Biocatalytic Asymmetric Dihydroxylation of Conjugated Mono- and Poly-alkenes to Yield Enantiopure Cyclic *cis*-Diols

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Abstract: Dioxygenase-catalysed asymmetric dihydroxylation, of a series of conjugated monoalkenes and polyenes, was found to yield the corresponding monols and 1,2-dihydrodiols. The diol metabolites were obtained from monosubstituted, *gem*-disubstituted, *cis*-disubstituted, and trisubstituted alkene substrates, using whole cells of *Pseudomonas putida* strains containing toluene and naphthalene dioxygenases. Dioxygenase selection and alkene type were established as important factors, in the preference for dioxygenase-catalysed 1,2-dihydroxylation of conjugated alkene or arene groups, and monohydroxylation at benzylic or allylic centres. Competition from allylic hydroxylation of methyl groups was observed only when naphthalene dioxygenase was used as biocatalyst. The structures, enantiomeric excess values and absolute configurations of the bioproducts, were determined by a combination of stereochemical correlation, spectroscopy (NMR and CD) and X-ray diffraction methods. *cis*-1,2-Diol metabolites from arenes, cyclic alkenes and dienes were generally observed to be enantiopure (>98% ee), while 1,2-diols from acyclic alkenes had lower enantiomeric excess values (<88% ee). The enantiopure *cis*-diol metabolite of a *gem*-disubstituted fulvene was used as precursor in a new chemoenzymatic route to a novel C_2-symmetrical ketone.

Keywords: allylic/benzylic hydroxylation; asymmetric alkene/arene dihydroxylation; chemoenzymatic synthesis; dioxygenases

Introduction

Ring hydroxylating dioxygenase enzymes, present in mutant strains of the soil bacterium *Pseudomonas putida*, have been found to catalyse the formation of a wide range of enantiopure *cis*-dihydrodiol metabolites, from the corresponding arene substrates. To date, more than three hundred arene *cis*-dihydrodiols have been obtained using toluene dioxygenase as biocatalyst, and these have been extensively used as chiral precursors in synthesis.[1–8] Benzene dioxygenase (BDO), toluene dioxygenase (TDO) and naphthalene dioxygenase (NDO) enzymes have also been reported to catalyse the dihydroxylation of a small number of alkenes.[9–23] The considerable potential of enantiopure alkene bioproducts as synthetic precursors has yet to be exploited.

Simple models and guidelines are now available to predict the regio- and stereochemical outcome of dioxygenase-catalysed *cis*-dihydroxylation of substituted arenes.[5] Similar guidelines have not yet been established for dioxygenase-catalysed 1,2-dihydroxylations of all possible substituted alkene types. Thus, it is difficult to predict the regioselectivity and stereoselectivity of the biocatalytic asymmetric dihydroxylation reactions of alkene substrates.

It should be emphasised that non-conjugated acyclic alkenes are generally very poor substrates for dioxygenase-catalysed 1,2-dihydroxylation, and few examples of this type have been reported.[24,25] However, the asymmetric 1,2-dihydroxylation of several acyclic substrates bearing a monosubstituted alkene group has been observed.[13–15] It is noteworthy that in these examples the alkene was conjugated to an aryl group, e.g., oxidation of chlorostyrene **1c** to yield arene-*cis*-dihydrodiol **2c** and alkene-1,2-diol **3c** (Scheme 1).[14]

Similar studies have been reported on the dioxygenase-catalysed 1,2-dihydroxylation of the benzo-fused cyclic alkenes **6a–c** to give the corresponding 1,2-diols

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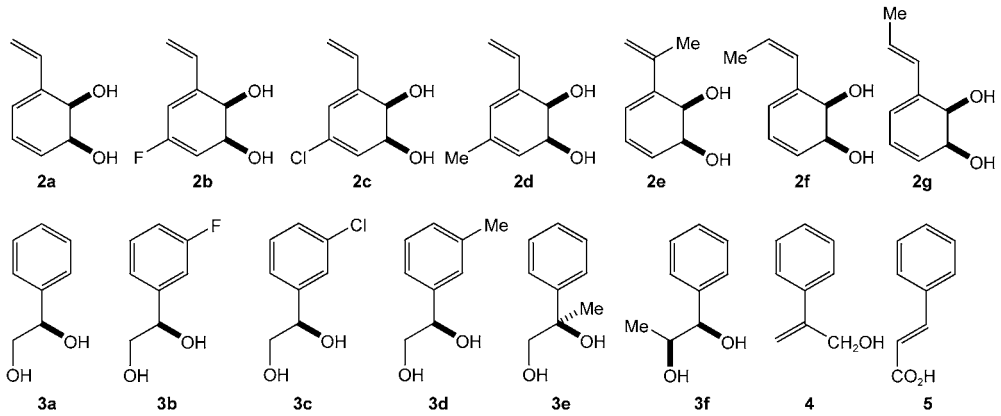
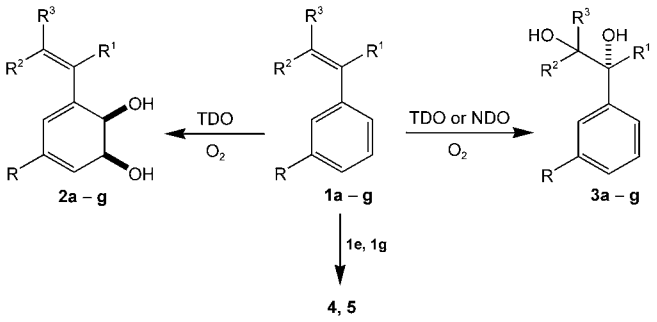




