Chemoenzymatic Synthesis of (*S*)-(−)-Frontalin Using Bacterial Epoxide Hydrolases

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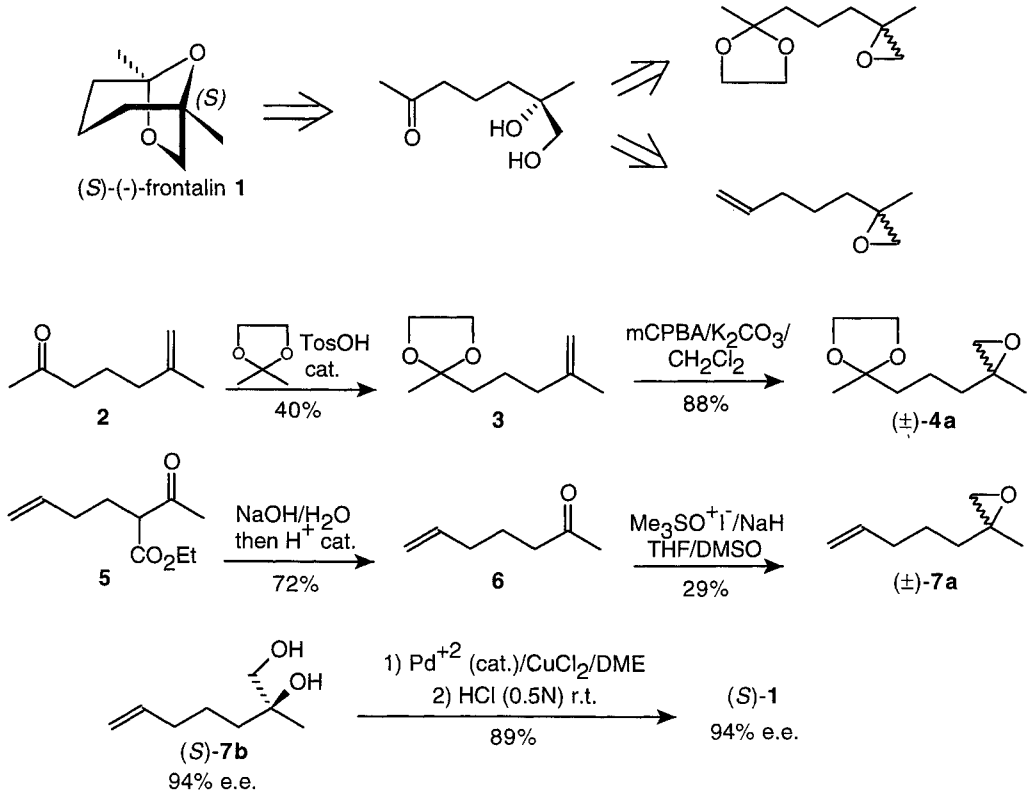
The pheromone (*S*)-(−)-frontalin (**1**) was synthesized in five steps in 94% ee via a chemoenzymatic route. In the key step, 2-methyl-2-(pent-4-enyl)oxirane [(±)-**7a**] was resolved employing lyophilized cells of *Rhodococcus equi* IFO 3730 (E = 39).

1,5-Dimethyl-6,8-dioxabicyclo[3.2.1]octane (**1**; frontalin) is one of the aggregation pheromones of pine beetles of the *Dendroctonus* family. It is released by the insects in order to cause effective colonization once a particular tree has been chosen as breeding ground and it serves to assure an optimal ratio of the sexes.[1] Although the (*S*)-enantiomer[2] is usually the predominant isomer, its enantiomeric purity depends on the species. Whereas the male of *Dendroctonus brevicomis* develops enantiopure (*S*)-frontalin, the pheromone from the females of *Dendroctonus frontalis* shows 70% ee.[3] As a consequence, the demand for (*S*)-frontalin is larger for field experiments with pheromone traps.[4] About thirty different syntheses have been realized so far for the title compound.[5] Among them, syntheses starting from methyl-α-D-glucopyranoside have been found to be rather tedious,[6] whereas asymmetric syntheses – in particular employing Sharpless' asymmetric epoxidation of allylic alcohols – are definitely more elegant.[7,18]

