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Enzymatic transesterification of racemic lavandulol: preparation of the two enantiomeric alcohols and of the two enantiomers of lavandulyl senecioate

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Abstract—(*R*) and (*S*)-lavandulol are important compounds in the cosmetics industry and in pheromone research. The senecioyl ester of (*S*)-lavandulyl has recently been identified as the sex pheromone of the vine mealybug, a significant pest in vineyards. We herein report the preparation of the two enantiomers of lavandulol and lavandulyl senecioate, starting from racemic lavandulol. The preparation is based on a two-cycle enzymatic transesterification of racemic lavandulol with vinyl acetate using *Porcine pancreas* lipase. High enantioselectivity was achieved while the preparation yielded (*R*)-lavandulol with 96.7% ee and (*S*)-lavandulol with 92.6% ee.

1. Introduction

Lavandulol is an important terpene constituent in plants and has also been found in insects. The (*R*)-form is a constituent of French lavender oil, which is used in the perfume chemistry. (*R*)-Lavandulol has recently been found to be a component of the aggregation pheromone of the strawberry blossom weevil *Anthonomus rubi* Herbst.[1] (*S*)-Lavandulol, as the senecioyl ester, has also been recently identified as the sex pheromone of the vine mealybug, *Planococcus ficus*,[2–4] which is a serious pest in vineyards. Lavandulol, with unknown configuration, is also a defensive pheromone of the carrion beetle, *Necrodes surinamensis*.[5]

Due to the importance of lavandulol as an additive in the perfume industry, much effort has been made in the synthesis of racemic and chiral lavandulol.[6] Most of the syntheses involve several steps or use expensive chiral reagents. We were interested in developing a simple procedure to prepare the two enantiomers. Racemic lavandulol is a commercial compound; therefore we decided to pursue the enzymatic methodology for its resolution. It should be noted that this is not the first attempt to apply a biocatalytic route for the separation of lavandulol,[7,8] but so far only low enantioselectivity has been achieved.[8]

We studied, both the lipase-mediated transesterification of lavandulol and the hydrolysis of a number of lavandulyl esters. The best results were achieved by a two-cycle transesterification with vinyl acetate that used *Porcine pancreas* lipase. Both enantiomers of lavandulol were obtained, with high chemical yield and enantiomeric excess.

2. Results and discussion

Eleven lipases were investigated for transesterification of lavandulol with vinyl acetate in *t*-butylmethyl ether (Scheme 1), but only *P. pancreas* lipase exhibited promising activity and selectivity (Table 1, entry 6). The reaction was monitored by GC analysis on a chiral column. Both lavandulol and lavandulyl acetate were base-line separated on the Hydrodex-β-6-TBDM chiral column; 25 m×0.25 mm (Macherey-Nagel); kept at 90 °C for 15 min, and then programmed at 5 °C/min to 170 °C and held for 10 min. The retention times of (*R*)-lavandulyl acetate and (*S*)-lavandulyl acetate were 20.6 and 20.8 min and of (*S*)-lavandulol and (*R*)-lavandulol were 21.6 and 22.3 min, respectively. The activity of this lipase was studied in two additional organic solvents that are regularly used in enzymatic transesterification,

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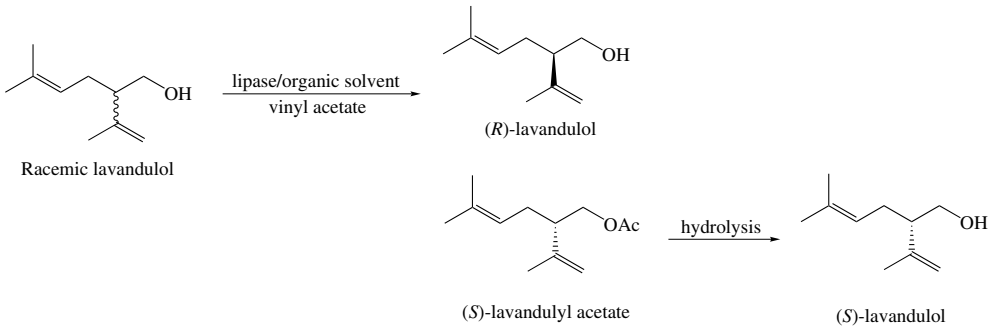


Scheme 1. Enzymatic separation of lavandulol using lipase in organic medium.

