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Microbial Oxidation with *Bacillus stearothermophilus* : High Enantioselective Resolution of 1-Heteroaryl and 1-Aryl Alcohols

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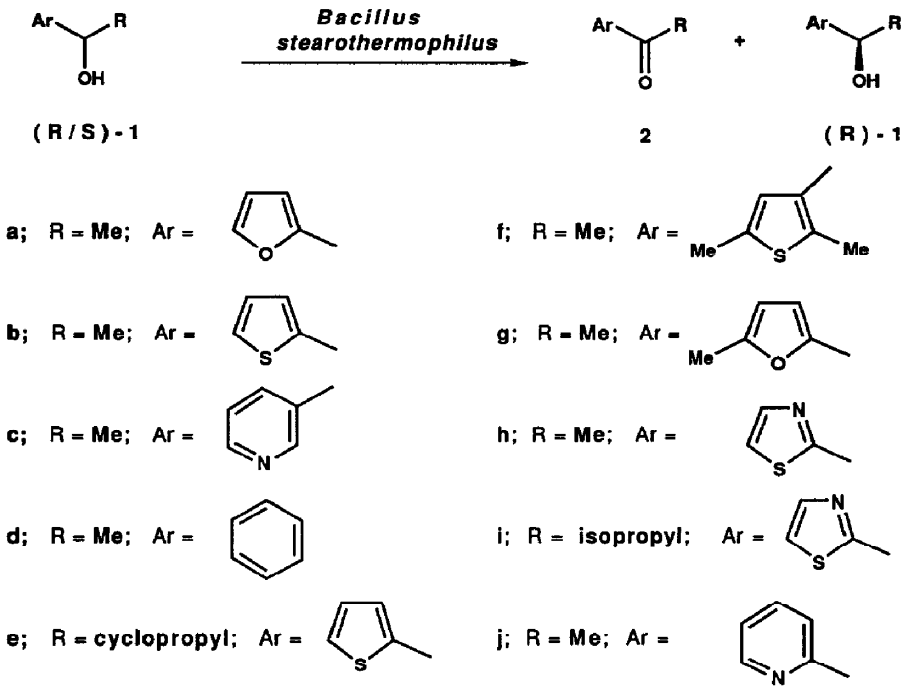
Abstract: The enantioselective resolution of the racemic 1-heteroaryl- and 1-arylethanols **1a-h** *via* microbial oxidation with *Bacillus stearothermophilus* to give the *R*-enantiomers with high enantiomeric excesses is described. The kinetic resolution of 1-(2-thienyl)ethanol **1b** by oxidation with *Acinetobacter calcoaceticus anitrat* to *S*-enantiomer (ee 100%) is also reported.

Enantiomerically pure secondary alcohols are useful chiral auxiliaries in organic chemistry both for analytical and synthetic applications.[1] In particular chiral heteroaryl secondary alcohols are synthesized with the aim of unmasking a hidden functionality after the desired transformations.[2] The methodologies to obtain these chiral compounds are mainly the reduction of the corresponding ketones by: i) catalytic asymmetric hydrogenation; [3] ii) treatment with a hydride reagent modified with a chiral auxiliary;[3] iii) Baker's yeast[4] or other microorganisms.[5,6] On the other hand, few examples are reported of the lipase catalyzed resolution of the racemic alcohols via esterification[7] or hydrolysis.[8] Although, compared with the reduction of ketones, there have been few reports of microbial oxidation of alcohols[9] because in general their oxidation is thermodynamically unfavourable and inhibited by the products formed,[10] recently we reported the kinetic resolution of racemic 1-heteroaryl - and 1-arylethanols *via* oxidation with whole Baker's yeast cells[11] as a new method to afford the R-enantiomer in comparison with the S-enantiomer generally obtained by reduction. These results encouraged a further application of this methodology utilizing bacterium species and strains that are widely employed in oxidation reactions.[9]

In this paper we report the enantioselective resolution of various 1-aryl- and 1-heteroaryl ethanols **1a-h** *via* oxidation with *Bacillus stearothermophilus* 2027 (Scheme).

