# Chemoenzymatic Synthesis of Homochiral (*R*)- and (*S*)-Karahanaenol from (*R*)-Limonene

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Terpinolene oxide, a monoterpene belonging to the *p*-menthane group, is easily derived from naturally abundant (*R*)-limonene. It was isomerized with montmorillonite clay catalyst to karahanaenone (2,2,5-trimethylcyclohept-4-en-1-one) by ring enlargement. The enantiomers of the corresponding alcohol, karahanaenol (2,2,5-trimethylcyclohept-4-en-1- ol), known for their individual organoleptic properties, were resolved through *Pseudomonas cepacia* lipase mediated enantiospecific alcoholysis of its acetate derivative.

**Keywords:** *Karahanaenone; karahanaenol; lipase; Pseudomonas cepacia; enantiospecific alcoholysis*

## INTRODUCTION

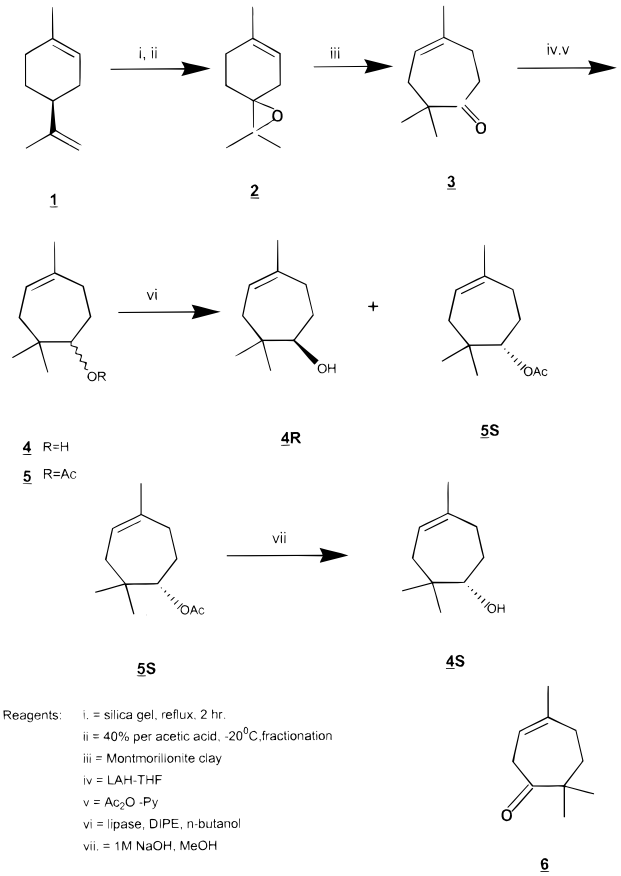
A large number of monoterpene hydrocarbons are readily available from natural sources, and these are often used as starting material for the synthesis of a wide variety of oxygenated derivatives that mostly find application as aroma chemicals (Ohloff, 1994). A common approach toward such functionalization is the epoxidation of the respective double bond and rearrangement of the resulting three-membered highly strained epoxide ring (oxiran) system under the influence of various acid, base, and heterogeneous catalysts (Smith, 1984). The main rearrangement products thus obtained are usually alcohols, carbonyl compounds, and skeletally rearranged products, respectively. On the other hand, direct addition of small molecules such as haloacid, alcohol, and water, is also possible across the double bond in the presence of a suitable catalyst (Erman, 1985). Following these methods, earlier workers from this laboratory have shown that (*R*)-limonene (**1**), a major citrus byproduct, can be utilized as a potential source for preparation of some aroma chemicals (Ravindranath, 1983). Now, I report the synthesis of the enantiomers of karahanaenol in this paper.

Karahanaenol (**4**) (Scheme 1) is an optically active monoterpene alcohol and usually obtained in racemic form by chemical reduction of karahanaenone (**3**), a constituent of hop oil (Naya and Kotake, 1968). Recently, the enantiomers of **4** have been obtained by asymmetric reduction of **3** using various microbial cultures. It was reported that the individual enantiomers differ in their odor (Miyazawa et al., 1995). In the present case, racemic **4** was resolved through lipase-catalyzed enantiospecific alcoholysis (transesterification) in organic solvent.

## MATERIALS AND METHODS

(*R*)-(+)-Limonene was obtained from commercial sources and fractionated. Terpinolene oxide (**2**) was prepared from (*R*)-limonene following the standardized procedure (Roy and Gurudutt, 1997). Racemic karahanaenol was obtained by reduction of karahanaenone with lithium aluminum hydride in dry tetrahydrofuran. All of the solvents were distilled prior to their use and stored over molecular sieves. *Candida rugosa* and porcine pancreas lipases were purchased from Sigma, whereas the remaining lipases were gifts from Amano and Novo Nordisk, respectively. The gas chromatographic analysis was carried out on an HP-5734 instrument equipped with an HP-3380A reporting integrator using a capillary column (6 ft × 0.125 in. o.d.) packed with 3% OV-17 under the following conditions: injection port temperature, 200 °C; detector ($H_2$, FID) temperature, 250 °C; temperature program, 90 °C (5 min

**Scheme 1**

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hold), raised at 4 °C/min to 180 °C (5 min hold); carrier gas, nitrogen; flow rate, 40 mL/min. Mass analysis was performed on a Shimadzu QP 5000 gas chromatography/mass spectroscopy instrument using a capillary DB-Wax column (30 m × 0.25 mm i.d.) under similar conditions. The optical rotation was measured on a Perkin-Elmer 243 polarimeter at 20 °C. $^1$H NMR analysis was performed on a Bruker AMX 300 MHz, instrument using TMS as internal standard. The enantiomeric excess was determined from the resolution of geminal methyl group shift using chiral shift reagent Pr(hfc)$_3$ (Sullivan, 1979), and absolute configuration of the enantiomers was assigned by comparing the optical rotation measurement with literature values (Miyazawa et al., 1995).