Table 1. Enzymes tested for the lipase-mediated transesterification of lavandulol with vinyl acetate in *t*-butylmethyl ether

Lipase	Time	Conversion (%)	Substrate alcohol ee_s[a] (%)	Product acetate ee_p[a] (%)	E[b]
A. niger	17 d	0	—	—	—
A. oryzae	4 d	18	[c]	[c]	—
C. antarctica	8 d	32	*S*, 54	*R*, 60	5.2
C. cylindracea	6 h	37	*R*, 54	*S*, 60	5.6
C. rugosa	10 d	9	[c]	[c]	—
P. pancreas	3 h	53	*R*, 74	*S*, 84	41.7
P. cepacia	22 h	20	[c]	[c]	—
P. fluorescens	3 h	58	*R*, 67	*S*, 69	19.8
P. stutzeri	20 d	43	*R*, 63	*S*, 72	10.5
R. oryzae	22 h	17	[c]	[c]	—
Wheat germ	2 d	0	—	—	—

[a] Enantiomeric excess determined by chiral GC.
[b] Enantiomeric ratio, $E = \ln[1 - C(1 + ee_p)]/\ln[1 - C(1 - ee_p)]$.
[c] No preference.

namely hexane and tetrahydrofuran (THF) (Table 2). Hexane gave the highest E value for the conversion and was selected for all further experiments.

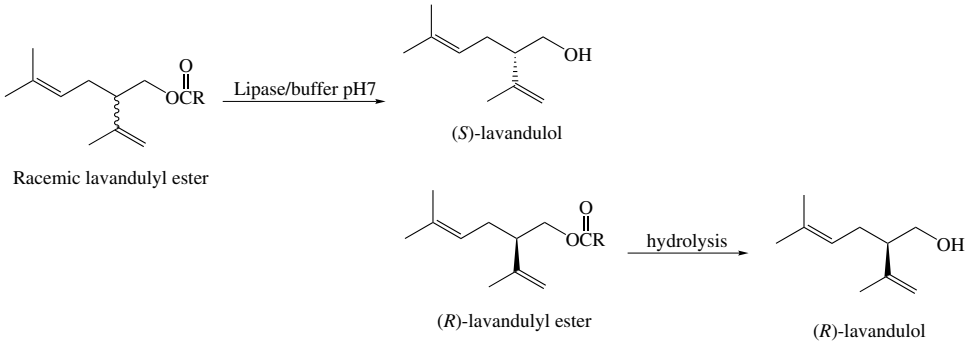
Table 2. *P. pancreas* lipase-catalyzed acetylation of lavandulol with vinyl acetate as the acyl donor in organic solvents

Solvent	Time	Conversion	Ee_s (%)[a]	Ee_p (%)[a]	E[b]
Hexane	3 h	49.6	75	86	37.8
t-Butylmethyl ether	5 h	47.8	73	85	28.9
THF	48 h	45.9	70	83	22.6

[a] Enantiomeric excess determined by chiral GC.
[b] Enantiomeric ratio.

The lipase-mediated hydrolysis of various lavandulyl esters, with *P. pancreas* as the lipase, was also examined (Scheme 2). Six esters of lavandulol were prepared and tested; they were lavandulyl acetate, lavandulyl propionate, lavandulyl 2-phenylbutyrate, lavandulyl pivalate, lavandulyl trifluoro acetate and lavandulyl valerate. Except for lavandulyl acetate and propionate, attempts to resolve these esters on different chiral GC columns failed. Also, the enantioselectivities of the hydrolyses were all low (Table 3).

