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Enantiocomplementary preparation of (*S*)- and (*R*)-1-pyridylalkanols via ketone reduction and alkane hydroxylation using whole cells of *Pseudomonas putida* UV4

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Abstract—A previously unreported alcohol dehydrogenase enzyme in the mutant soil bacterium *Pseudomonas putida* UV4 catalyses the reduction of 2-, 3- and 4-acylpyridines to afford the corresponding (*S*)-1-pyridyl alkanols, with moderate to high e.e., whilst under the same conditions 2,6-diacetylpyridine is readily converted to the corresponding enantiopure C_2-symmetric (*S*,*S*)-diol in one step. In contrast, the toluene dioxygenase enzyme in the same organism catalyses the hydroxylation of 2- and 3-alkylpyridines to (*R*)-1-(2-pyridyl) and (*R*)-1-(3-pyridyl)alkanols. This combination of oxidative and reductive biotransformations thus provides a method for preparing both enantiomers of chiral 1-pyridyl alkanols using one biocatalyst.

In recent years, bacterial dioxygenase enzymes have been used to produce a wide range of arene *cis*-dihydrodiols[1,2] many of which have been used as chiral raw materials for asymmetric synthesis.[2–5] More recently, some of the other oxidative reactions catalysed by dioxygenase enzymes have been investigated and exploited synthetically.[6–8] These include sulfoxidations, alkene dihydroxylations, dehydrogenations, *N*-, *O*- and *S*-dealkylations and benzylic hydroxylations. Part of our continuing research into dioxygenase-catalysed oxidations has been aimed at understanding the criteria that influence the oxidation pathway observed in situations where there is potential competition between reaction sites in the substrate. In one approach towards this end we investigated the biotransformation of alkyl pyridines with *Pseudomonas putida* UV4, a mutant strain of a soil bacterium containing a toluene dioxygenase (TDO) enzyme but lacking the diol dehydrogenase enzyme which catalyses the next step in normal bacterial arene degradation. It has already been observed that carbocyclic arenes are more readily oxidised by dioxygenase enzymes than nitrogen-containing heterocycles[1] and we anticipated that alkyl pyridines would be preferentially hydroxylated at the side chain rather than on the ring.

In the course of these investigations we have discovered a new alcohol dehydrogenase enzyme (ADH) in *P. putida* UV4 which catalyses the asymmetric reduction of acyl pyridine substrates. The asymmetric reduction of ketones to chiral secondary alcohols is one of the cornerstones of biotransformations in synthetic organic chemistry but the vast majority of alcohol dehydrogenases (ADHs) employed to produce synthetically useful quantities of chiral alcohols are to be found in yeasts and mammalian systems. While many bacterial ADHs have been characterised and investigated mechanistically,[9] relatively few have been used in biotransformations for chemical synthesis. There has been considerable recent interest in the synthetic applications of chiral 1-pyridylethanols. Examples include chiral ligands in the zinc-catalysed asymmetric alkylation of aldehydes[10] and involvement in asymmetric hydroboration reactions.[11] Generally these alcohols are prepared by enzymatic or non-enzymatic asymmetric reductions of the corresponding ketones or by lipase-catalysed kinetic resolution of racemic esters.[12] Such enzymatic reductions, e.g. using bakers' yeast, generally allow the preparation of only one enantiomer of the alcohol, and similarly, many kinetic resolution processes are also found to be more efficient for the production of one enantiomer.

The starting point for this study was the attempted use of a heterocyclic ring (pyridine) to disfavour arene oxidation and promote the hydroxylation of alkyl side

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chain substituents. The biotransformation of 2-ethylpyridine **1a** resulted in the production of the anticipated (*R*)-1-(2-pyridyl)ethanol, (*R*)-**2a**, as the major product. The unoptimised isolated yield of the alcohol was only moderate (29%) and the enantiopurity of the product was good (e.e. = 83%) but not quantitative. Detailed HPLC analysis of the products from the biotransformation of **1a** indicated that small amounts of other metabolites were present which were determined by NMR and mass spectroscopy to be ring hydroxylated products and the ketone **3a** (Scheme 1). Five other alkyl pyridines (**1b**, **4a**, **4b**, **6a** and **6b**) were also biotransformed to give products with variable yields and enantiopurities (Table 1). The 4-alkylpyridine substrates **6a** and **6b** produced only the ring-hydroxylated products **7a** and **7b**.