Scheme



The choice of this microorganism has been made on the basis of screening of a series of bacteria species and strains[11] in the oxidation reactions of the alcohols **1a-d**. The data of the screening, obtained only on an analytical scale, are not reported because most of the bacteria were ineffective in the oxidation reactions and so in the kinetic resolutions of the alcohols **1a-d** (for the procedure see the experimental part). The only exception was *Bacillus stearothermophilus* 2027 which gave good results with all these substrates. On this basis, the reactions were repeated on a preparative scale utilizing other 1-heteroaryl alcohols (Scheme) and the results are summarized in Table 1.

The substrate was added to a growing culture as a concentrated solution in DMF or DMSO and the incubation was prolonged for the appropriate time at 38° C. As showed in Table 1, all the reactions afforded the ketone **2a-h** leaving the unreacted R-enantiomers of the corresponding alcohols. The enantiomeric excesses are normally very high except for 1-(2,5-dimethyl-3-thienyl)ethanol **1f** (ee 20%). Surprisingly, *Bacillus stearothermophilus* gave no oxidation products with the heteroaryl ethanols **1i** and **1j**. On the other hand, from the above screening we obtained interesting results with the 2-thienyl derivative **1b**. Among all the substrates tested, this is the only one that was oxidized by other bacteria than *Bac. stearotherm.* and the results are summarized in Table 2.

Table 1. Kinetic resolution of the racemic 1-aryl alcohols **1a-h** with *Bacillus stearothermophilus* 2027.

Alcohol	Product[a]	Time (days)	% ratio[b]	% ee[b]	Configuration[c]
1a	**2a**	6	47	--	--
	1a		53	95	*R*
1b	**2b**	4	50	--	--
	1b		50	100	*R*
1c[d]	**2c**	6	70	--	--
	1c		30	90	*R*
1d	**2d**	6	42	--	--
	1d		58	92	*R*
1e	**2e**	6	45	--	--
	1e		55	95	*R*
1f	**2f**	6	14	--	--
	1f		86	20	*R*
1g[d]	**2g**	6	49	--	--
	1g		51	95	*R*
1h[d]	**2h**	5	40	--	--
	1h		60	90	*R*

[a] The mixture of the products **1** and **2** was purified on a short column of silica gel (recovered material 80-90%) [b] Determined by GLC on chiral column containing permethylated β-cyclodextrin. [c] Determined by GLC by comparison with the racemic compound and/or with the optical rotation. [d] The substrate was added in DMSO.

Table 2. Kinetic resolution *via* microbial oxidation of the racemic 1-(2-thienyl)ethanol **1b**.

Microorganism[a]	time (days)	**1b** % [b]	ee (abs. conf.)[c]
Pseudomonas cepacia. MR38	2	70	24(*R*)
Pseudomonas vescicularis MR 16	6	56	30(S)
Acinetobacter calcoaceticus. anitrat	4	41	100(*S*)
Pseudomonas picketti	4	60	54(*S*)
Actynomices B	4	62	52(*R*)

[a] The bacteria culture belong to DPVA collection. [b] Yields are determined by GLC. [c] Determined by GLC by comparison with the racemic compound; absolute configuration in parenthesis.

The oxidation with *Acinetobacter calc. anitrat* afforded the corresponding ketone **2b** leaving unreacted the S-enantiomer of the alcohol **1b** (41%, ee 100%). Also other bacteria gave the S-enantiomer but with lower enantiomeric excesses.

The use of *Bacillus stearothermophilus* in oxidation reactions of heteroaromatic and aromatic ethanols is a new method of kinetic resolution for this series of compounds. The recovered alcohols are always the R-enantiomers while the S-enantiomers can be obtained easily in most of cases *via* microbial reduction, i.e. with Baker's yeast. On the other hand, the 2-thienyl derivative showed an higher affinity to bacteria species and can be oxidized with various microorganisms. In this case we obtained different enantiomers depending on the bacteria species and strains.