Lipase-mediated reactions depend mainly on the type and origin of the enzyme. Among all the lipases that were screened for the separation of racemic lavandulol, the best results were achieved by transesterification with *P. pancreas*. However, the highest ee values were in the range of 74–84%, and therefore not sufficient for further biological work, particularly for the preparation of the



Scheme 2. Lipase-mediated hydrolysis of racemic lavandulyl esters in a phosphate buffer.

Table 3. *P. pancreas* lipase-catalyzed hydrolysis of different lavandulyl esters in phosphate buffer (pH 7) at 25 °C

Lavandulyl ester	Time	Conversion (%)	Substrate ester ee_s[a] (%)	Product alcohol ee_p[a] (%)	E[b]
Lavandulyl acetate	0.5 h	29	*R*, 62	*S*, 61	5.2
Lavandulyl 2-phenylbutyrate	4 d	0	—	—	—
Lavandulyl pivalate	42 h	5.8	[c]	*S*, 58	8.9
Lavandulyl propionate	1 h	56.4	*R*, 55	*S*, 57	7.5
Lavandulyl trifluoroacetate	1 h	54.5	[c]	*R*, 51	5.5
Lavandulyl valerate	1 h	24.3	[c]	*S*, 57	4.4

[a] Enantiomeric excess determined by chiral GC.
[b] Enantiomeric ratio.
[c] Not separated by GC analysis on a chiral column.

enantiomers of the vine mealybug sex pheromone. Therefore, a second cycle of transesterification was applied to the partially separated lavandulol enantiomers. This two-step enzymatic process yielded (*R*)-lavandulol with 96.7% ee and (*S*)-lavandulol with 92.6% ee. The two lavandulol enantiomers were esterified with senecioyl chloride to provide (*R*)-lavandulyl senecioate and (*S*)-lavandulyl senecioate, which is the sex pheromone of the vine mealybug. Aliquots of these two esters were hydrolyzed to the corresponding lavandulol enantiomers in order to verify that no racemization had occurred during the esterification. Direct chiral analysis of the enantiomeric senecoyl esters failed because they could not be separated on the available chiral GC columns. The recovered lavandulol enantiomers had the same enantiomeric excess as before the esterification. The identities of the lavandulol enantiomers and the senecioyl esters were confirmed by GC and GC–MS comparison with authentic samples from our previous work on the identification of the sex pheromone of the vine mealybug.[4]

3. Conclusion

Screening of 11 lipases for the transesterification of racemic lavandulol with vinyl acetate indicated that the best enantioselectivity could be obtained with *P. pancreas*. Approximately the same enantiomeric separation was achieved in hexane, *t*-butylmethyl ether and THF. Hexane was used for further work, which yielded the two enantiomers at 74–84% ee in gram quantities. A second cycle of transesterification with the partially separated lavandulol enantiomers yielded (*R*)- and (*S*)-lavandulol in high chemical and enantiomeric excess. The reverse reaction of the lipase-mediated hydrolysis of several lavandulyl esters gave very low enantioselectivity and hence is not suitable for the chiral separation of racemic lavandulol.

4. Experimental

4.1. General methods and materials

4.1.1. Analytical methods. GC analysis was performed on an HP6890 gas chromatograph equipped with a flame ionization detector. Enantiomeric purity was determined on a 25 m×0.25 mm, Hydrodex-β-6-TBDM column (Macherey-Nagel, Düren, Germany), which was held at 90 °C for 15 min and then programmed to 170 °C at a rate of 5°/min. Analysis of the racemic esters and monitoring of the reactions were conducted on a 30 m×0.25 mm×250 μm HP5 column (Hewlett–Packard, USA), which was held at 60 °C for 2 min, programmed to 170 °C at 15°/min, held at 170 °C for 3 min and then programmed to 220 °C at the same rate. Helium was used as the carrier gas and the temperature of the injector and detector held at 220 °C. Analysis on the chiral column was conducted in the split mode at a ratio of 10:1 while the analysis on the HP5 column was conducted in the splitless mode with the purge valve being opened after 1 min. Optical rotations were determined on a Jasco P-1010 polarimeter at a wavelength 589 nm, cell length 0.5 dm.