1a R - R^3 = H; **1b** R = F, (R^1 - R^3) = H; **1c** R = Cl, (R^1 - R^3) = H; **1d** R = Me, (R^1 - R^3) = H; **1e** R^1 = Me, (R, R^2, R^3) = H; **1f** R^2 = Me, (R, R^1, R^3) = H; **1g** R^3 = Me, (R, R^1, R^2 = H)

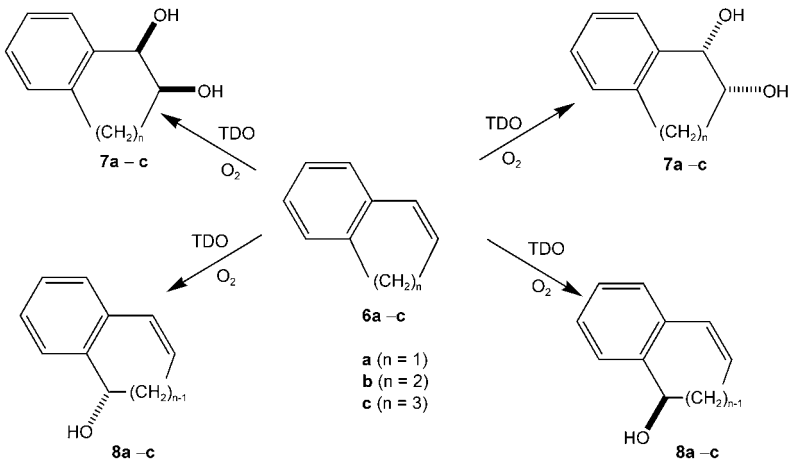
Scheme 1. TDO- and NDO-catalysed dihydroxylation of monosubstituted alkenes **1a**–**g**.

7a–**c** of opposite absolute configurations (enantiocomplementarity) using TDO and NDO (Scheme 2).[10–12,18,19]

Alkenediols **7a**–**c** were also accompanied by enantiocomplementary benzylic monohydroxylation products **8a**–**c**, but without any evidence of the corresponding arene-*cis*-dihydrodiols.

The dioxygenase-catalysed 1,2-dihydroxylation of conjugated dienes, in common with acyclic alkene substrates, has to date also received little attention. Isoprene, a typical example of a conjugated diene, is freely liberated into the environment from plants and, in view of the large quantities produced, exerts an important influence on atmospheric chemistry. This observation prompted our recent study of the BDO-catalysed 1,2-dihydroxylation of acyclic C_4 and C_5 dienes including butadiene and the methyl-substituted derivatives isoprene, *cis*-piperylene and *trans*-piperylene using *P. putida* ML2.[22] Our preliminary results from the dioxygenase-catalysed 1,2-dihydroxylation of several conjugated cyclic C_5-C_8 dienes and C_7 trienes, have been reported.[21]

Chemical methods are currently available for catalytic asymmetric dihydroxylation of all six alkene types (mono-, *gem*-di-, *cis*-di-, *trans*-di-, tri- and tetrasubstituted alkenes).[26] Enantomeric excess (ee) values of diols, synthesised by using the most commonly employed chiral dihydroxylating agents, e.g., $K_2OsO_4(OH)_4$, $K_3Fe(CN)_6$, $(DHQ)_2PHAL$ (AD-mix-β), are generally



Scheme 2. TDO- and NDO-catalysed mono- and di-hydroxylation of alkenes **6a**–**c** to yield *cis*-diols **7a**–**c** and monoalcohols **8a**–**c**.

high (>95% ee) for five of the alkene types; the degree of stereoselectivity for the sixth, *cis*-disubstituted alkenes, is lower (20–80% ee).[26] During this study, particular emphasis has been placed on: (i) a systematic evaluation of the regioselective and stereoselective potential of dioxygenase-catalysed 1,2-dioxygenation when challenged with substrates containing all six alkene types (ii) establishing general trends that would facilitate prediction of the facial and regiochemical aspects of dioxygenase-catalysed 1,2-dihydroxylation of aryl-substituted alkenes **1a–g**, **9** (Schemes 1 and 3) and conjugated cyclic dienes **14a–i** (Scheme 4) and (iii) demonstrating the value of dioxygenase-catalysed asymmetric dihydroxylation of *cis*-disubstituted alkenes, in comparison with chemical asymmetric dihydroxylation, by using the enantiopure alkene 1,2-dihydrodiol bioproduct **15f** as precursor in a chemoenzymatic synthesis of the useful C_2-symmetrical ketone **21** (Scheme 5).

Results and Discussion

Non-conjugated monoalkenes appear to be very poor substrates for bacterial dioxygenase-catalysed 1,2-dihydroxylation, based on the few literature reports available.[24,25] This premise is supported by our unpublished observations of very low isolated yields of the corresponding alkene-1,2-diols from as series of non-conjugated acyclic alkene (e.g., mono-, di- and trisubstituted allylbenzenes) and cyclic alkene substrates (e.g., cyclohexene and 1,4-dihydronaphthalene) in the presence of TDO biocatalyst. The remit of the present study, to elucidate factors that influence dioxygenase-catalysed alkene mono- and dihydroxylation *versus* other oxidation types, was therefore, confined to conjugated alkenes.