We wish to report a short synthesis of (*S*)-frontalin via a chemoenzymatic approach. Retrosynthetic analysis suggested that 2,2-disubstituted epoxides (±)-**4a** and (±)-**7a** might serve as suitable substrates for our recently developed biocatalytic kinetic resolution via enzymatic hydrolysis catalyzed by bacterial epoxide hydrolases[8,9] (Scheme 1). Both compounds possess a 2-alkyl-2-methyloxirane framework which ensures excellent chiral recognition by bacterial epoxide hydrolases. Furthermore, from our working model,[8] it was anticipated that the (*S*)-configurated diol required for the synthesis of the biologically more active enantiomer of frontalin would be formed.

Compound (±)-**4a** was prepared from ketone **2**[10] via acetalization followed by epoxidation using conventional methodology (Scheme 1). Substrate (±)-**7a** was obtained by an analogous approach: α-alkylation of ethyl acetoacetate using 1-bromobut-3-ene followed by hydrolysis of the ester **5** and decarboxylation furnished the ketone **6**.[19] The latter was treated with trimethylsulfoxonium ylide in THF/DMSO[20] to afford (±)-**7a**. The comparatively low yield was due to the extreme volatility of the epoxide which impeded purification.

After a screening of several bacteria,[11] three strains were selected for the transformation of substrates (±)-**4a** and (±)-**7a** on a preparative scale (Scheme 2). To our disappointment, (±)-**4a** was only slugglishly accepted by the



Scheme 1

most promising candidate *Rhodococcus* NCIMB 11216.[12] However, with *Rhodococcus equi* IFO 3730 substrate (±)-**4a** was consumed, but the product(s) formed arose mainly from decomposition and only traces of the desired diol were formed.[13] The only strain which produced diol **4b** in a clean reaction was *Mycobacterium paraffinicum* NCIMB 10420. However, the selectivity of the reaction, expressed as the Enantiomeric Ratio[14] proved to be too low for preparative purpose[15] (E = 10).

On the other hand, the unsaturated oxirane (±)-**7a** was enzymatically hydrolyzed by all of the strains to yield diol **7b** without side reactions. However, *Rhodococcus* NCIMB 11216 and *Mycobacterium paraffinicum* NCIMB 10420 were shown to be quite unselective with E-values not exceeding 10. Finally, lyophilized cells of *Rhodococcus equi* IFO 3730 gave the desired results: At 18% conversion, diol **7b** having the required (*S*)-configuration was isolated in 94% enantiomeric purity. The latter material was transformed into the target compound (*S*)-**1** in a one-pot reaction via Wacker-oxidation and subsequent ketalization without loss of enantiomeric purity (Scheme 1).

In summary, (*S*)-frontalin **(1)** (94% ee) was prepared in five steps via a chemoenzymatic route. The key step consists of a novel biocatalytic resolution of 2-methyl-2-(but-3-enyl)oxirane **(7a)** employing lyophilized cells of *Rhodococcus equi* IFO 3730 as a convenient and highly selective source for bacterial epoxide hydrolase(s).

