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A convenient resolution of racemic lavandulol through lipase-catalyzed acylation with succinic anhydride: simple preparation of enantiomerically pure (*R*)-lavandulol

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Abstract—(*R*)- and (*S*)-lavandulol and their esters are important compounds in perfumery and have recently become significant in pheromone research. (*R*)-Lavandulol and its esters, as well as the esters of (*S*)-lavandulol have been identified as sex and aggregation pheromones in two mealybugs, in thrips and in weevils. We report a convenient resolution of racemic lavandulol through lipase-catalyzed acylation with succinic anhydride. This method does not require tedious chromatographic separation and is particularly suitable for the preparation of enantiomerically pure (*R*)-lavandulol with 98% ee in one resolution cycle. The (*S*)-lavandulol with 90% ee can be obtained by a second resolution cycle.

1. Introduction

Lavandulol and its simple esters, mainly the acetate, are minor but important constituents of essential oils, particularly that of lavender. They are common ingredients in the cosmetic industry. Recently, (*R*)-lavandulol and the different esters of this enantiomer have been identified as sex or aggregation pheromone components in several insects. (*R*)-Lavandulyl (*S*)-methylbutanoate is a component of the female sex pheromone of the hibiscus mealybug,[1] (*R*)-lavandulyl acetate is a component of the male produced sex pheromone of the western flower thrips[2] and (*R*)-lavandulol is a component of the aggregation pheromone of the strawberry blossom weevil.[3] The (*S*)-enantiomer, as the senecioate and isovalerate esters, has been identified in the female sex pheromone of the vine mealybug.[4,5]

We have recently developed a two stage enzymatic separation of commercial (±)-lavandulol using vinyl acetate as the acylating reagent to provide the two enantiomers in good enantiomeric excess.[6] The drawback of this method is the need of lengthy column chromatography in order to separate the unreacted alcohol from the formed acetate. Due to our continuing interest in the lavandulol enantiomers and the recent discovery of pheromones comprising of (*R*)-lavandulol and its esters in different insects, we herein report a simplified enzymatic resolution of (±)-lavandulol, which avoids chromatography through the use of succinic anhydride as the acylating reagent, particularly for preparation of the (*R*)-enantiomer. In addition, easy access to the two lavandulol enantiomers is also of importance in perfumery because they may display different pharmacological activities and certainly different fragrances[7] and odor thresholds.

2. Results and discussion

The enzymatic transesterification of alcohols is a widespread method for the separation of racemic alcohols. The acylating agents may vary, but enol-esters are usually the first choice. Several years ago, succinic anhydride was introduced as an acylating agent,[8,9] but since then has not been used extensively. Thus, acylation converts one enantiomer of the alcohol into the succinic half acid, which can be separated from the unreacted alcohol by extraction with aqueous sodium carbonate, avoiding tedious chromatography.

Application of this method to the resolution of (±)-lavandulol **1** gave directly, in one step, (*R*)-lavandulol **2**

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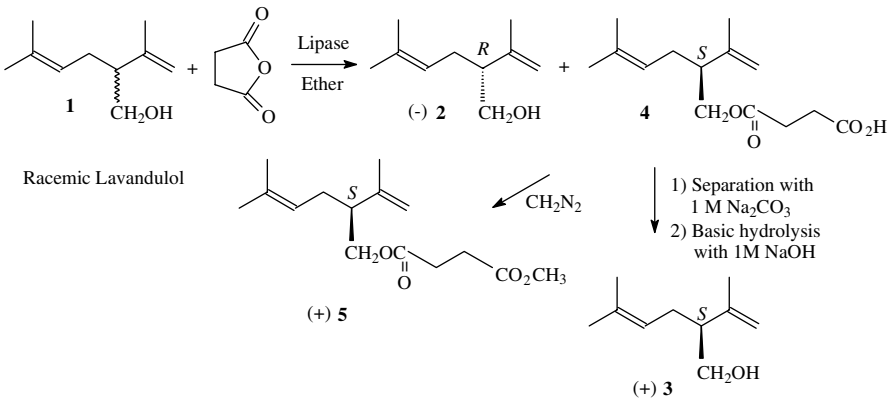

Scheme 1. Enzymatic resolution of (±)-lavandulol with succinic anhydride mediated by *Hog pancreas* lipase in ether.