Styrene **1a** and a series of *meta*-substituted styrene substrates **1b–d** were added to whole cells of both *P. putida* UV4 (a mutant strain containing TDO without diol dehydrogenase activity), and *P. putida* NCIMB 8859 (a wild-type strain containing NDO and a diol dehydrogenase) (Scheme 1). Substrates **1b–d** were selected on the basis of our earlier observations that *meta*-disubstituted benzenes were, generally, poorer substrates for TDO-catalysed arene *cis*-dihydroxylation, in comparison to *ortho*- and *para*-disubstituted benzenes. It was anticipated that this factor might favour the formation of monosubstituted alkene-1,2-diols **3b–d** over arene-*cis*-dihydrodiols **2b–d**. The other styrene substrates **1e–g** were chosen as typical examples of *gem*-disubstituted **1e**, *cis*-disubstituted **1f** and *trans*-disubstituted **1g** alkenes.

Each of the styrene substrates **1a–g** was biotransformed under the catalytic influence of TDO into the corresponding (1*S*,2*R*)-arene-*cis*-dihydrodiols **2a–g** (Table 1). Arene-*cis*-diols **2a–d** were accompanied by the corresponding alkene-1,2-diols **3a–d**. After PLC separation and purification, the ee values and absolute configurations of the *cis*-dihydrodiols **2a–g** (2–42% yield) were determined using both (*R*) and (*S*) enantiomers of 2-(1-methoxyethyl)-phenylboronic acids (MEPBAs) to form the corresponding diastereoisomeric boronates according to the reported method[27–30] and by comparison of CD spectra. The *cis*-dihydrodiol bioproducts **2a–g** were all found to be enantiopure (>98% ee) and compounds **2a**, **2e–g** showed the typical CD spectra associated with a (1*S*,2*R*) absolute configuration ($\lambda = 218–235$ nm [$\Delta\varepsilon$ − ve], 280–282 nm [$\Delta\varepsilon$ + ve]); in accord with expectations from earlier biotransformations of monosubstituted and *meta*-disubstituted benzene substrates. The earlier report [14] of TDO-catalysed dihydroxylation (*P. putida* 39/D) of *meta*-chlorostyrene **1c** to yield *cis*-dihydrodiol **2c** with a lower enantiopurity value (1% yield, 54% ee), was not reproduced during this study using *P. putida* UV4.

While the monosubstituted (1*R*)-alkenediols **3a–d** were consistently formed (3–22% yield) using TDO as

Table 1. Arene- (**2a–g**) and alkene (**3a–f**)-diol metabolites of styrenes (**1a–g**).

Alkene	Arene-*cis*-1,2 dihydrodiol[a, b]			Alkene-1,2-diol[b] ( )[c]			
		Yield[b]	Config.[d]		Yield	ee	Config.[d]
1a	**2a**	32	1*S*,2*R*	**3a**	3 (60)	88 (80)	1*R*
1b	**2b**	27	1*S*,2*R*	**3b**	22 (14)	62 (62)	1*R*
1c	**2c**	2	1*S*,2*R*	**3c**	14 (18)	42 (56)	1*R*
1d	**2d**	22	1*S*,2*R*	**3d**	8 (12)	48 (56)	1*R*
1e	**2e**	24	1*S*,2*R*	**3e**, **4**	(20), (12)[e]	(46)	1*R*
1f	**2f**	42	1*S*,2*R*	**3f**	(15)	(82)	1*R*,2*S*
1g	**2g**	37	1*S*,2*R*	**5**	(52)[f]	–	–

[a] >98% ee.
[b] Using TDO.
[c] (Using NDO).
[d] Absolute configuration.
[e] Yield of allylic alcohol **4**.
[f] Yield of α,β-unsaturated carboxylic acid **5**.

biocatalyst, no evidence was found for the alkene-1,2-diol metabolites **3e**–**g** from the corresponding disubstituted alkenes **1e**–**g** under these conditions. The ee values and absolute configurations of the alkene-1,2-diol bioproducts **3a**–**d** were determined by: (i) comparison of optical rotation values with those reported in the literature and (ii) formation of the corresponding diastereoisomeric di-MTPA esters using (*R*) and (*S*) forms of 2-methoxy-2-phenyltrifluoromethylacetyl chloride, followed by their ^{1}H NMR spectral analysis. Based on this method, the alkene-1,2-diols **3a**–**d** were all found to have an excess of the (1*R*) enantiomer (42–88% ee). The earlier observation[14] that 1,2-diol **3c** (2% yield, 95% ee), a metabolite of *meta*-chlorostyrene **1c** using *P. putida* 39D (TDO), had an excess of the (1*S*) configuration, is at variance with the consistent (1*R*) trend found using *P. putida* UV4.

