Microbial Deracemization of 1-Aryl and 1-Heteroaryl Secondary Alcohols

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Received May 18, 2001

Chiral secondary alcohols are widely used as synthetic intermediates, chiral auxiliaries, and analytical reagents and can be obtained by resolution or asymmetric reduction processes. The deracemization of racemic alcohols is an attractive approach since there is, in principle, no need to discard or recycle an unwanted enantiomer. Biocatalytic deracemization processes are an efficient method by which chiral secondary alcohols can be obtained.[1] The combination of transition metal-catalyzed racemization with an enantioselective lipase-catalyzed esterification has been reported by several groups.[2] Bäckvall has used a ruthenium catalyst, 4-chlorophenyl acetate, and *Candida antarctica* lipase (Novozyme 435) to deracemize secondary alcohols and obtained, in most cases, essentially enantiomerically pure (>99% ee) acetates in up to 88% yield.[3] Faber has used a lipase-mandelate racemase two-enzyme system to achieve deracemization of mandelic acid.[4] We and other groups have reported the deracemization of alcohols and diols using microbial systems containing redox enzymes.[5] Nakamura has reported the deracemization of 1-aryl ethanols with *Geotrichum Candidum* IFO 5767.[6] It is also possible to use two isolated dehydrogenase enzymes with matched selectivity to achieve similar transformations. More attractive is the use of an oxidase, which does not require the addition of nicotinamide cofactors, to catalyze the initial enantioselective oxidation in combination with a dehydrogenase for the second enantiocomplementary reduction leading to overall deracemization. In fact the second reduction step need not be selective and could be performed with a chemical reagent compatible with the oxidase enzyme as described by Faber.[7]

In connection with our search for enantioselective alcohol oxidase enzymes we surveyed the literature for microorganisms displaying high enantioselectivity for the oxidation of secondary alcohols. The bacterium *Sphingomonas paucimobilis* has been successfully used by Fogagnolo for the resolution of 1-heteroaryl and 1-aryl-2-propanols where the (*S*)-alcohol is oxidized to the ketone with good selectivity.[8] *Bacillus stereothermophilus* was used by the same group to enantioselectively oxidize aryl and heteroaryl ethanols with excellent selectivity.[9] On screening aryl and heteroaryl alcohols **1a**−**p** with *Sphingomonas paucimobilis* NCIMB 8195, we made the fortuitous discovery that this organism catalyzes the efficient deracemization of a wide range of this class of substrates leading to up to 90% yield of the (*R*)-alcohol (Scheme 1, Table 1). The corresponding ketones **2a**−**p** were formed at various levels during each of the biotransformations, indicating that stereoinversion of the (*S*)-alcohol proceeds by sequential oxidation and reduction. Substrates **1a**−**e**, with a steady increase in steric demand

Scheme 1. Secondary Alcohols To Undergo Deracemisation

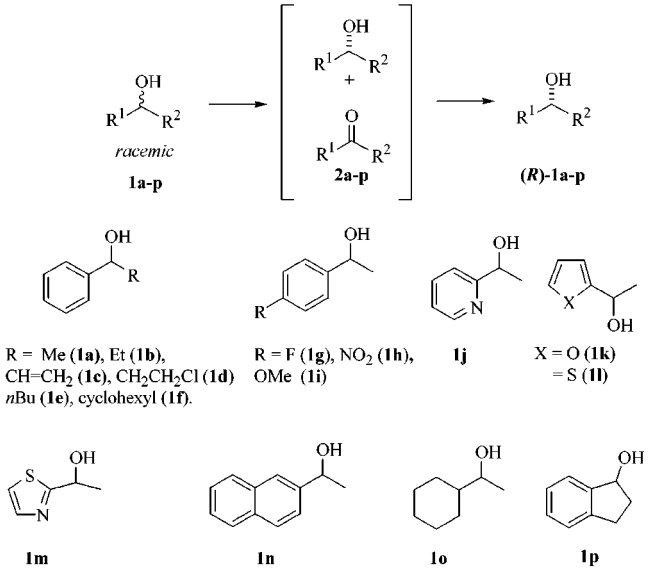

Table 1. Results of the Deracemization of Secondary Alcohols

(*R*/*S*) substrate[a]	R^1	R^2	time (days)	% ketone	% alcohol	% ee (*R*)[10]
1a	Ph	Me	6	16	84	96[9]
1b	Ph	Et	5	8	90	99[3]
1c	Ph	$CH{=}CH_2$	5	9	90	45[15]
1d[b]	Ph	CH_2CH_2Cl	5	21	79	99[11]
1e	Ph	*n*Bu	5	11	89	67[16]
1f[17]	Ph	cyclohexyl	5	0	100	0
1g	*p*-FC_6H_4	Me	5	21	79	99[18]
1h	*p*-$NO_2C_6H_4$	Me	5	17	81	89[19]
1i	*p*-$MeOC_6H_4$	Me	5	43	56	99[3]
1j[9]	2-pyridyl	Me	5	0	100	0
1k	C_4H_3O	Me	4	19	81	99[9]
1l	C_4H_3S	Me	4	21	78	99[9]
1m	C_3H_2NS	Me	5	14	85	97[9]
1n	$C_{10}H_7$	Me	5	15	85	99[3]
1o	C_6H_{11}	Me	4	27	73	98[3]
1p	indan-1ol	-	5	71	29	99[3]

[a] Reactions were performed using a substrate concentration of 1 mg/mL in the growth media (ref 13). [b] Substrate concentration 0.5 mg/mL. Yields and ee's were determined by HPLC using a Chiracel OB-H column using a quantitated method for determination of yield.