with an enantiomeric excess of 96–98%. The second enantiomer, (*S*)-lavandulol **3** could be recovered after hydrolysis of lavandulyl succinate half acid **4** with aqueous sodium hydroxide (Scheme 1). The enantiomeric excess of this enantiomer was only 54–74%, depending on the reaction time. Three solvents were tested, *tert*-butylmethyl ether, diethyl ether, and hexane and two similar lipases were used, *Porcine pancreas* from Sigma or *Hog pancreas* from Fluka. These specific enzymes were chosen on the basis of our previous screening of different lipases for the resolution of (±)-lavandulol.[6] The conversion rate using the *Hog pancreas* was higher than that with the *Porcine pancreas* and the enantioselectivity in diethyl ether was higher than that in *tert*-butylmethyl ether (Table 1). In a comparative experiment using the two enzymes in ether, a conversion rate of 50% was obtained after 24 h with the *Hog pancreas* as compared with a 42% conversion rate after 53 h with the *Porcine pancreas*. The reaction in hexane was extremely slow, probably due to the low solubility of succinic anhydride in this solvent. Accordingly, all other experiments were conducted in diethyl ether with the *Hog pancreas* lipase.

Table 1. *Hog pancreas* lipase-catalyzed acylation of (±)-lavandulol with succinic anhydride in organic solvents

Solvent	Time (h)	Conversion (%)	ee (%)[a] of (*R*)	ee (%)[a] of (*S*)
tert-Butylmethyl ether	24	48	74.0	54.0
Diethyl ether	5	25	64.0	74.0
Diethyl ether	24	55	95.0	59.0
Diethyl ether	48	85	96.4	54.0

[a] As determined by GC on a chiral capillary column.

The resolution of **1** was monitored via the formation of succinic half acid **4** by GC on the conventional Rtx-5SILMS column. Succinic half acid **4** was isolated in one case and characterized as its methyl ester **5** by NMR and MS analyses. The (*S*)-enantiomer **3** reacted much faster than (*R*)-enantiomer **2**. The progress of the reaction was determined by measuring the ratio **A** by GC of the formed half acid **4** to the remaining alcohol **1**. At 50% conversion, ratio **A** should be 1.65, which is the molecular weight of **4** divided by the molecular weight of **1**. Reactions were terminated after 24–48 h, when the ratio was about 2, in order to obtain the (*R*)-enantiomer **2** in a high enantiomeric excess. Attempts to obtain the (*S*)-enantiomer with an enantiomeric excess of more than 74% in one step, in short reaction times or by using an excess of (±)-lavandulol, did not succeed (Table 1).

The main advantages of the presented method is the convenient separation between the unreacted (*R*)-enantiomer **2** and succinic half acid **4**, without tedious column chromatography, and the very high enantiomeric excess (96–98%) of the resolved (*R*)-lavandulol. (*S*)-Lavandulol with a moderate enantiomeric excess was recycled by the same resolution method to yield (*S*)-lavandulol with a high enantiomeric excess of 90%. The chemical purity of the commercial (±)-lavandulol was 95%; the residual 5% consisted of several additional small peaks shown in the GC analysis. All the impurities concentrated into the (*R*)-enantiomer, after the resolution, yielding this enantiomer in 90% chemical purity. One sample of the (*R*)-enantiomer was purified by flash chromatography providing **2** in a chemical purity of 98% and enantiomeric excess of 98%. All samples of the *S*-enantiomer were chemically pure (~99%), due to the isolation as sodium salt of half acid **4**. Both purified enantiomers displayed a very high specific rotation of *R* with $[\alpha]_D^{27} = -11.1$ and *S* with $[\alpha]_D^{27} = +10.7$.

3. Conclusion

The enzymatic transesterification of (±)-lavandulol with succinic anhydride as the acylating agent mediated by *Hog pancreas* lipase provides an efficient and convenient method for the resolution of the racemate, and particularly for the preparation of enantiomerically pure (*R*)-lavandulol. The (*R*)-enantiomer was obtained in one step with an enantiomeric excess purity of 96–98%. The remaining (*S*)-enantiomer only had a moderate enantiomeric excess of 75–88%, however, it could be submitted to a second cycle of resolution to provide this enantiomer in 90% enantiomeric excess.