NDO had previously been found to catalyse the production of *cis*-dihydrodiols of naphthalene, and biphenyl (a monosubstituted benzene).[9] One disadvantage of NDO was its inability to catalyse the formation of *cis*-dihydrodiols from any monosubstituted benzene substrates except for biphenyl.[9] However, this limitation was turned to advantage, when styrene substrates **1a**–**g** were used in the present study with NDO. Thus, in the absence of arene-*cis*-dihydroxylation, the alkene-diol bioproducts **3a**–**f** were formed exclusively (12–60% yield, Table 1), thereby avoiding any separation problem of alkene- and arene-1,2-diols. Furthermore, both TDO and NDO enzyme systems yielded alkene-1,2-diols **3a**–**f** with an excess (42–88% ee) of the (1*R*) absolute configuration, but failed to catalyse the formation of the 1,2-diol from *trans*-alkene **1g**.

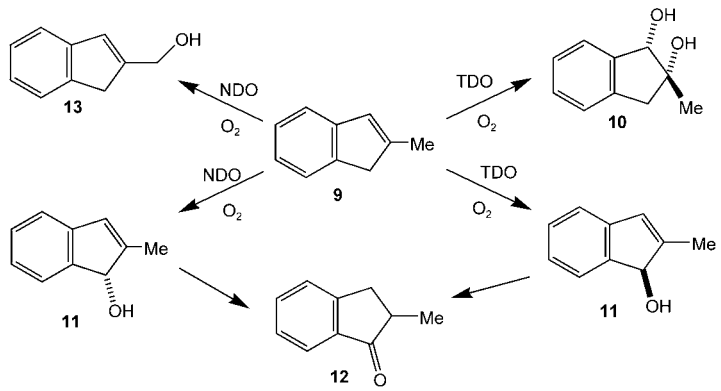
When α-methylstyrene **1e**, was added as substrate to whole cells of *P. putida* NCIMB 8859, the expected alkene-1,2-diol **3e** (20% yield), and an unusual allylic monohydroxylation product **4** (12% yield) were isolated. It was interesting to note that *trans*-β-methylstyrene **1g** showed no evidence of 1,2-dihydroxylation and instead gave the α,β-unsaturated carboxylic acid **5** (52% yield) as the sole metabolite, presumably as a result of initial allylic hydroxylation of the methyl group followed by further oxidation. NDO-catalysed allylic hydroxylation appears to be without precedent.[8,9] However, allylic hydroxylation of substrates **1e** and **1g** in the presence of NDO was observed to be the preferred metabolic pathway, when the rate of 1,2-dihydroxylation was either slowed (e.g., on the *gem*-disubstituted alkene **1e**) or halted (e.g., on the arene rings of styrenes **1e** and **1g** and the *trans*-alkene group of styrene **1g**).

The yields of acyclic alkene-1,2-diols, obtained from a earlier biotransformation study of methyl-substituted butadienes with a wild-type strain (ML2) of *P. putida*, were used as a crude indicator of the relative rates of dioxygenase-catalysed dihydroxylation of four alkene types.[22] Due to further biotransformations of the 1,2-diol bioproducts also occurring, only tentative conclusions could be drawn. The present study of TDO and NDO-catalysed 1,2-dihydroxylation of styrene substrates **1a**–**g**, supported the earlier observed trend that dioxygenase-catalysed 1,2-dihydroxylation occurred more readily in the sequence: monosubstituted alkene > *cis*-disubstituted alkene > *gem*-disubstituted alkene > *trans*-disubstituted alkene.

In view of the total preference shown for TDO-catalysed *cis*-dihydroxylation of the phenyl ring over the disubstituted alkene group in styrene substrates, **1e**–**g** (Scheme 1), neither trisubstituted nor tetrasubstituted alkene analogues of these compounds were considered as appropriate substrates. A precedent for 1,2-dihydroxylation of an acyclic trisubstituted alkene bond was found when isoprene was oxidised using BDO as biocatalyst, although this was only a minor pathway relative to 1,2-dihydroxylation of the monosubstituted alkene bond.[22]

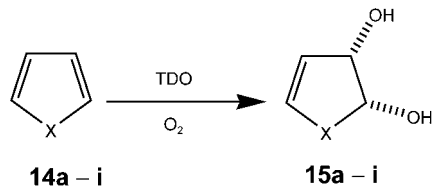
Benzo-fused cyclic alkenes, e.g., **6a**–**c**, in the presence of TDO and NDO, had been known to give the corresponding 1,2-diols without arene *cis*-dihydroxylation (Scheme 2).Thus, as a logical extension of our earlier studies of the biotransformation of substituted indenes[23] and indanes,[31] the methyl-substituted derivatives of bicyclic alkenes **6a** (2-methyl-, 3-methyl- and 2,3-dimethylindenes) and **6b** (3-methyl- and 4-methyl-1,2-dihydronaphthalenes) were used as substrates with TDO and NDO. Unfortunately only one of the substrates, 2-methylindene **9**, which contains a cyclic trisubstituted alkene bond, was found to give the corresponding alkene 1,2-diol metabolite (using *P. putida* UV4, TDO, Scheme 3).