**Preparation of Karahanaenone (3).** To a solution of 1.52 g (0.01 mol) of distilled terpinolene oxide in 60 mL of benzene was added 200 mg of K-10 montmorillonite clay (Sigma), and the mixture was stirred at 30 °C for 6 h. The clay material was filtered, and the solvent was removed through rotary evaporation in vacuo. The residual product was purified by column (length 30 cm × 20 mm i.d.) chromatography over silica gel (Glaxo, 60−120 mesh). Isolated yield of **4**: 1.05 g (70%); $^1$H NMR (TMS) $\delta$ 1.02 (s, 3H), 1.03 (s, 3H), 1.67 (s, 3H), 2.69 (m, 2H), 1.99−2.31 (m, 4H), 5.58 (m, 1H); MS 152 [M$^+$], 137 (15), 109 (52), 95 (100), 81(46), 41(43).

**Lipase-Catalyzed Enantioselective Alcoholysis of Karahanaenol Acetate (5).** In a 100 mL stoppered Erlenmeyer flask was dissolved 400 mg of **5** in 15 mL of a diisopropyl ether/*n*-butanol mixture (3:1), and 100 mg of *P. cepacia* lipase was added. The mixture was incubated at 35 °C, 200 rpm, and progress of the reaction was monitored by GC at regular intervals. After 70 h, the enzyme was filtered off and solvent removed by rotary evaporation. The residual oily mass was separated and purified over a silica gel (Glaxo, 60−120 mesh) column (30 cm × 20 mm i.d.) with an ethyl acetate/hexane (1−10%) solvent gradient. Yield of **4**(*R*): 164 mg (82%); $[\alpha]^{20} = 36.2°$ (*c* 0.87, $CHCl_3$); enantiomeric excess 94%. Recovered unreacted **5**(*S*): 190 mg.

**Alkaline Hydrolysis of 5.** One hundred milligrams of the unreacted **5**(*S*) was dissolved in 2 mL of 1 M methanolic NaOH solution and stirred magnetically at 0−5 °C for 6 h. Usual workup and purification led to 84% of **4**(*S*): $[\alpha]^{20} = -28.4°$ (*c* 0.53, $CHCl_3$); enantiomeric excess 91%.

## RESULTS AND DISCUSSION

Terpinolene oxide [*p*-menth-1-en-4(8)-oxide] is a tetrasubstituted spiro oxiran (2,2,6-trimethyl-1-oxa-spira-[2.5]oct-ene, CAS Registry No. 4584-23-0), and the inherent reactivity of the molecule has made it an important intermediate for the synthesis of many flavor and fragrance chemicals. Rearrangement with Lewis acids such as zinc bromide, lithium perchlorate, magnesium bromide, and borontrifluoride etherate has led to a number of products including ring-enlarged karahanaenone (**3**) and its isomer isokarahanaenone (**6**) in almost equal amounts (Roy and Gurudutt, 1997). Attempts to increase the formation of **3** and/or **6** by altering the reaction condition or solvent did not work. Montmorillonite clay is a noncorrosive solid Lewis acid in use for a wide variety of organic reactions (Laszlo, 1987). Isomerization of **2** with K-10 montmorillonite gave **3** in 71% yield. This prochiral ketone with its characteristic fragrance is very important in the flavor industry, and the individual enantiomers of the corresponding reduced alcohol **4** are known to possess varying degrees of fruity and woody odor.

Resolution of racemic alcohols using biocatalysts is now considered to be one of the best methods. Hydrolases are by far the most important class of enzymes preferred for the preparation of chiral alcohols, and their application in organic synthesis has developed extensively during the past two decades. Initial efforts to resolve **4** through the lipase-catalyzed irreversible acyl transfer technique did not show any encouraging results. The lipases screened were from *C. rugosa*, porcine pancreas, *Mucor miehei*, *P. cepacia*, *Pseudomonas* sp., and an esterase isolated from hog liver. Only *C. rugosa* and *P. cepacia* indicated some selectivity (48−56% ee). However, the best results were obtained by carrying out the alcoholysis of the acetate derivative (Bevinakatti and Banerji, 1991; Shkolnik and Gutman, 1994) (**5**) in a diisopropyl ether/*n*-butanol (3:1) mixture employing *P. cepacia* lipase. The marked increase in enantiospecificity in changing the reaction conditions from acylation of **4** to alcoholysis of **5** could be due to steric reasons. The presence of geminal methyl groups at the C-2 position may facilitate the *deacylation* process more than the corresponding *acylation* at the chiral center by virtue of their spatial arrangements.

## CONCLUSION

The work reported here offers a direct access to the enantiomers of karahanaenol starting from a naturally abundant and inexpensive terpene hydrocarbon such as (*R*)-limonene. Similarly, α-terpineol derived from the same hydrocarbon was resolved via lipase catalysis, and the details of this work will be the subject of another paper soon.

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