4. Experimental

4.1. Chemicals and enzymes

(±)-Lavandulol of 95% purity (Fluka), hexane (Merck), anhydrous *tert*-butylmethyl ether (*t*-BuOMe) (Aldrich) and succinic anhydride, 99% pure (Aldrich) were used without further purification. Diethyl ether (Biolab) was dried by reflux over sodium and benzophenone. Lipase from *Porcine pancreas* (41 U/mg) and lipase from *Hog pancreas* (23.9 U/mg) were purchased from Sigma and Fluka, respectively. Diazomethane was prepared in ether from *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine and 40% aqueous KOH according to Fieser and Fieser[10] and was used without distillation. Flash chromatography was carried out on Silica gel 70–230 mesh, 60 Å (Aldrich) with hexane and ether. (*R*)-Lavandulol, as standard, isolated from lavandin oil was available from previous work.[5]

4.2. Analytical methods

Analysis of chemical purity and monitoring of the reactions were conducted on a Carlo Erba, Mega gas chromatograph equipped with a flame ionization detector and equipped with a Rtx-5SILMS (30 m × 0.32 mm × 0.5 μm df, Resteck, Bellefonte, PA, USA) column was kept at 80 °C for 2 min and then programmed to 190 °C at a rate of 20 °C/min. The chiral analysis was performed on a HP6890 gas chromatograph equipped with a flame ionization detector and equipped with a cyclodextrin Rt-βDEXsm (30 m × 0.25 mm ID, 0.25 μm df, Resteck, Bellefonte, PA, USA) column kept at 100 °C for 5 min and then programmed to 180 °C at a rate of 5 °C/min. Helium was used as a carrier gas and the temperature of the injector and detector was kept at 220 °C on both machines. Analysis on the chiral column was conducted in the split mode at a ratio of 50:1 and the analysis on the Rtx-5 column was conducted in the splitless mode, the purge valve was opened after 1 min. Optical rotations were determined in methanol on a Jasco P-1010 polarimeter, at a wave length of 589 nm and temperature of 27 °C, in a 1 ml cell of 0.5 decimeter length. Proton NMR was recorded on a Brucker 600 MHz spectrometer in $CDCl_3$ with TMS as internal standard. The electron impact (EI) mass spectrum was recorded on an Agilent 6890N GC–MS instrument equipped with a HP5MS 30 m by 0.25 column. The high-resolution mass spectrum for the determination of the molecular weight of compounds **2**, **3** and **5** were recorded by electron spray ionization (ESI) on a Waters-Micromass Q-TOF Premier Mass Spectrometer (Milford, MA, USA). The CH analyses were carried out in the elemental analysis laboratory of the Hebrew University in Jerusalem.

4.3. Lipase-mediated resolution of (±)-lavandulol

A mixture of (±)-lavandulol (2.31 g, 15 mmol), succinic anhydride (3 g, 30 mmol) and lipase (1.115 g, Fluka, 1777 U/mmol of (±)-lavandulol) was stirred in 50 ml ether at room temperature. Stirring was stopped periodically and aliquots of the supernatant solution analyzed by GC on an Rtx-5SILMS column in order to monitor the progress of the transesterification. The Rt of (±)-lavandulol **1** was 6.26 min and of half acid **4** was 15.90 min. The reaction was terminated after 48 h by filtration of the enzyme and dilution with 20 ml ether. The ethereal mixture was stirred with 1 M Na_2CO_3 solution (60 ml) for 30 min. The basic aqueous layer was extracted with additional 20 ml of ether. The combined organic fractions were extracted with more 1 M Na_2CO_3 solution (20 ml), dried over $MgSO_4$ and the solvent removed to give 0.68 g of the (*R*)-enantiomer **2** with an enantiomeric excess of 98% and chemical purity of 90%. The combined basic aqueous fractions were hydrolyzed by stirring with 1 M NaOH solution (50 ml) for 5 h and then the product was extracted with ether (3 × 60 ml), dried over $MgSO_4$ and the solvent removed to give 0.80 g of the (*S*)-enantiomer **3** with an enantiomeric excess of 54% and chemical purity of 99%. The combined yield of both enantiomers was 64%. The reaction was repeated a number of times and the *S*-enantiomer **3** with higher optical purity, up to 74%, could be obtained by terminating the reaction after 5 h.