Asymmetric dihydroxylation of 2-methylindene **9** thus occurred to give the *cis*-diol **10** (26% yield, Scheme 3). The diol **10** was found to be enantiopure and of the (1*S*,2*R*) configuration, based on the ^{1}H NMR spectral analysis of the corresponding di-MTPA esters. The major bioproduct (57% yield) was identified as the racemic 2-methyl-1-indanone **12**, which was assumed to have been formed during the biotransformation or isolation/purification phases, *via* a facile isomerisation of the unstable benzylic alcohol metabolite **11** followed by racemisation of the indanone product **12**. This was confirmed by the isolation of 2-methylindenol **11** as an unstable minor metabolite (1% yield) which readily isomerised to ketone **12**. A repeat experiment again gave ketone **12** with a slight enrichment (7% ee) of the (2*R*) enantiomer consistent with a partial racemisation process. It was possible to form an MTPA ester of indenol **11** for determination of its enantiopurity (>98% ee). A (2*R*) configuration was assigned to indenol **11** by analysis of its CD spectrum. It is noteworthy that both the mono- and dihydroxylation products **11** and **10**, obtained using TDO, had identical absolute configurations to those obtained earlier (**8a**–**c** and **7a**–**c**) using the same biocatalyst and the corresponding unsubstituted benzocycloalkenes **6a**–**c** (Scheme 2).[10,12,13,18,19]



Scheme 3. TDO-and NDO-catalysed mono- and dihydroxylation of 2-methylindene **9**.

When the biotransformation of 2-methylindene **9** was repeated using *P putida* NCIMB 8859 (NDO) , benzylic hydroxylation was found to be dominant and (1*S*)-2-methylinden-1-ol **11** was isolated in good yield (67%, >98% ee) without isomerisation to ketone **12**. Isomerisation of the enantiopure compound, under mild basic conditions, yielded ketone **12** which was again found to have racemised spontaneously. Allylic hydroxylation of the methyl group was also observed to give 2-hydroxymethylindene **13** as a minor metabolite (5% yield). When considered with the earlier NDO metabolites **4** and **5**, bioproduct **13** provided a third example of NDO-catalysed allylic hydroxylation. In the absence of NDO-catalysed 1,2-dihydroxylation of either the alkene or arene groups in substrate **9**, it is evident that monohydroxylation is preferred at the cyclic benzylic/allylic (C-1) centre rather than at the acyclic allylic (Me) centre.



14a – i **15a – i**

a [X: CH_2], **b** [X: $(CH_2)_2$], **c** [X: $(CH_2)_3$], **d** [X: $(CH_2)_4$], **e** or **e'** [X: $CH{=}CHCH_2$ or $CH_2CH{=}CH$], **f** [X: $C{=}CMe_2$], **g** [X: $C{=}CEt_2$], **h** [X: $C{=}C(CH_2)_4$], **i** [X: $C{=}C(CH_2)_5$]

Scheme 4. TDO-catalysed 1,2-dihydroxylation of dienes **14a–i**.

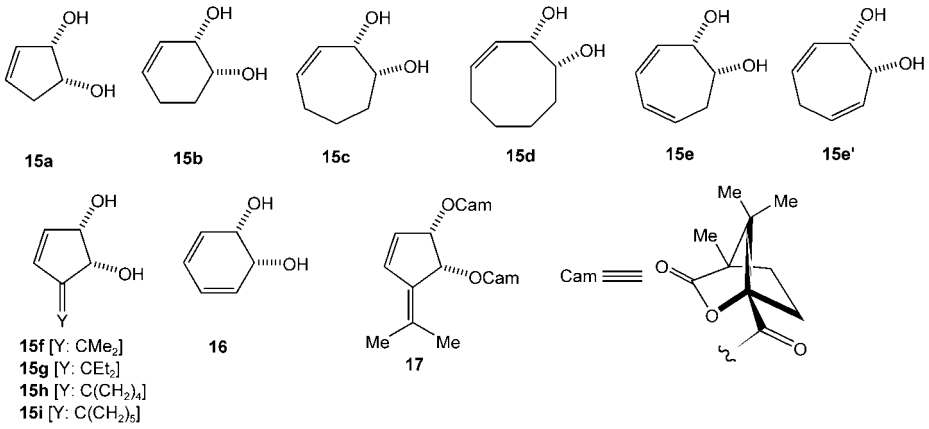
The last part of the study was focused on the biotransformation of four cyclic dienes **14a–d** and five trienes **14e–i**, using TDO and NDO as biocatalysts (Scheme 4, Table 2). Enantiomerically enriched samples (5–38% ee) of the alkene-1,2-diols **15a–f** had been obtained earlier from the corresponding dienes **14a–f**, using AD-mix-$[DHQD)_2$-PHAL].[26,32] The absolute configurations of *cis*-diols **15a–f** were tentatively assigned on the basis of the mnemonic device proposed by Sharpless.[26,32] In a preliminary report of the present work, using TDO as biocatalyst,[21] it was shown that several of these cyclic polyenes yielded the corresponding enantiopure *cis*-diols; the absolute configurations of these were based solely on the earlier tentative assignments.[32] As the preliminary results indicated a marked increase in the ee values of alkene 1,2-diols **14b, 14c**,**14f** and **14i**, obtained by the biocatalytic route over the chemical method,[32] both these and other polyene substrates were investigated further using TDO and NDO.

Cyclopentadiene **14a**, was biotransformed, by *P. putida* strains containing either TDO or NDO, to give the corresponding *cis*-diol metabolite **15a** with a relatively

Table 2. *cis*-Diol metabolites (**15a–i**) of dienes (**14a–d**) and trienes (**14e–i**).