Experimental

^{1}H NMR spectra were obtained on a 300 MHz Gemini 300 Varian spectrometer. Chemical shifts were given in parts per million from Me_4Si as internal standard. Optical rotations were measured on a Perkin Elmer Model 241 polarimeter. Gas chromatographic analyses were performed on a Carlo Erba HRGC 5160 Mega series. The racemic alcohols **1a-j** were obtained by reduction with sodium borohydride of the corresponding commercially available ketones.

Separation by GLC. Enantiomer separations were obtained on a Megadex 1 column (25 m X 0.32 mm) containing permethylated β-cyclodextrin in OV 1701 from Mega s.n.c.. For compound **1a**: helium, 0.45 atm; temp 110-200° C (1.5° C/min); retention time in min 5.64 (**2a**), 8.03 (**R-1a**), and 8.28 (**S-1a**). For compound **1b**: helium, 0.5 atm; temp: 150-200° C (2° C/min); retention time in min: 4.41 (**2b**), 5.38 (**R-1b**), and 5.54 (**S-1b**). For compound **1c**: helium, 0.5 atm; temp: 150-200° C(2° C/min); retention time in min: 4.96 (**2c**), 9.10 (**R-1c**), and 9.33 (**S-1c**). For compound **1d**: helium, 0.5 atm; temp: 150° C; retention time in min: 4.15 (**2d**), 5.82 (**R-1d**), and 6.04 (**S-1d**). For compound **1e**: helium, 0.5 atm; temp: 150-200° C (1° C/min); retention time in min: 9.81 (**2e**), 13.14 (**R-1e**), and 13.43 (**S-1e**). For compound **1f**: helium, 0.5 atm; temp: 150-200° C(1° C/min); retention time in min: 6.02 (**2f**), 8.92 (**R-1f**), and 9.46 (**S-1f**). For compound **1g**: helium, 0.5 atm; temp: 110-200° C (1.5° C/min); retention time in min: 3.88 (**2g**), 9.32 (**R-1g**), and 9.51 (**S-1g**). For compound **1h**: helium, 1 atm; temp: 100-200° C (2.5° C/min); retention time in min: 6.61 (**2h**), 14.4 (**R-1h**), and 14.6 (**S-1h**). For compound **1i**: helium, 1 atm; temp: 100-200° C (2.5° C/min); retention time in min: 9.39 (**2i**), 19.4 (**R-1i**), and 19.7 (**S-1i**). For compound **1j**: helium, 0.6 atm; temp: 110-200° C (2° C/min); retention time in min: 6.42 (**2j**), 11.11 (**R-1j**), and 11.26 (**S-1j**). The absolute configurations of the compounds **1a**,[13] **1d**,[14] and **1h**[6] were determined to be R from their specific rotations. For the other compounds the absolute configuration is determined on the basis of the retention time, assuming that if in a homologous series the R-isomer has the lowest retention time; this is a standard feature in all series.

Nutrient broth for microbial transformations. The composition of the nutrient broth was as follows: bactotryptone, 5 g; yeast extract, 2.5 g; and glucose, 1 g in 1 L of distilled water.

Microbial oxidations of compounds 1a-d. General procedure for the screening. A sterilized nutrient broth (8 mL) was inoculated with a loopful of the selected bacterium. The mixture was incubated for 2 days at 30° C on a reciprocatory shaker in the presence of small amounts of the appropriate substrate (0.02 mL from a solution of 10 mg of the substrate in 0.1 mL of DMF). To the resulting suspension of grown cells a further solution of the substrate (0.08 mL) was added and the incubation was continued at 30° C. Aliquots were

withdrawing periodically at 2, 4, and 6 days respectively.The mixture was extracted with diethyl ether and dried over anhydrous Na_2SO_4. The crude oxidation products are analyzed by GLC on a chiral column.