4.4. Chemical purification of (*R*)-lavandulol

(*R*)-Lavandulol (200 mg), 90% pure (from Section 4.3) was submitted to flash chromatography on silica gel (15 cm × 1.5 cm). Elution with hexane + 10% ether gave 134 mg of (*R*)-lavandulol 98% chemically pure and 25 mg 85% pure. The pure (*R*)-lavandulol (98%) (ee = 98%, by GC) displayed $[\alpha]_D^{27} = -11.1$ (*c* 2.28, MeOH) (lit.[11] $[\alpha]_D^{20} = -10.7$) whereas the 90% (*R*)-lavandulol displayed a lower specific rotation of $[\alpha]_D^{27} = -9.4$ (*c* 2.20, MeOH). The exact molecular weight was determined by ESI as $C_{10}H_{19}O$ $(MH)^+$, found 155.1450 calculated 155.1436; in addition the ion of $C_{10}H_{17}$ corresponding to $(MH-H_2O)^+$ was found to be 137.1336 calculated 137.1331. CH analysis: Found: C, 77.41; H, 11.93. Calcd for $C_{10}H_{18}O$: C, 77.92; H, 11.69%.

4.5. Second cycle of lipase-mediated transesterification of partially resolved (*S*)-lavandulol

Reaction of partially resolved (*S*)-lavandulol (462 mg), containing 80% *S*, with succinic anhydride (600 mg, 6 mmol) and lipase (462 mg, Fluka) in 15 ml was terminated after 18 h and worked-up as described in Section 4.3. Hydrolysis of the corresponding half acid **4** gave 130 mg (*S*)-lavandulol, 99% pure with a ratio of 95% *S* and 5% *R* (ee = 90%, by GC) and $[\alpha]_D^{27} = +10.7$ (*c* 2.28, MeOH) (lit.[11] $[\alpha]_D^{20} = +10.1$). The exact molecular weight was determined by ESI as $C_{10}H_{19}O$ $(MH)^+$, found 155.1441 calculated 155.1436; in addition the ion of $C_{10}H_{17}$ corresponding to $(MH-H_2O)^+$ was found to be 137.1321 calculated 137.1331. CH analysis: Found: C, 77.01; H, 11.83. Calcd for $C_{10}H_{18}O$: C, 77.92; H, 11.69%.

4.6. Lipase-mediated resolution of (±)-lavandulol using an excess of lavandulol; isolation of half acid 4 as the methyl ester 5

The reaction of a mixture of (±)-lavandulol (2.77 g, 18 mmol), succinic anhydride (0.6 g, 6 mmol) and

lipase (432 mg, Fluka) in 25 ml was terminated after 48 h and worked-up as described in Section 4.3. The ethereal phase gave 1.7 g lavandulol. The aqueous Na_2CO_3 fraction was cooled in an ice bath, stirred and acidified with 1 M HCl. Half acid **4** was extracted with 2×30 ml ether, dried over $MgSO_4$ to yield 1.03 g **4** as oil. GC analysis indicated 98% of **4** with 2% of lavandulol. A sample of **4** (0.85 g) was dissolved in 10 ml ether and was methylated by a slow addition of ethereal diazomethane, until the yellow color persisted. The reaction mixture was dried over $MgSO_4$ and the solvent removed to give 0.87 g of crude lavandulyl methyl succinate **5** containing a number of impurities, including traces of **4** and a few percent of lavandulol. Purification by column chromatography on silica gel (18 cm × 1.5 cm) and elution with hexane + 5% ether gave 0.56 g of pure **5** (98.5% by GC; Rt of 14.47 min) and additional 0.1 g of material with 1% of lavandulol. ^{1}H NMR (600 MHz, $CDCl_3$): δ 5.06 (tt, 1H), 4.82 (dq, 1H), 4.73 (m, 1H), 4.07 (d, 2H), 3.69 (s, 3H), 2.62 (s, 4H), 2.39 (q, 1H), 2.15 (dt, 1H), 2.07 (dt, 1H), 1.69 (m, 6H), 1.60 (s, 3H). The exact molecular weight was determined by ESI as $C_{15}H_{24}O_4Na(MNa)^+$, found 291.1570 calculated 291.1572; EI-MS: main ions at *m/z* (relative abundance) were 268 (M^+, trace), 136 (M^+-methyl succinate, 29), 115 (71), 93 (100), 69 (86). CH analysis: Found: C, 67.05; H, 9.19. Calcd for $C_{15}H_{24}O_4$: C, 67.16; H, 8.96%.

4.7. Hydrolysis of methyl ester 5 to (*S*)-lavandulol

Ester **5** (89 mg) was hydrolyzed with 1 M KOH/MeOH (2 ml) for 4 h at room temperature. Dilution with 20 ml of water and extraction with ether (2×20 ml) gave, after drying and solvent removal, 44 mg of (*S*)-lavandulol with 68% enantiomeric excess by GC.

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