Polyene	*cis*-Diol	Yield [%][a]	Config.[b]	Polyene	*cis*-Diol	Yield [%]	Config.[b]
14a	**15a**	32[c] (14)[d]	1*R*,2*S*	**14e**	**15e′**	15 (0)	
14b	**15b**	12[e] (8)	1*R*,2*S*	**14f**	**15f**	19 (7)	1*R*,2*S*
14c	**15c**	20 (24)	1*R*,2*S*	**14g**	**15g**	7	1*R*,2*S*
14d	**15d**	4 (4)	1*R*,2*S*	**14h**	**15h**	5	1*R*,2*S*
14e	**15e**	29 (24)	1*R*,2*S*	**14i**	**15i**	5	1*R*,2*S*

[a] Using TDO; (using NDO).
[b] >98% ee.
[c] 20% ee.
[d] 40% ee.
[e] *cis*-1,2-Dihydroxycyclohexa-3,5-diene also formed (10% yield).



low excess of the (1*R*,2*S*) enantiomer (20% and 40% ee, respectively). The other monocyclic diene substrates **14b**–**d** similarly yielded the corresponding *cis*-diols **15b**–**d**, but these were found to be enantiopure, based on ^{1}H NMR spectral analyses of their diastereoisomeric MEPBA boronates and di-MTPA esters. The latter derivatives provided two independent methods for confirming the (1*R*,2*S*) configuration tentatively assigned earlier.[32]

In addition to the expected *cis*-1,2-dihydrodiol **15b** (12% yield), TDO-catalysed oxidation of 1,3-cyclohexadiene **14b** also gave *cis*-1,2-dihydroxycyclohexa-3,5-diene **16** (10% yield). Diol **16** resulted from a TDO-catalysed desaturation of 1,3-cyclohexadiene **14b** to yield benzene followed by its *cis*-dihydroxylation. An earlier precedent for this TDO-catalysed desaturation/*cis*-dihydroxylation sequence was the formation of *cis*-1,2-dihydroxy-1,2-dihydronaphthalene from 1,2-dihydronaphthalene.[18]

The ability of the TDO system to stereodifferentiate between different alkene faces and groups was evaluated with the help of the triene substrates **14e**–**i**. Biotransformation of cycloheptatriene **14e** with *P. putida* UV4 resulted in TDO-catalysed dihydroxylation at two different *cis*-disubstituted alkene bonds to yield an inseparable mixture of chiral diol **15e** (29% yield) and achiral diol **15e**′ (15% yield). The former chiral diol contained a conjugated diene group and reacted with 4-phenyl-1,2,4-triazoline-3,5-dione to yield a cycloadduct, while diol **15e**′ remained unaffected. Formation of di-MTPA ester diastereoisomers from the cycloadduct confirmed that diol **15e** was enantiopure (>98% ee). NDO-catalysed *cis*-dihydroxylation of cycloheptatriene **14e** using *P. putida* NCIMB 8859 gave the enantiopure chiral diene **15e** as the sole metabolite of identical (1*R*,2*S*) configuration to that isolated using TDO. The lack of NDO-catalysed allylic hydroxylation products from any of the cyclic diene substrates **14a**–**i** despite the availability of doubly activated allylic carbon atoms in two substrates (**14a** and **14e)**, indicated that this type of oxidation was particularly slow at cyclic allylic centres compared with 1,2-dihydroxylation of an alkene bond.

The fulvenes **14f**–**i**, containing both *cis*-disubstituted and tetrasubstituted alkene groups, presented the TDO enzyme with an opportunity to differentiate between alkene bond types and prochiral faces. The corresponding single enantiomer *cis*-dihydrodiols **15f**–**i** were formed exclusively (5–19% yield). Thus, the TDO enzyme showed a marked preference for dihydroxylation of a *cis*-disubstituted alkene over a tetrasubstituted alkene, in accord with expectations based on the earlier results obtained using indene and 2,3-dimethylindene substrates.

As the assignment of absolute configuration of the fulvene-*cis*-diol metabolite **15f**, derived from the Sharpless mnemonic device method, [26] was described as tentative,[32] an X-ray crystal structure analysis of the crystalline dicamphanate derivative **17** was used to provide an unequivocal determination of absolute configuration, relative to the *known* (1*S*) configuration of the camphanate groups (Figure 1). This analysis confirmed the (1*R*,2*S*) absolute configuration originally proposed[26] and thus provided an anchor structure for the other ful-

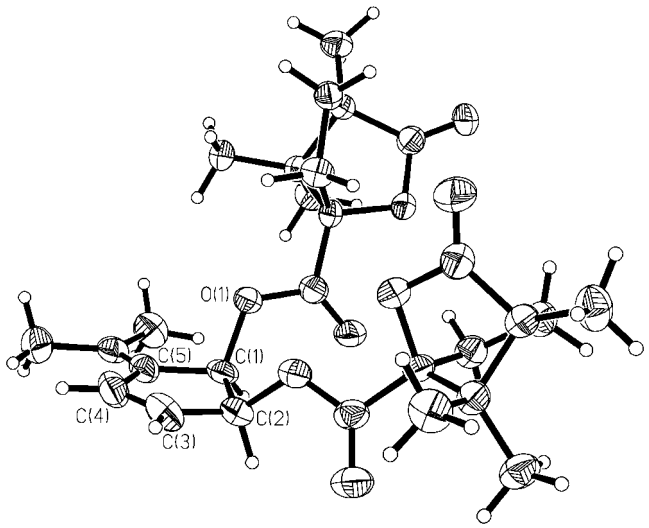


Figure 1. X-ray crystal structure of compound **17**.