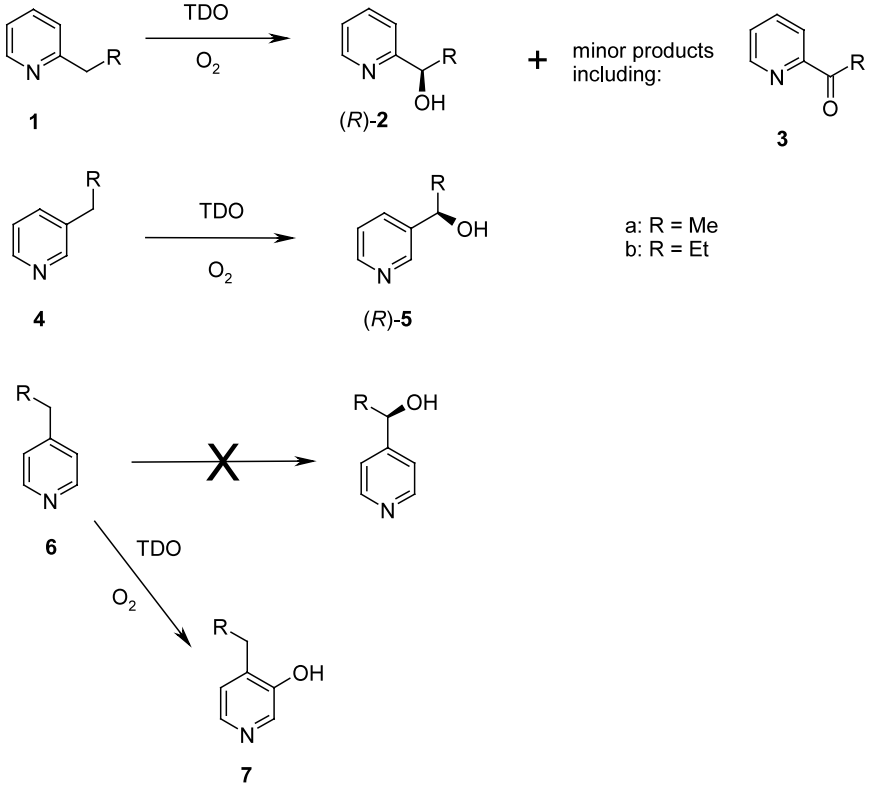
When ketone **3a** was fed as a substrate to the organism there was rapid biotransformation to the alcohol (*S*)-**2a** with the opposite configuration to that obtained on oxidation of 2-ethylpyridine. To our knowledge, the enzyme responsible for these reductions (presumably an alcohol dehydrogenase) has not been reported previously and some exploratory studies into substrate range for this enzyme were carried out. Biotransformation of a small series of acylpyridines (**3b**, **8a**, **8b**, **9a** and **9b**, Scheme 2) gave modest isolated yields but good enantioselectivities in most cases (Table 2) but acetophenone and simple, ring-substituted analogues of acetophenone were not reduced. However, 2,6-diacetylpyridine **11** proved to be an excellent substrate and was reduced rapidly to the C_2-symmetric *S*,*S*-diol **12** with no other detectable stereoisomers. This diol has been obtained by yeast reduction of the diketone **11**[13,14] but the process requires a two-stage biotransformation for optimum conversion and enantioselectivity. With *P. putida* UV4 as biocatalyst, the intermediate keto alcohol may be intercepted during the biotransformation but only as a mixture with substrate and the diol product.

The stereoselectivities of these ketone reductions using *P. putida* UV4 vary, and bioproduct enantiomeric excesses range from 71% (for (*S*)-1-(3-pyridyl)ethanol, (*S*)-**5a**) to >99% (for (*S*)-1-(4-pyridyl)propanol, (*S*)-**10a** and the *S*,*S*-diol **12**). All of the chiral alcohols reported here have been prepared previously as single enantiomers and the absolute configurations of these bioproducts were determined by comparison of the stereochemical data with literature values. The enantiomeric excesses of the products (Table 1) were determined by chiral stationary phase (CSP) HPLC analysis

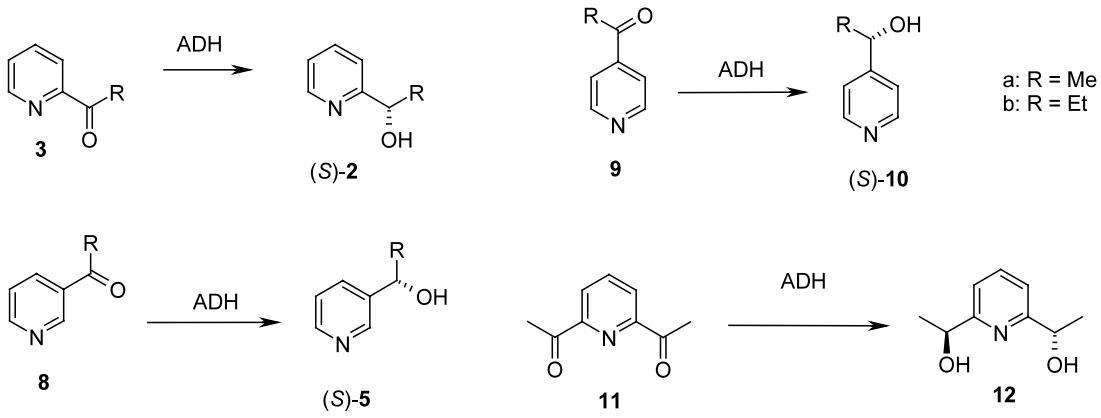
Table 1. Oxidation of alkylpyridines with *P. putida* UV4

