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# Stereoselective Dioxygenase-Catalysed Benzylic Hydroxylation at Prochiral Methylene Groups in the Chemoenzymatic Synthesis of Enantiopure Vicinal Aminoindanols.

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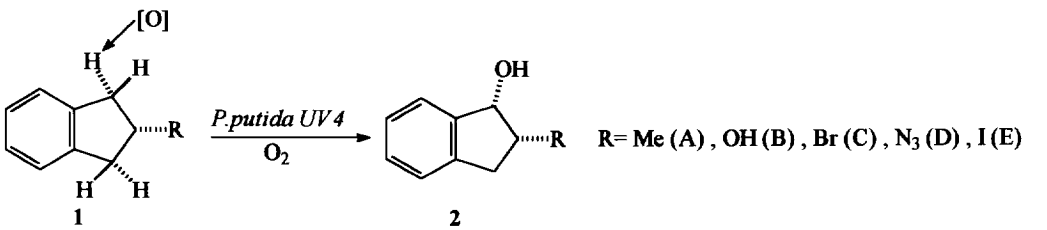
**Abstract**: Enantiopure benzylic alcohols containing two stereogenic centres in a *cis*-relationship result from stereoselective monohydroxylation of achiral 2-substituted indans in cultures of *Pseudomonas putida* UV4 and are used in the chemoenzymatic synthesis of both *cis*- and *trans*-aminoindanol enantiomers. Copyright © 1996 Elsevier Science Ltd

Previous studies have shown that dioxygenase enzymes present in the soil bacterium *Pseudomonas putida* can catalyse benzylic monohydroxylation[1-6] involving stereoselective replacement of one prochiral hydrogen atom and creation of a new stereogenic centre in bioproducts of variable enantiopurity. The results now presented differ from the earlier reports[1-6] since they demonstrate an exclusive stereopreference for one prochiral hydrogen atom *and* one prochiral methylene group during dioxygenase-catalysed benzylic monohydroxylation with the concomitant creation of two new chiral centres. This phenomenon has been observed during oxidation of the series of achiral 2-substituted indan substrates **1A-1D** to yield enantiopure *cis*-2-methyl-1-indanol **2A**, *cis*-1,2-dihydroxyindan **2B**, *cis*-2-bromo-1-indanol **2C**, and *cis*-2-azido-1-indanol **2D** respectively.