venediols **15g–i**. A similar (1*R*, 2*S*) configuration was, therefore, assigned to the other fulvene-*cis*-diol metabolites **15g–i**, by comparison of their CD spectra ($\lambda =$ 205–210 nm [$\Delta\varepsilon$ – ve], 238–242 nm [$\Delta\varepsilon$ + ve]). Further evidence of identical absolute configurations and enantiopurity values (> 98% ee) for each of the fulvenediols **15f–i** was obtained from ^{1}H NMR analysis of the corresponding boronates using (*R*) and (*S*)-MEPBA.

The final phase of the study was linked to our current interest in using dioxygenase bioproducts for the synthesis of chiral ligands and auxiliaries. The (1*R*,2*S*)-diol metabolite **15f** of dimethylfulvene **14f** was employed as a precursor of the potentially useful chiral ketone **21** that was required in these and other laboratories (Scheme 5).[33] (1*R*,2*S*)-Diol metabolite **15f** was protected as the acetonide derivative **18** prior to subjecting it to the reaction sequence: osmylation (**18** → **19**), bis-acetonide formation (**19** → **20**) and ozonolysis (**20** → **21**). The chemical dihydroxylation of the *cis*-disubstituted alkene bond of acetonide **18** to yield diol **19**, in common with the TDO-catalysed dihydroxylation of the cyclic alkene bond in fulvene **14f** to yield diol **15f**, occurred without evidence of dihydroxylation of the corresponding tetrasubstituted alkene bond.

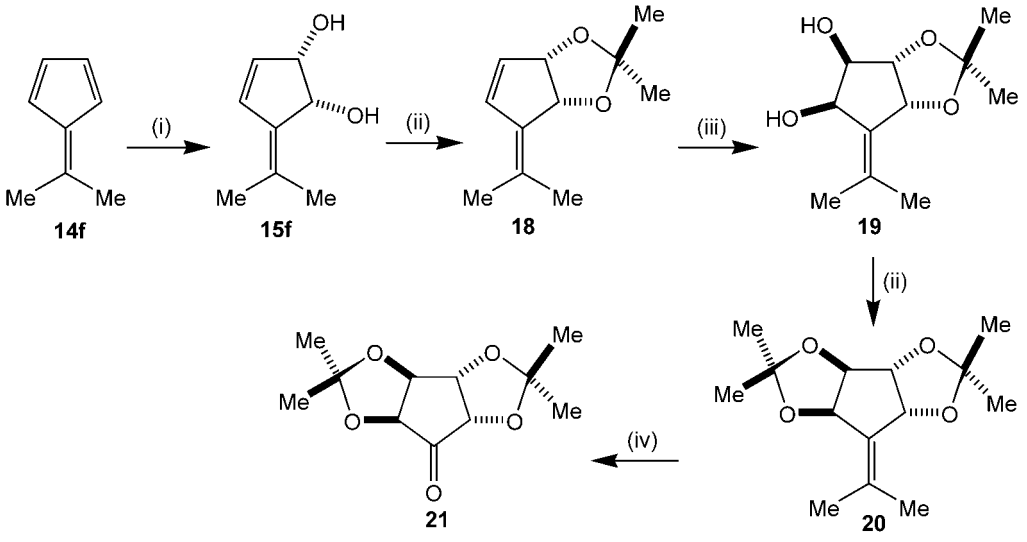
This chemoenzymatic approach to ketone **21** (Scheme 5) has advantages over the earlier chemical method [33] from *n*-heptylfulvene in terms of the higher stereoselectivity during the biocatalytic asymmetric dihydroxylation (> 98% ee compared with 78% ee using AD-mix) and regioselectivity for the *cis*-disubstituted alkene bond during the chemical dihydroxylation step (> 98% compared with 48%, at the cyclic disubstituted alkene bond).

Conclusion

Examples of dioxygenase-catalysed asymmetric 1,2-dihydroxylation of monosubstituted (**1a–d**; with TDO and NDO), *gem*-disubstituted (**1e**; with NDO), *cis*-disubstituted (**1f**, **14a–i**; with NDO) and trisubstituted alkenes (**9**; with TDO) have been found during the current study. Neither the *trans*-disubstituted alkene **1g** nor the tetrasubstituted alkene bonds in fulvenes **14f–i** were dihydroxylated using TDO or NDO. From an estimate of the product yields obtained, the relative ease of 1,2-dihydroxylation of the six alkene types, using BDO, TDO and NDO, appears to be in the order: mono-substituted alkene > *cis*-disubstituted alkene > *gem*-disubstituted alkene > trisubstituted alkene > *trans*-disubstituted > tetrasubstituted alkene.

