), 5.63 (1H, m, 6-H), 5.80 (1H, dd, $J_{4,5}$ = 5.4 Hz, $J_{4,2}$ = 0.6 Hz, 4-H), 5.92 (1H, ddd, $J_{5,4}$ = 5.4 Hz, $J_{5,6}$ = 4.7 Hz, $J_{5,1}$ = 2.7 Hz, 5-H); ^{13}C NMR (125 MHz): δ = 29.77, 35.42, 60.82, 72.70, 117.94, 124.24, 130.35, 149.92; EI-MS: *m/z* = 168 (M^+, 30 %), 150 (64), 94 (100).

(−)-*cis*-(1*S*,2*R*)-3-*t*-butyl-3-cyclohexene-1,2-diol D (R = *t*-Bu)

White solid. mp 77–79 °C; $[\alpha]_D$: −65 (*c* 1.00, MeOH); MS: *m/z* = 170.1325 [M^+], calcd. for $C_{10}H_{18}O_2$: 170.1307; ^{1}H NMR (300 MHz): δ = 1.12 (9H, s, *t*-Bu), 1.73 (2H, m, 6-H_A, 6-H_B), 2.20 (4H, m, 5-H_A, 5-H_B, 2OH), 3.59 (1H, m, 1-H), 4.21 (1H, d, $J_{2,1}$ = 3.0 Hz, 2-H), 5.69 (1H, dd, $J_{4,5A}$ = 2.8 Hz, $J_{4,5B}$ = 4.6 Hz, 4-H); ^{13}C NMR (125 Hz): δ = 24.29, 29.17, 30.34, 35.49, 66.83, 71.56, 123.61, 145.82; EI-MS: *m/z* = 170 (M^+, 1 %), 111 (100).

(−)-*cis*-(1*S*,2*R*)-3-cyano-3-cyclohexene-1,2-diol D (R = CN)

Viscous oil; $[\alpha]_D$: −64 *c* 1.12, MeOH); MS: *m/z* = 139.0626 [M^+], calcd. for $C_7H_9NO_2$: 139.0633; ^{1}H NMR (500 MHz): δ = 1.59–1.79 (2H, m, 6-H_A, 6-H_B), 2.04 (1H, m, 5-H_A), 2.20 (1H, m, 5-H_B), 3.91 (1H, m, 1-H), 4.14 (1H, d, $J_{2,1}$ = 5.2 Hz, 2-H), 6.43 (1H, dd, $J_{4,5A}$ = 2.6 Hz, $J_{4,5B}$ = 4.2 Hz, 4-H); ^{13}C NMR (125 MHz): δ = 25.12, 26.92, 68.78, 73.91, 119.03, 132.14, 141.11; EI-MS: *m/z* = 139 (M^+, 2 %), 121 (4), 95-(100).

3-Trifluoromethyl-1,2-benzenediol C (R = CF_3)

White solid; mp 39–42 °C; MS: *m/z* = 178.0240 [M^+], calcd. for $C_7H_5O_2F_3$: 178.0241; ^{1}H NMR (300 MHz): δ = 5.86 (2H, br s, OH), 6.88 (1H, m, 5-H), 7.07 (2H, m, 4-H, 6-H); ^{13}C NMR (125 MHz): δ = 117.94, 118.47, 118.54, 119.07, 120.90, 142.37, 144.98; EI-MS: *m/z* = 178 (M^+, 100 %), 159 (17), 158 (82).

Biotransformation Using *P. putida* UV4 to Yield *cis*-Dihydrodiols B

Biotransformation procedures employed for arene substrates **A** (including 1,5-D_2- and D_8-toluene) with whole cells of *P. putida* UV4, a proprietary constitutive mutant strain derived from the wild type strain *P. putida* NCIB 11767, were identical to those reported earlier for the production of *cis*-dihydrodiols **B**.[3,12,35,36]

Biotransformation Using *E. coli nar* B to Yield Catechols C

E. coli nar B was grown at 37 °C in Luria broth (LB) medium. Antibiotic ampicillin (0.1 mg cm^{-3}) was added to the culture medium to maintain plasmid-harbouring cells. *iso*-Propyl-β-D-thiogalactopyranoside (IPTG), an enzyme-inducer, was added (0.05 mg cm^{-3}) before inoculation of the liquid LB-media for the cell-growth. Cells were harvested, in late exponential growth phase, by centrifugation, washed and re-suspended (shake flasks; OD_{600} = 5–10) in potassium phosphate buffer (50 mM, pH 7.2) prior to the biotransformation at 30 °C. Substrates (0.5–2.0 mg cm^{-3}) were added separately and the reaction was terminated after 18 h. The catechols were extracted from the aqueous medium using EtOAc and were purified by flash column chromatography (silica gel, 25 % EtOAc in hexane).