Substrate	Product	Yield (%)	e.e. (%)
1a	(*R*)-**2a**[a]	29	83
	3a	5	–
1b	(*R*)-**2b**[a]	28	>99
4a	(*R*)-**5a**	30	90
4b	(*R*)-**5b**	70	>99
6a	**7a**	15	–
6b	**7b**	17	–

[a] Ca. 10% ring hydroxylation products also isolated.



Scheme 1. Oxidation of alkyl pyridines by *P. putida* UV4.



Scheme 2. Reduction of acyl pyridines by *P. putida* UV4.

Table 2. Reduction of acylpyridines with *P. putida* UV4

Substrate	Product	Yield (%)	e.e. (%)
3a	(*S*)-**2a**	45	95
3b	(*S*)-**2b**	51	95
8a	(*S*)-**5a**	51	71
8b	(*S*)-**5b**	19	79
9a	(*S*)-**10a**	83	>99
9b	(*S*)-**10b**	86	98
11	(*S*,*S*)-**12**	23	>99

and confirmed by ^{1}H NMR spectroscopic analysis of diastereoisomeric MTPA ester derivatives. It is not clear at this stage whether the variation in product enantiopurity is due to an inherent lack of selectivity of the enzyme or whether there is competition from other reductase enzymes of opposite stereopreference in the organism. The chemical yields of the reduction reactions have not been optimised, but complete conversion is observed for the better substrates (e.g. **9a** and **9b**) at reaction concentrations up to 0.5% w/v using a single addition of glucose, gluconate or pyruvate as the carbon source, and cells in the resting phase.

The identification of the ketone **3a** as a transient metabolite during the biotransformation of 2-ethylpyridine suggested that there was oxidation of the alcohol metabolite (*R*)-**2a**. The subsequent reduction of the ketone to the opposite enantiomer of the alcohol (*S*)-**2a** would also provide an explanation for the observed mixture of enantiomers of **2a** in the isolated product. The conversion of the *R*-alcohol into the *S*-alcohol must follow an equilibrium pathway similar to that outlined in Scheme 3. The oxidation of the *R*-alcohol to the ketone **3a** must be a relatively slow process (compared with the initial oxidation) for the observed enantiomeric excess of the *R*-alcohol to be as high as 83% after 24 hours contact with the biocatalyst. The enantioselectivity of the alcohol dehydrogenase enzyme in acyl pyridine reductions is extremely high (>95% e.e.) when the acyl group is in the 2- or 4-position on the pyridine ring. The enantioselectivity is lower for the reduction of the 3-acyl pyridines **8a** and **8b** (70–80% e.e.) suggesting either a poorer fit in the active site or the concurrent operation of other reduction pathways.

The enantioselectivities of the dioxygenase-catalysed hydroxylations of the alkyl pyridines are very high except when 2-ethylpyridine was the substrate. This suggests a high level of enantioselectivity in the initial oxidation reaction and that conversion to the opposite enantiomer through the ketone intermediate (Scheme 3) is only significant for this one example. As with all dioxygenase-catalysed oxidations of substituted arenes, there is regioselective competition for the type of oxidation observed. For 4-alkylpyridines, ring hydroxylation predominates (probably via 2,3-*cis*-dihydroxylation followed by rapid dehydration to the 3-pyridinol) with no observable side-chain oxidation. For the 2-alkylpyridines the balance is closer, with a ratio of side-chain to ring oxidation of about 3:1. For the 3-alkylpyridines only side-chain hydroxylation is observed. This inhibition of ring oxidation is consistent with a general phenomenon observed in these laboratories in which arene rings with a *meta* substitution pattern are much poorer substrates for dioxygenase enzymes.

We have demonstrated that control of biotransformation conditions and choice of substrate enables a single biocatalyst to be used to prepare both enantiomers of a series of chiral 1-pyridyl alkanols by either alkane

Scheme 3. Potential for partial racemisation of 1-(2-pyridyl)ethanol via alcohol dehydrogenase redox pathway.

hydroxylation or ketone reduction. The enantioselectivities of both types of bioprocess are good to excellent and unoptimised yields are moderate to good. The *P. putida* UV4 organism is used regularly to carry out multi-gram and even multi-kilogram biotransformations and we anticipate that synthetically useful quantities of the bioproducts reported here will be accessible with minimal process development.

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