Competing dioxygenase-catalysed arene dihydroxylations often occurred simultaneously with monosubstituted alkene 1,2-dihydroxylation, e.g., TDO-catalysed arene-*cis*-dihydroxylation was also observed with acyclic alkenes bearing an aryl substituent **1a–g**. With a cyclic trisubstituted alkene (e.g., **9**) competition from monohydroxylation (benzylic and allylic) was observed without any evidence of arene dihydroxylation. Allylic hydroxylation of both a *gem*-disubstituted alkene (e.g., **1e**) and a *trans*-disubstituted alkene (e.g., **1g**) was again observed using NDO. The TDO enzyme was assumed to be responsible for the competing tandem desaturation of 1,2-cyclohexadiene **14b** and *cis*-dihydroxylation of the resulting benzene intermediate to yield *cis*-dihydrodiol **16**.

As shown herein, dioxygenase selection has a marked effect on the proportion of alkene 1,2-dihydroxylation relative to alternative oxidation pathways. The locations of substituents on the alkene substrate also exert a



Scheme 5. Chemoenzymatic synthesis of chiral ketone **21** from fulvene **14f**.

strong influence on the regioselectivity. Thus, the proportion of TDO-catalysed alkene dihydroxylation, relative to arene dihydroxylation of styrene substrates, was generally found to be higher when a *meta*-substituent was present on the arene ring (e.g., **1b–d**) and lower when the alkene had a methyl substitutent (e.g., **1e–g**).

The synthetic potential of the dioxygenase-catalysed asymmetric dihydroxylation of alkenes depends upon the availability of 1,2-diol bioproducts in enantiopure form and of known absolute configuration. While the acyclic alkene substrates **1a–g** yielded enantiopure arene-*cis*-dihydrodiols **2a–g**, of identical configuration (>98% ee, 1*S*,2*R*), the corresponding acyclic alkene 1,2-diols **3a–f** had lower ee values and similar configurations (42–88%, 1*R*) using both TDO and NDO. Based on the results obtained with acyclic alkenes, the dioxygenase-catalysed asymmetric dihydroxylation route does not appear to have any significant advantages over the chemical method.[26] However, with the notable exception of cyclopentadiene **14a**, the other cyclic *cis*-disubstituted alkenes (**9** and **14b–i**), were all found to undergo TDO-catalysed asymmetric 1,2-dihydroxylation to give *cis*-diols **10** and **15b–i** as single enantiomers having similar (*S*)-configurations at the benzylic and allylic centres.

The successful biocatalytic formation of enantiopure *cis*-diols (**7b**, **7c**, **10** and **15b–i**), from the corresponding cyclic *cis*-disubstituted alkenes, provides an attractive alternative route to the reported chemical asymmetric dihydroxylation method where ee values obtained were much lower. Initial studies to realise the synthetic potential of these enantiopure 1,2-diols include the use of *cis*-diol **15f** as a precursor of enantiopure ketone **21**.

Experimental Section

Characterisation data for all bioproducts is available in the Supporting Information.

Wild-type strains of *P. putida* (NCIB 11767 and NCIMB 8859) used in this work were obtained from the National Collections of Industrial and Marine Bacteria, 23 St. Machar Drive, Aberdeen, Scotland, UK. The UV4 mutant strain of *P. putida* was obtained from Dr. S. C. Taylor, Avecia Pharmaceuticals, Billingham, Cleveland, UK. The UV4 mutant strain, containing TDO, was derived from the NCIB 11767 wild-type strain using the methods described in the following patents: EP 76606, EP 253,485 and US 5,073,640 and in the literature.[42] The mutant strain *P. putida* 39/D (ATCC 700008) also contains TDO and was generally found to give comparable results for a wide range of substrates in these and other laboratories.[8] Comparative experimental details for the biotransformations of arenes using TDO, NDO and other dioxygenases with a range of *P. putida* strains have recently been reported.[8]

Typical Small-Scale Biotransformation Procedures using *P. putida* NCIMB 8859 and *P. putida* UV4

The *P. putida* NCIMB 8859 wild-type inducible strain was grown on naphthalene (10 g L^{-1}) as sole carbon source and inducer. Small-scale biotransformations were carried out using whole cells in the late exponential phase of growth. The excess naphthalene was filtered off, the filtrate was centrifuged and the collected cells were resuspended in potassium phosphate buffer (50 mM) at pH 7.2. Sodium pyruvate (0.5%) was added as co-substrate with the substrates (0.2–1.0 g L^{-1}) which were added in a minimum volume of ethanol to 500-mL Erlenmeyer flasks. These were in turn incubated at 30 °C on an orbital shaker (200 rpm) for 24 h. The contents of the flasks were centrifuged (9000 rpm, 10 min). The aqueous supernatant was decanted off and the bioproducts harvested by concentration under reduced pressure and solvent extraction (EtOAc) of the sodium chloride-saturated solution.

The *P. putida* UV 4 constitutive mutant strain required that the most TDO-active colonies (determined by the indole test)[43] be selected before use since reversion to the wild-type state occurred readily. The whole cells were again grown in Erlenmeyer flasks containing minimal salts growth medium (100 mL) and sodium pyruvate (0.5%) and substrates added to cell suspensions in phosphate buffer. The biotransformation (24 h) and isolation of bioproducts (concentration and EtOAc extraction) was as described for the *P. putida* NCIMB 8859 strain.

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