Typical Heterogeneous Catalysed Reaction of *cis*-Dihydrodiols B and Recycling of Catalyst

The heterogeneously catalysed reactions were carried out, using a magnetically stirred, sealed, 50-cm^3 round-bottom flask fitted with a gas reservoir. The reservoir was filled with gas from a gas manifold consisting of a number of independent calibrated mass flow controllers, feeding the gases into a mixing chamber. The gases were purged through the system to ensure the correct concentrations and good mixing before being filling the reservoir.

Unless stated otherwise, the catalyst 10 % wt Pd/C (0.005–0.04 = Pd/substrate) was placed in the reactor with methanol (*ca.* 18 cm^3) and heated to 30 °C. After purging the solution and headspace of the reactor for 20 min with gas from the reservoir, a solution of *cis*-dihydrodiol in methanol (2 cm^3) was injected into the vessel so that the final substrate concentration was 80 mM. The reaction mixture was stirred at 1000 rpm. For benzene *cis*-dihydrodiol precursors **B** (R = Br, I), the catalyst was pre-activated by stirring (2 h) in an atmosphere of hydrogen.

In the recycling of catalyst experiments, after completion of each cycle, the catalyst was filtered from the reaction mixture, washed several times with small volumes of methanol, dried and weighed. In each case, the repeat reaction was conducted using a slightly reduced weight of substrate, to take into account the small loss of catalyst occurring during sampling and recovery procedures.

Tandem (One-Pot) Biocatalytic/Chemocatalytic Procedure for Catechol C Formation

The 10 wt% Pd/C catalyst (0.04 = Pd/substrate) was either added directly to the aqueous biotransformation medium (200 cm^3 in a 2-L conical flask) along with the 10 mM aromatic substrate **A** (R = F) or with a delay period of 3 or 6 h, after addition of the substrate. The flasks, containing the reaction mixtures under air atmosphere, were mounted on a rotary shaker (110 rpm) maintained at 30 °C. In all experiments, the bacterial cell debris was removed by centrifugation after 24 h and the products were extracted from the aqueous supernatant.

Effect of the Presence of Cells and Culture Medium on the Rate of Catechol C Formation Using a Pd/C Catalyst

To determine effect of solvent, the culture medium and the cells on rate of the reaction, the dehydrogenation reactions of *cis*-dihydrodiols **B** were separately performed in water, the culture medium and in the medium after the cells were removed. The reaction conditions employed were: *cis*-dihydrodiol **B** (R = F, 8 mM) and 10 wt% Pd/C (0.04 = Pd/substrate) in culture medium or water (200 cm^3), under air atmosphere, maintained at 30 °C on a rotary shaker (110 rpm) for 24 h.

Enzyme Assay of Naphthalene *cis*-Dihydrodiol Dehydrogenase (*cis*-NDD)

The enzymatic activity of the *cis*-NDD_R was measured spectrophotometrically at 340 nm, in cell-free extracts, by measuring the initial rate of reduction of NAD^+, as described by Patel and Gibson[33]. The protein concentration was determined by the Pierce BCA assay (Pierce, USA).

The reaction mixture (2.0 cm^3) contained, 180 μmol of KH_2PO_4 buffer (pH 7), 5.3 μmol of NAD^+, 0.66 μmol of *cis*-dihydrodiol substrate **B** and the enzyme (1.125–1.640 mg). In these experiments, the specific activity in crude cell-free extracts was typically between 0.017 and 0.030 μmol $min^{-1}mg^{-1}$. The reaction was initiated by addition of NAD^+ and the absorbance followed over a period with different substrates. Tangents drawn to the curves, obtained during the first minute of the reaction, were used to calculate the initial reaction rate. Reactions were performed in duplicate. For these experiments, one unit of enzyme activity was defined as the amount of enzyme required for the reduction of 1 μmol of NAD^+ per minute. Specific activities were expressed in activity units per milligram of protein.

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