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10.1021/jo015770n CCC: $20.00
Published on Web 08/23/2001

Table 2. Results of the Deracemization of 1-Aryl Propan-2-ols

substrate[a]	R^1	R^2	% ketone	% alcohol	% ee alcohol	ee_{max} (E)
3a	C_6H_5	CH_3	40	60	82	66%
3b	C_4H_3S	CH_3	0	100	0	-
3c	C_4H_3O	CH_3	22	78	31	22%
3d	*m*-$CF_3C_6H_4$	CH_3	74	26	99	(9)

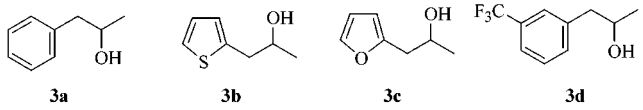
[a] Reactions were run over 5 days at substrate concentration of 0.5 mg/mL in growth media (ref 13).

of the R^2 group, show that deracemization of substrates with a saturated chain proceeds well after 5−6 days.[10]

The allylic alcohol **1c** undergoes a slower deracemization, perhaps reflecting the lower reactivity of the intermediate α,β-unsaturated ketone **2c**. The alcohol **1d** is of particular interest as an intermediate for the synthesis of Prozac.[11] This substrate was toxic to the cells at 1 mg/mL concentration so the biotransformation was carried out at half the normal concentration. Substrate **1e** contains the *n*Bu group which is obviously too sterically demanding for high selectivity. With two large cyclic groups flanking the carbinol center in **1f** there was no deracemization. Introduction of *para* substituents on the aromatic ring showed a marked difference in outcome for the substrate **1i**, possessing the electron-donating methoxy group, compared to the substrates **1g** and **1h** with electron-withdrawing substituents. Substrate **1i** gave a much higher level of residual ketone **2i**, perhaps a reflection of the higher reduction potential of this alcohol.[12]

In the heterocyclic series tested, the pyridyl alcohol **1j** was not transformed whereas the 2-furanyl, 2-thiophenyl and 2-thiazyl alcohols **1k**−**m** were deracemized almost equally efficiently. The naphthyl alcohol **1n** shows that larger aromatic groups can be accepted. Substrate **1o** was the only substrate tested with no aromatic ring meaning that the alcohol is significantly less activated. However this substrate was deracemized with reasonable efficiency, giving the alcohol with 98% e.e. The last susbtrate, indan-1-ol **1p** underwent kinetic resolution giving **1p** in 99% ee after 71% conversion ($E = 10$). Incubation of (*R*)-**1a** as the substrate led to no conversion to ketone. Incubation of pure (*S*)-**1a** resulted in a slower conversion compared to the racemic substrate giving 70% ee for (*R*)-**1a** and 16% residual ketone **2a** after 7 days.

Fogagnolo used an unspecified noncommercially available strain of *Pseudomonas paucimobilis* for the kinetic resolution of 1-aryl and 1-heteroaryl propan-2-ols.[8] Since we had used a strain listed as *Sphingomonas (Pseudomonas) paucimobilis* NCIMB 8195, we were conscious that we may well have used a related strain containing different enzymes so we tested a small range of 1-aryl propan-2-ols **3a**−**d**, including 1-*m*-trifluoromethylphenyl propan-2-ol **3d**, tested by Fogagnolo, to determine if they underwent kinetic resolution or deracemization (Table 2).



3a **3b** **3c** **3d**

Figure 1. 1-Aryl propan-2-ols studied.