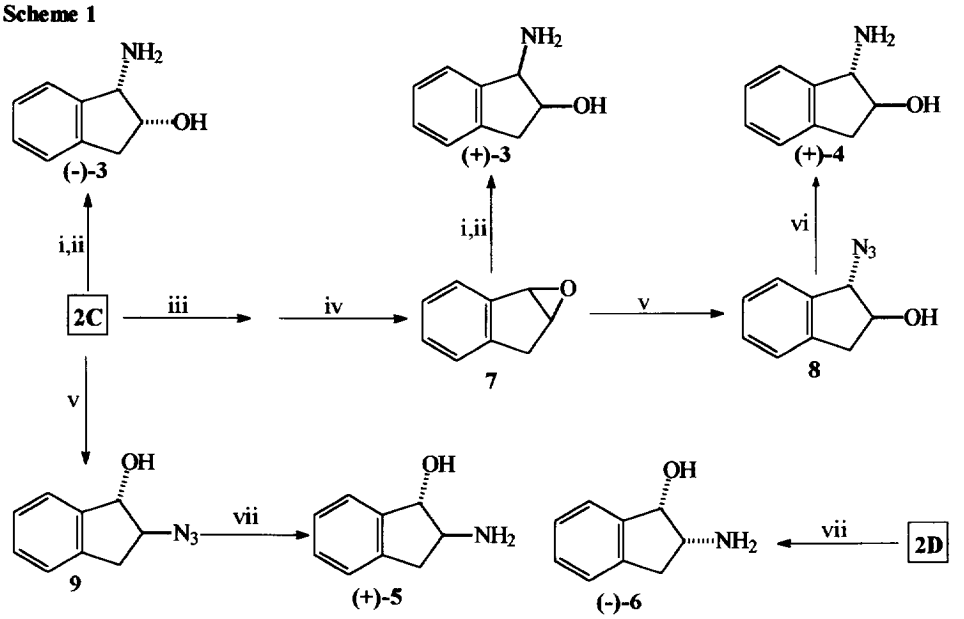



**1** **2**

Addition of 2-methyindan **1A** as substrate to growing shake flask cultures of *P.putida* UV4, using standard conditions previously reported,[2,6] gave [1R,2R]-2-methyl-1-indanol (**2A**, 21% isolated yield, $\alpha]_D^{25}$ -38, $CHCl_3$, >98% e.e. by MTPA ester formation; lit.[7] $\alpha]_D^{25}$ +30, $CHCl_3$). Addition of 2-indanol **1B**, 2-bromoindan **1C**, and 2-azidoindan **1D** as substrates under standard biotransformation conditions in each case yielded the corresponding enantiopure 1-indanols *i.e.* [1S,2R]-**2B** (50% isolated yield, $[\alpha]_D^{25}$ -48, $CHCl_3$,

>98% e.e. by MTPA ester formation; lit.[8] $[\alpha]_D^{25}$ -51, $CHCl_3$), [1S,2R]-**2C** (35% isolated yield, $[\alpha]_D^{25}$ -61, $CHCl_3$, >98% e.e. by MTPA ester formation), [1S,2R]-**2D** ( 70 % isolated yield, $[\alpha]_D^{25}$ -111, $CHCl_3$, >98% e.e.; lit.[9] $[\alpha]_D^{25}$ -111, $CHCl_3$ ). The absolute configurations of enantiopure samples of compounds **2A, 2B,** and **2D** have been reported.[7-9] The [1S,2R] absolute configuration assigned to metabolite (-)-**2C** was based on the stereochemical correlation sequence shown in **Scheme 1**. The substituted 1-indanol metabolites **2A-2D** had the same absolute configuration at C-1 (shown in structure **2**) as that found in the parent 1-indanol derived by toluene dioxygenase (TDO)-.catalysed hydroxylation of indan [2]

**Scheme 1**




Reagents ( yield ): i $CH_3CN$ / $H_2SO_4$ (70-85%); ii KOH (90%); iii $MeSO_2Cl$ ; iv KOH (80%); v $NaN_3$(70%); vi $LiAlH_4$ (60%) ; vii $H_2$/Pd/C (80%);

Evidence that the monohydroxylation reactions were catalysed by a TDO enzyme was obtained with a recombinant *E.coli* strain expressing the toluene dioxygenase gene from *P.putida* NCIMB 11767 on plasmid pKST11.[10] The monol **2A,** enantiopurity *ca.* 91% e.e., of identical absolute configuration and comparable isolated yield (40%) to that obtained using *P.putida* UV4, was again obtained from biotransformation of substrate **1A** using the *E.coli* pKST11 clone. When the parent *E.coli* strain was used as a control, the bioproduct **2A** was not detected. The mechanism of the TDO-catalysed oxidation is presently unknown, however, the structures of the metabolites obtained when 2-iodoindan **1E** was used as substrate with *P.putida* UV4, were consistent with involvement of an initially formed benzylic radical. Thus, compound **1E** was assumed to have been biotransformed, *via* indene as intermediate, to yield 1R-indenol (8% isolated yield , $[\alpha]_D^{25}$ -249, $CHCl_3$, >98% e.e.) and *cis*-1S,2R- dihydroxyindan ( **2B,** $[\alpha]_D^{25}$ -11, $CHCl_3$, 20% isolated yield , *ca.*20% e.e.) of identical configuration and enantiopurity to those found as metabolites of indene using *P.putida* UV4.[11] Since the C-I bond in **1E** is weaker than any of the C-R bonds in substrates **1A-1D,**

homolytic cleavage of the C-I bond β to a radical centre will be extremely rapid.[12] It is probable that in compound **1E** this homolysis occurs preferentially to yield indene prior to hydroxylation occurring (only traces of the hydroxylation product **2E** were found). A similar mechanism involving a carbon centred benzylic radical has recently been postulated for the benzylic hydroxylation of 1,2- and 1,4-dihydronaphthalenes.[5,6] Preliminary studies from these laboratories, using specifically labelled precursors, have also shown that the TDO-catalysed benzylic oxidation process occurs with total retention of configuration.[13]

This enzyme-catalysed synthetic approach to *cis*-diol **2B** *via* benzylic monohydroxylation of 2-indanol **1B**, resulted in high enantiopurity ( >98% e.e.) and improved yield (50%) compared with the TDO-catalysed *cis*-dihydroxylation of indene in *P.putida*.[1,2,14] Samples of *cis*-diol **2B** of high enantiopurity (≥ 90% e.e.) have been obtained *via* a naphthalene dioxygenase-catalysed (NDO) asymmetric *cis*-dihydroxylation of indene or NDO-catalysed kinetic resolution of racemic *cis*-1,2-dihydroxyindan using *P.Putida* NCIMB 8859.[14] The ready availability of single enantiomers of the novel bioproducts *cis*-bromohydrin **2C**, and *cis*-hydroxyazide **2D** from the benzylic hydroxylation process prompted studies of their application in asymmetric synthesis.