Microbial oxidations with *Bacillus stearothermophilus*. General procedure for preparative scale. A sterilized 250 mL Erlenmyer flask containing 100 mL of the nutrient broth was inoculated with a loopful of *Bacillus stearothermophilus*. The mixture was incubated for 2 days at 38° C on a reciprocatory shaker. To the resulting suspension of grown cells the appropriate racemic alcohols (1 mmol) in DMF or DMSO (1 mL) (see Table 1) was added and the incubation was continued under the same conditions for the appropriate time (monitored by GLC). The reaction mixture was extracted four times with 50 mL portion of diethyl ether. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The products were purified by column chromatography (silica gel, petroleum ether/diethyl ether 80:20) to give the ketones and the unreacted alcohols (total yield 85-95%). The **2:1** molar ratios from GLC are given in Table 1.

(R)-1-(2-Furyl)ethanol (1a) [13]: ee = 95%; $[\alpha]_D$ = 22 (c 2.7, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.55 (d, 3 H), 1.68 (s, 1 H), 4.88 (m, 1 H), 6.22 (d, 1H), 6.31 (m, 1 H), 7.38 (d, 1 H).

(R)-1-(2-Thienyl)ethanol (1b): ee = 100%; $[\alpha]_D$ = 24.2 (c 5, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.6 (d, 3 H, *J* = 6.3 Hz), 2.28 (br, 1 H), 5.12 (q, 1 H, *J* = 6.3 Hz), 7.0 (m, 2 H), 7.25 (m, 1 H).

(R)-1-(3-Pyridyl)ethanol (1c): ee = 90%; $[\alpha]_D$ = 26.7 (c 2, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.50 (d, 3 H, *J* = 6.5 Hz), 3.10 (br s, 1 H), 4.95 (q, 1 H, *J* = 6.5 Hz), 7.28 (dd, 1 H, *J* = 4.8 and 7.9 Hz), 7.74 (d, 1 H, *J* = 7.9 Hz), 8.45 (dd, 1 H, *J* = 1.5 and 4.8 Hz), 8.53 (s, 1 H).

(R)-1-Phenylethanol (1d)[14]: ee = 92%; $[\alpha]_D$ = 41 (c 5.1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.47 (d, 3 H, *J* = 6.9 Hz), 2.25 (s, 1 H), 4.79 (q, 1 H, *J* = 6.9 Hz), 7.28 (m, 5 H).

(R)-1-(2-Thienyl)cyclopropanemethanol (1e): ee =95%; $[\alpha]_D$ = -51.5 (c 4.1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.43 (m, 1 H), 0.52 (m, 1 H), 0.65 (m, 2 H), 1.3 (m, 1 H), 2.22 (s, 1 H), 4.27 (d, 1 H, *J* = 8.3 Hz), 6.98 (dd, 1 H, *J* = 3 and 4.8 Hz), 7.05 (m, 1 H), 7.25 (dd, 1 H, *J* = 1.8 and 4.8 Hz).

(R)-1-(2,5-Dimethyl-3-thienyl)ethanol (1f): ee = 20%; ^1H NMR ($CDCl_3$) δ 1.44 (d, 3 H, *J* = 6.4 Hz), 1.62 (br s, 1 H), 2.35 (s, 3 H), 2.40 (s, 3 H), 4.90 (q, 1 H, *J* = 6.4 Hz), 6.70 (s, 1 H).

(R)-1-(5-Methyl-2-furyl)ethanol (1g): ee = 95%; $[\alpha]_D$ = 8.5 (c 2.2, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.52 (d, 3 H, *J* = 6.4 Hz), 2 27 (s, 3 H), 2.42 (br s, 1 H), 4.8 (q, 1 H, *J* = 6.4 Hz), 5.89 (d, 1H, *J* = 3 Hz), 6.09 (d, 1H, *J* = 3 Hz).

(R)-1-(2-Thiazolyl)ethanol (1h)[6]: ee = 90%; $[\alpha]_D$ = 18.6 (c 1.2, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.62 (d, 3 H, *J* = 8 Hz), 5.12 (q, 1 H, *J* = 8 Hz), 7.17 (d, 1 H, *J* = 3.2 Hz), 7.62 (d, 1 H, *J* = 3.2 Hz).

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