Of the three substrates that reacted, substrates **3a** and **3c** reacted more slowly than the corresponding aryl ethanols **1a** and **1k** but still gave ee's higher than the theoretical maximum at the degrees of conversion shown. Substrate **3d** reacted at a faster rate although more slowly than with the *P. paucimobilis* strain used by Fogagnolo and with much lower selectivity (*E* value of 9 compared with around 200). These results suggest that the *Sphingomonas paucimobilis* NCIMB 8195 strain we have used is indeed a different strain to that used by Fogagnolo. Our strain gives efficient deracemization of eleven of the secondary aryl and heteroaryl ethanols tested and partial slow deracemization of two (hetero)-aryl propan-2-ols and resolution of another with low enantioselectivity. The deracemization of 1-phenyl ethanol **1a** was scaled up starting from 220 mg to provide 179 mg of the (*R*)-**1a** in 81% yield, 97% ee with 14% residual ketone **2a** after flash column chromatography. Similarly from 250 mg of thiophenyl ethanol **1l** we obtained the (*R*)-**1h** with a 99% ee in 70% isolated yield along with the ketone (24%).[13]

There is currently no evidence that *Sphingomonas paucimobilis* NCIMB 8195 contains an alcohol oxidase enzyme. This has been established from negative ABTS/horseradish peroxidase plate tests which give a purple coloration in response to hydrogen peroxide production by sugar and alcohol oxidases.[14] The likelihood is therefore that the deracemization is driven by dehydrogenase enzymes within the cells which may be compartmentalized and have different cofactor requirements (i.e., NADH and NADPH), thus providing the thermodynamic driving force necessary for deracemization to occur. If this is the case, then the reaction is unlikely to be viable in vitro since satisfying these conditions in the absence of the cell is likely to be difficult. We are therefore currently focusing our efforts on the identification and isolation of novel alcohol oxidases which can catalyze the irreversible oxidation of secondary alcohols and do not require nicotinamide cofactors. These enzymes will be coupled with compatible chemical reductants for deracemization reactions.

Experimental Section

Racemic alcohols **1a** and **1n** were purchased from Aldrich Chemical Co. All other 2-substituted ethanols and alcohol **3d** were synthesized by sodium borohydride reduction of the commercially available ketones and spectroscopic data were consistent with those reported in the literature.

General Procedure for the Synthesis of 1-Aryl-2-propanols 3a−c. To a stirred solution of aryl bromide (20 mmol) in dry THF (100 mL) at −30 °C was added *n*-butyllithium (22 mmol). The solution was stirred for 30 min at −30 °C whereupon

(10) The absolute configurations of alcohols **1a, 1b**, and **1l** were proved by chiral GC (Lipodex A) correlation with standards prepared by resolution with Lipase SAM-2, Laumen, K.; Schneider, M. P. *J. Chem. Soc., Chem Commun.* **1988**, 598. The absolute configuration of alcohols **1e**, **1i**, **1n**, **1o**, and **1p** were proved by chiral HPLC (Chiralcel OB-H) correlation with standards prepared by resolution with Novozyme 435 and vinyl acetate as reported by Bäckvall and co-workers (ref 3).

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(13) *Sphingomonas paucimobilis* was obtained from the National Collections of Industrial and Marine Bacteria Ltd. (NCIMB), Aberdeen, Scotland, and was maintained on malt extract agar slopes. *Sphingomonas paucimobilis* was grown in liquid culture on a nutrient media composed of glucose (20 g/L), yeast extract (5 g/L), malt extract (5 g/L), nutrient broth (10 g/L), and K_2HPO_4 (0.4 g/L). All media components were purchased from Sigma-Aldrich Co. Ltd.

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propylene oxide (20 mmol) was added and the solution allowed to warm to room temperature and stirred for a further 1 h. The reaction was quenched by the addition of saturated ammonium chloride (50 mL) and diethyl ether (100 mL) added. The organic extract was washed with water and dried ($MgSO_4$) and the solvent removed under reduced pressure to yield the crude products which were purified by flash column chromatography on silica (eluant petroleum ether:ethyl acetate 2:1) to yield the pure 1-aryl propan-2-ols **3a**−**c**.

1-Phenyl propan-2-ol (3a) was isolated as a colorless oil: yield 68%. Spectral data was consistent with that reported previously.[20]

1-Thienyl propan-2-ol (3b) was isolated as a colorless oil: yield 61%. Spectral data was consistent with that reported previously.[21]

1-Furyl propan-2-ol (3c) was isolated as a pale yellow oil: yield 77%. Spectral data was consistent with that reported previously.[22]

Representative Biotransformation Procedure. Bacteria were grown in 250 mL of sterile liquid media,[13] after inoculation with an aseptic loop (30 μL), for 2 days at 32 °C with vigorous aeration (200 rpm, New Brunswick orbital incubator). The alcohol **1l** (250 mg) was added in DMF (1 mL) and the reaction shaken for 4 days. The cells were removed by centrifugation (3000 rpm for 5 min), and the supernatant was extracted with ethyl acetate (3 × 200 mL) and dried over magnesium sulfate to afford, after purification by flash column chromatography (ethyl acetate:petroleum ether; 2:8), the ketone **2l** (60 mg, 24%) and the (*R*)-alcohol **1l** (175 mg, 70%); $[\alpha]^{25}_D = +25.0$ (*c* 5.0 $CHCl_3$) (lit.[9] +24.3 *c* 5.0 $CHCl_3$); ee > 99% by chiral HPLC (Chiralcel OB-H).

Acknowledgment. We thank the BBSRC for a PDRA to G.R.A.

JO015770N

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