Considerable interest has recently been shown in the synthesis[15-17] and use of enantiopure *cis*-1-amino-2-hydroxyindan **3** and isomers as chiral catalysts in reductions,[18,19] in alkyl zinc addition reactions,[20] as chiral ligands in Diels-Alder cycloadditions,[20] and as chiral intermediates in the synthesis of HIV inhibitors.[21] The application of enantiopure 1-indanol metabolites **2C** and **2D** to the synthesis of *cis*-[1S,2R]- and *cis*-[1R,2S]-1-amino-2-hydroxyindan **3**, *trans*-[1S,2S]-1-amino-2-hydroxyindan **4**, *trans*-[1S,2S]-2-amino-1-hydroxyindan **5** and *cis*-[1S,2R]-2-amino-1-hydroxyindan **6** is shown in **Scheme 1**.

Using Ritter reaction conditions similar to those reported,[15] for the conversion of epoxide **7** to the *cis*-aminoindanol **3**, the enantiopure sample of *cis*-bromohydrin **2C** was converted to *cis*-[1S,2R]-1-amino-2-hydroxyindan **3** ($[\alpha]_D^{25}$ -65, $CHCl_3$; lit.[22] $[\alpha]_D^{25}$ -65, $CHCl_3$ ). Similarly the derived [1R,2S]-epoxide **7** ($[\alpha]_D^{25}$ -55, $CHCl_3$; lit.[8] $[\alpha]_D^{25}$ -55, $CHCl_3$), available *via* synthesis and inversion of configuration at C-1 of the *cis*-bromomesylate intermediate, was converted into enantiomerically pure *cis*-[1R,2S]-1-amino-2-hydroxyindan **3** ($[\alpha]_D^{25}$ +65, $CHCl_3$). Nucleophilic attack of azide at the benzylic C-1 position of the [1R,2S]-epoxide **7** yielded the *trans*-1-azido-2-hydroxyindan **8** ($[\alpha]_D^{25}$ +75, $CHCl_3$ ). The enantiopurity (>98%e.e.) and absolute configuration [1S,2S] of the latter compound was assumed to follow from the precursor [1R,2S]-epoxide **7** and was sterechemically correlated with the derived *trans*-[1S,2S]-1-amino-2-hydroxyindan **4** ($[\alpha]_D^{25}$ +22, $CHCl_3$) following $LiAlH_4$ reduction. Nucleophilic substitution of the bromine atom in the [1S,2R]-*cis*-bromohydrin **2C** using sodium azide in DMF gave [1S,2S]-*trans*-1-hydroxy-2-azidoindan **9** ($[\alpha]_D^{25}$ +32, $CHCl_3$; lit.[9] $[\alpha]_D^{25}$ +32, $CHCl_3$). Catalytic hydrogenolysis of the hydroxyazides **9** and **2D** yielded *trans*-[1S,2S]-2-amino-1-hydroxyindan **5** ($[\alpha]_D^{25}$ +16, $CHCl_3$; lit.[9] $[\alpha]_D^{25}$ +13, $CHCl_3$) and *cis*-[1S,2R]-2-amino-1-hydroxyindan **6** ($[\alpha]_D^{25}$ -63, $CHCl_3$; lit.[9] $[\alpha]_D^{25}$ -61, $CHCl_3$) respectively. The aminoindanol enantiomers **(+)-3** and **(-)-3**, **(+)-5** and **(-)-6** derived from enantiopure metabolites were found to have similar $[\alpha]_D^{25}$ values to those previously synthesised by alternative methods, and are assumed to have the reported [9,22] absolute configurations. In conclusion, stereoselective TDO-catalysed benzylic hydroxylation of 2-substituted indans allied to chemical synthesis has provided a new chemoenzymatic route to the corresponding *cis*-1-

hydroxyindan enantiomers which have been utilized in the synthesis of a range of enantiopure *vicinal* aminoalcohols.

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