2-(4-Methylpent-4-enyl)-2-methyl-1,3-dioxolane **(3)** was obtained by ketalization of **2**[10] (2,2-dimethyl-1,3-dioxolane, TsOH cat., r.t.) in 40% yield; bp 85°C/20 Torr (for spectroscopic data, see reference 16). Epoxidation of alkene **3** using *m*-chloroperbenzoic acid (CH_2Cl_2, powdered K_2CO_3) provided (±)-**4a** in 88% yield (for spectroscopic data, see reference 17). Diol **4b** was identified by comparison of spectroscopic data with literature values.[18] Hept-6-en-2-one **(6)**[19] was converted into (±)-**7a** in 29% yield by treatment with trimethylsulfoxonium ylide.[20]

(±)-**7a**:

^{1}H NMR ($CDCl_3$): δ = 1.24 (s, 3H, CH_3), 1.4–1.6 (m, 4H, CH_2CH_2C), 1.93–2.08 (m, 2H, $CH_2CH{=}CH_2$), 2.48 (d, J = 5 Hz, 1H, oxirane-CH_2), 2.53 (d, J = 5 Hz, 1H, oxirane-CH_2), 4.8–5.0 (m, 2H, $CH_2{=}CH$), 5.6–5.82 (m, 1H, $CH_2{=}CH$).

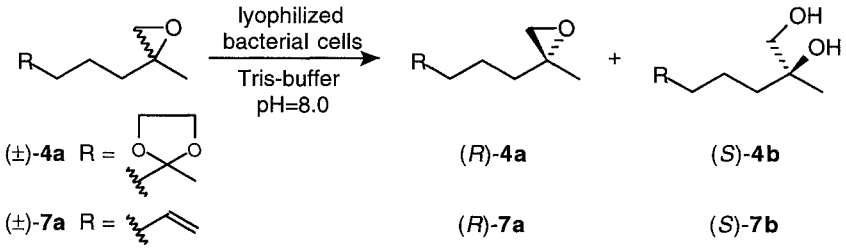
^{13}C NMR ($CDCl_3$): δ = 20.86 ($CH_2CH_2CH_2$), 24.46 (CH_3), 33.62 ($CH_2{=}CHCH_2$), 36.09 (CH_2C), 53.76 (oxirane-CH_2), 56.77 (quart. C), 114.73 ($CH_2{=}CH$), 138.3 ($CH_2{=}CH$).

Growth of Bacteria:

Rhodococcus sp. NCIMB 11216 was grown as previously reported.[8] *Mycobacterium paraffinicum* NCIMB 10420 and *Rhodococcus equi* IFO 3730 were grown in 1 L shake flasks (250 mL medium, 100 rpm, 30°C) on a complex medium: NaH_2PO_4 (1.3 g/L), K_2HPO_4 (4.4 g/L), $(NH_4)_2SO_4$ (1.7 g/L), $MgSO_4 \cdot 7H_2O$ (0.2 g/L), Fe(III)NH_4-citrate (0.05 g/L), $CaCl_2 \cdot 2H_2O$ (0.02 g/L), glucose (11 g/L), yeast extract (1 g/L) and trace element solution[24] (1 mL/L). After 48 h the cells were harvested by centrifugation, washed (2 × Tris-buffer, pH 8.0, 0.05M) and lyophilized.

Biocatalytic Hydrolysis of (±)-7a; Typical Procedure:

Lyophilized cells of *Rhodococcus equi* IFO 3730 (270 mg) were rehydrated in Tris-buffer (15 mL, 50 mM, pH 8.0) for 1 h. Then (±)-**7a** (300 μL) was added and the mixture was shaken at r.t. for 24 h.



Substrate	Biocatalyst	Conversion [%]	e e [%] Epoxide	Diol	Selectivity (E)
(±)-**4a**	*Rhodococcus* NCIMB 11216	no reaction	--	--	--
(±)-**4a**	*Rhodococcus equi* IFO 3730	decomposition	--	--	--
(±)-**4a**	*Mycobacterium paraffinicum* NCIMB 10420	21	20	79	10
(±)-**7a**	*Rhodococcus* NCIMB 11216	50	61	60	7.3
(±)-**7a**	*Mycobacterium paraffinicum* NCIMB 10420	29	20	49	3.5
(±)-**7a**	*Rhodococcus equi* IFO 3730	18	20	94	39

Scheme 2

The reaction was quenched by addition of acetone (6 mL), centrifuged and the products were extracted with EtOAc. After evaporation of the solvent, diol **7b** was separated from the remaining **7a** by chromatography on silica gel (Merck 60, petroleum ether/EtOAc, 1:1); yield: 46 mg (13%); $[\alpha]_D^{20} - 2.1$ ($c = 0.92$, EtOH).