4.1.2. Chemicals and enzymes. Lavandulol was obtained from Fluka (Buchs, Switzerland), hexane from Merck (Darmstadt, Germany), anhydrous *t*-butylmethyl ether (*t*-BuOMe) from Aldrich (Milwaukee, WI), THF from Fluka, senecoyl chloride, 2-phenylbutyryl chloride, propionyl chloride, valeryl chloride and trifluoroaceticanhydride from Aldrich, pivaloyl chloride from Fluka, acetic anhydride from Merck, anhydrous pyridine from Aldrich and KH_2PO_4/Na_2HPO_4 buffer pH 7.00 from Riedel-de Haën (Seelze, Germany); all were used without further purification. Molecular sieves type 4 Å from BDH (Dorset, England) were oven-dried overnight at 150 °C. Vinyl acetate (Aldrich) was distilled before use. *Aspergillus niger*, *Aspergillus oryzae*, *Candida antarctica* (immobilized in sol–gel-AK), *Candida cylindracea*, *Candida rugosa*, *Pseudomonas stutzeri*, *Rhizopus oryzae* and *wheat germ* lipases were obtained from Fluka. *P. pancreas* lipase (type II, crude; Sigma). *Pseudomonas cepacia* (lipase PS) and *Pseudomonas fluorescens* (lipase AK) lipases were a gift from Amano Enzyme (Japan). Flash chromatography was carried out with silica gel 70–230 mesh, 60 Å (Aldrich). (*R*)-Lavandulol, that had been isolated from lavandin oil,[4] was used as a standard for the determination of the absolute configuration of the lavandulol enantiomers.

4.1.3. Calculation of enantioselectivity. Both enantiomers of lavandulol (the substrate) and lavandulyl acetate (the product) were baseline separated in the GC analysis on the chiral column to obtain ee_s and ee_p. The t_R values for (*R*)- and (*S*)-lavandulyl acetate were 20.82 and

21.03 min, respectively, and those for (*S*)- and (*R*)-lavandulol were 21.84 and 22.52 min, respectively. The conversion (*C*) was calculated from the equation $C = ee_s/(ee_s+ee_p)$. Enantioselectivity was calculated by means of the equation

$$E = \ln[1 - C(1 + ee_p)]/\ln[1 - C(1 - ee_p)]$$

according to Chen et al.[9] In the enantioselective hydrolysis of the various lavandulyl esters only lavandulyl acetate and lavandulyl propionate were baseline separated. Therefore, the conversion (*C*) of the reactions of lavandulyl 2-phenylbutyrate, lavandulyl pivalate, lavandulyl trifluoroacetate and lavandulyl valerate were calculated from the relative amounts of the esters and alcohol. Unsuccessful attempts were made, to separate the higher esters on different chiral GC columns, such as 25 m×0.25 mm LipodexG (Macherey-Nagel, Düren, Germany), 30 m×0.25 mm×0.25 mm CDX-B (J & W Scientific, Folson, CA, USA), 30 m×0.25 mm×0.25 mm, Rt-βDEXsm (Restek, Bellefonte PA, USA).

4.1.4. Lipase-mediated resolution of racemic lavandulol. A mixture of the racemic lavandulol (20 mg), lipase (20 mg), vinyl acetate (1 mL) and *t*-BuOMe (3 mL) (or hexane or THF), with two or three pellets of molecular sieve was stirred at room temperature. Aliquots of 1 μL were withdrawn at intervals and analyzed by gas chromatography.

4.1.5. General procedure for the synthesis of racemic lavandulyl esters. To an ice-cooled, stirred solution of lavandulol (0.2 mL, 1.1 mmol) and pyridine (0.5 mL) in ether (3 mL), acyl chloride (2.2 mmol) was added dropwise. The reaction mixture was stirred until the disappearance of lavandulol, as determined by GC analysis. The reaction mixture was poured into cold HCl (1 M, 5 mL), and extracted twice with hexane (2 mL). The organic phase was washed with saturated $NaHCO_3$ solution (5 mL), brine (5 mL) and then dried over $MgSO_4$. After evaporation of the solvent under reduced pressure, the crude residue was purified by flash chromatography on silica (hexane/diethyl ether 95:5).