Diol **7b**:

^{1}H NMR ($CDCl_3$): $\delta = 1.1$ (s, 3H, CH_3), 1.35–1.48 (m, 4H, CH_2CH_2C), 1.93–2.08 (m, 2H, CH_2=CHCH_2), 3.33 (d, $J = 11$ Hz, 1H, CH_2OH), 3.40 (d, $J = 11$ Hz, 1H, CH_2OH), 4.83–5.03 (m, 2H, CH_2=CH), 5.6–5.85 (m, 1H, CH_2=CH).

^{13}C NMR ($CDCl_3$): $\delta = 22.99$ ($CH_2CH_2CH_2$), 23.07 (CH_3), 34.23 (CH_2=$CHCH_2$), 38.06 (CH_2C), 69.57 (quart. C), 73.18 (CH_2OH), 114.74 (CH_2=CH), 138.60 (CH_2=CH).

(−)-(*S*)-Frontalin (1):

Wacker-oxidation of (*S*)-**7b** (94% ee) using the procedure published in reference 21 provided (*S*)-**1** in 89% yield without racemization. Spectroscopic data of the product were in accord with the literature values.[16]

Determination of Enantiomeric Purities:

Epoxide **4a** was converted into methoxy derivative **4c** by treatment with NaOMe (0.2M in MeOH) at reflux for 6 h. The same derivative was obtained from **4b** by selective methylation of the primary hydroxyl group (MeI/KOH/DMSO).[22] Enantiomeric purities were analyzed on a Chirasil-DEX-CB column (25 m × 0.25 mm, 0.25 μm film, 1 bar H_2).

4c (120 °C iso): (*R*)-**4c** 9.2 min, (*S*)-**4c** 9.6 min; $\alpha = 1.06$, $k_1 = 12.4$, $k_2 = 13.2$, $R_S = 1.3$.

7a (55 °C iso) and **7b** (115 °C iso) were analyzed without derivatization: (*R*)-**7a** 6.4 min, (*S*)-**7a** 6.8 min, $\alpha = 1.07$, $k_1 = 6.3$, $k_2 = 6.8$, $R_S = 1.4$ (*R*)-**7b** 6.5 min, (*S*)-**7b** 6.1 min, $\alpha = 1.06$, $k_1 = 6.9$, $k_2 = 7.3$, $R_S = 1.2$.

Determination of Absolute Configuration:

Epoxide **4a** obtained from the enzymatic reaction was chemically transformed into the corresponding methoxy derivative **4c**. Compound **7a** was hydrolysed to give diol **7b** (aq NaOH 10%, reflux).[20] Chiral analysis of **4c** and **7b** thus obtained proved that the enzyme-catalyzed reaction proceeded with retention of configuration.[23,25] Diol **4b** ($[\alpha]_D^{20} - 1.39$, $c = 1$, Et_2O) was shown to be (*S*)-configurated by comparison with literature data[18] {$[\alpha]_D^{20} - 1.8$ ($c = 1.2$, Et_2O, ca. 95% ee)}. The latter compound was converted[21] to (*S*)-frontalin $[\alpha]_D^{20} - 52.6$ ($c = 0.9$, Et_2O). {Lit.[17] $[\alpha]_D^{20} - 50.9$, Et_2O, ee = 92%; Lit.[18] $[\alpha]_D^{25} - 51.5$ ($c = 1.2$, Et_2O)}.

Diol **7b** was hydrogenated (Pd on C, 5%) in MeOH to give (*S*)-2-methylheptane-1,2-diol of known absolute configuration.[8] In addition, frontalin obtained from **7b** proved to be (*S*)-configurated.

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