4.1.6. Lipase-mediated resolution of racemic lavandulyl esters. A mixture of racemic lavandulyl ester (20 mg) and *P. pancreas* lipase (20 mg) was dissolved in 5 mL of sodium phosphate buffer (50 mM, pH 7.00) and stirred at room temperature. Aliquots of 20 μL were withdrawn at intervals, dispersed into hexane (1 mL), dried over $MgSO_4$ and analyzed by gas chromatography.

4.2. Gram-scale lipase-catalyzed separation of lavandulol enantiomers

4.2.1. Enzymatic separation of racemic lavandulol by *P. pancreas* lipase. A mixture of lavandulol (5 g, 32 mmol), *P. pancreas* lipase (5 g), vinyl acetate (12 mL, 0.11 mol) and molecular sieve pellets (0.25 g) in hexane (300 mL) was stirred at room temperature. After 3 h, when the conversion had reached 50% (according to the GC analysis), the reaction was terminated by filtration and the solvent was evaporated. The residue was distilled under reduced pressure (75–107 °C/12 mm) to yield 4.65 g of a mixture of lavandulol and lavandulyl acetate. Flash chromatography of 2.25 g of the distilled mixture on silica (45 g) gave (*S*)-lavandulyl acetate (750 mg, ee = 82.7%), which was eluted with hexane/diethyl ether at a ratio of 98:2, followed by unreacted (*R*)-lavandulol (1.25 g, ee = 73%) and then with hexane/diethyl ether at a ratio of 9:1.

4.2.2. Enhancing the chiral purity of (*S*)-lavandulol.

4.2.2.1. Hydrolysis of (*S*)-lavandulyl acetate. (*S*)-Lavandulyl acetate (750 mg, 3.8 mmol, ee = 82.7%) was hydrolyzed by stirring in 15 mL of KOH/MeOH (0.5 M) for 1.5 h. The hydrolysis was monitored by GC analysis every 0.5 h. The reaction mixture was poured onto 10 g of ice and extracted with hexane (3 × 5 mL). The organic phase was washed with brine (5 mL), dried over $MgSO_4$ and evaporated under reduced pressure to yield 567 mg of (*S*)-lavandulol (ee = 82.7%) (3.7 mmol, recovery of 96%).

4.2.2.2. Second cycle of lipase-mediated transesterification of (*S*)-lavandulol. Transesterification was performed as described in Section 4.2.1, but without the distillation step. The 567 mg of (*S*)-lavandulol (ee = 82.7%) was converted into (*S*)-lavandulyl acetate, purified by chromatography and then hydrolyzed to give 331 mg (2.1 mmol) of (*S*)-lavandulol (ee = 92.6%, by GC). $[\alpha]_D^{25} = +10.1$ (*c* 1.13, MeOH) [lit.[10] = +9.94 (*c* = 1, MeOH)].

4.2.3. Increasing the chiral purity of (*R*)-lavandulol. A second cycle of enzymatic separation has been carried out in the same way as described above without distillation. (*R*)-Lavandulol (ee = 73%) (1.25 g) yielded 971 mg (*R*)-lavandulol (ee = 96.75%, by GC), recovery of 77%. $[\alpha]_D^{25} = -9.6$ (*c* 1.0, MeOH) [lit.[6] = −10.05 (*c* = 1.1, MeOH)].

4.3. Preparation of (*R*)-lavandulyl senecioate and (*S*)-lavandulyl senecioate

The syntheses were performed in the same manner as described in Section 4.1.5 to yield the (*R*)-enantiomer $[\alpha]_D^{25} = -8.3$ (*c* 1.2, hexane) and the (*S*)-enantiomer $[\alpha]_D^{25} = +9.1$ (*c* 0.99, hexane).

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