Lipase-catalyzed Separation of Geometrical Isomers: Geraniol–Nerol

Pankaj Gupta, Subhash C. Taneja,* Bhahwal A. Shah, Vijay K. Sethi, and Ghulam N. Qazi
Indian Institute of Integrative Medicine (CSIR), Canal Road, Jammu-180001, India

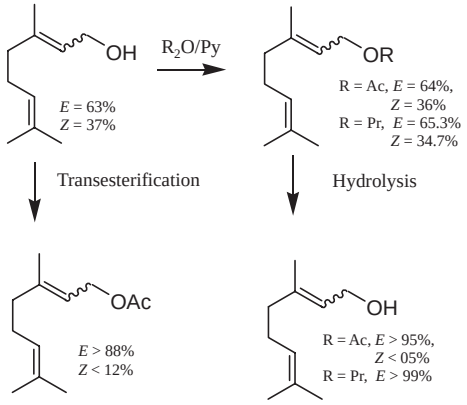
(Received June 4, 2007; CL-070594; E-mail: sc_taneja@yahoo.co.in)

The substrate/lipase ratio as well as pH of the buffer medium played important roles in the resolution of geometrical isomeric mixture of geraniol–nerol. Based on the results, an immobilized lipase from *Pseudomonas fluorescens* (PFL) was found effective in selective transesterifications whereas *Pseudomonas* sp. *Lipase* (PSL) was found to be useful in hydrolyzing the esters.

Geraniol and nerol (trans and cis isomers of 3,7-dimethyl-2,6-octadiene-1-ol) are acyclic monoterpene primary alcohols and the important constituents of a number of aromatic plants.[1] Besides, their use in food and flavour industry these alcohols are used for the synthesis of Vitamins A and E.[2] Geraniol also called rhodinol, is the primary constituent of the oil of rose and palmarosa, and is associated with a sweet rose-like odor, for which it is commonly used in perfumes. High content of geraniol is mainly responsible for the characteristic odor of palmarosa oil, which has its use as antiseptic, mosquito repellent, and pain relieving agent.[3] Different biological activities have been attributed to geraniol and its geometrical isomer nerol. The trans isomer has modest in vitro and in vivo immunosuppressive activity[4] and also inhibits the growth and polyamine biosynthesis in human colon cancer cells.[5,6] Geraniol reportedly inhibited MCF-7 proliferation in human breast cancer cells.[7] It also inhibited HMG-CoA (3-hydroxy-3-methylglutaryl Coenzyme A) reductase activity as the increase in HMG-CoA reductase activity is characteristic of a tumor type.[8] Geraniol is also capable of potentiating the anti tumor effect of 5-florouracil.[9]

The Z isomer nerol is a starting material for the synthesis of nerol oxide,[10] an important perfumery material. The small difference in boiling points (nerol 227–228 °C and geraniol 229–230 °C) makes their separation more cumbersome even if an efficient fractional distillation method may be used for their separation. Though, biocatalytic methods including hydrolases have extensively been used for the resolution of racemic mixtures, however, their application in the separation of geometrical isomers has been uncommon as well as less effective.[11] In the present study, we envisaged to exploit the biocatalytic route for the separation of the cis and trans isomers. An earlier attempt to resolve a mixture of geraniol and nerol, was achieved via selective acylation with acid anhydrides in the presence of Porcine pancreatic lipase (PPL) as a catalyst.[12] However, they were able to achieve only a partial separation resulting in enrichment level of 90:10 of E:Z isomers. In this communication, results of our attempts towards the development of a more efficient method of kinetic resolution of geometrical isomers are presented (Scheme 1).

For the kinetic resolution studies, a panel of lipases belonging to institute's repository as well as procured from commercial sources, were used for the selective hydrolysis of various acylates of natural geraniol–nerol mixture (63:37) under varying



Scheme 1. Lipase-catalyzed transesterification and hydrolysis.

experimental conditions of pH, temperature, and substrate:lipase ratios.

In the course of biocatalytic approach, we were able to achieve the separation of two geometrical isomers with moderate to high selectivity. The various microorganisms used in the present study include *Trichosporon beigilli* (*DSMZ 11829*), *Arthrobacter* sp. (*MTCC no. 5125*), *Aspergillus niger* (*Lipase AS, Amano*), *Mucor miehi* (*MM*), *Pseudomonas* sp. (*PS*), *Candida crusie* (*CC*), *Candida rugosa* (*CR*), etc.

Table 1. Enzyme-catalyzed hydrolysis of (E/Z) geranyl/neryl acylates using PSL*

Substrate	Solvent (1:5)	E:Z	Time /h	Convn /%
Geranyl	ACN:Buffer	95.6:4.4	71	42
acetate	Acetone:Buffer	87.6:12.4	71	50
(E/Z)	DMSO:Buffer	85:15	71	49
	Methanol:Buffer	90.2:9.8	71	60
Geranyl	ACN:Buffer	>99	71	35
propionate	ACN:Buffer[a]	82.3:17.7	71	45.7
(E/Z)	ACN:Buffer	96:4	120	36
	Acetone:Buffer	>99	71	38
	Acetone:Buffer	93:7	120	49.5
	Methanol:Buffer	90.1:9.9	71	45.7
Geranyl	ACN:Buffer	>99	41	18
butyrate (E/Z)	ACN:Buffer[a]	80:20	41	21.8

*All the reactions were performed at 20–22 °C, Quantity of lipase (w/w) = 1:49. a, 1:19. Product was analyzed by injecting a 0.5-μL aliquot in a split less mode into a gas–liquid chromatograph (GLC) equipped with a flame ionization detector. A BP-10 fused silica capillary column heated isothermally at 170 °C was used to separate and identify the products. Injector and detector temperatures were set at 250 and 260 °C respectively. Helium was the carrier gas with a total flow rate of 5 mL/m. Product yield and selectivity were calculated using peak area integration by an on-line computer.

Table 2. Enzyme-catalyzed transesterification reactions of geraniol/nerol using PFL

S.No	Solvent[f]	E:Z	Temp /°C	Time /h	Convn /%
1[a]	ACN	88:12	30–32	20	44
2[a]	ACN	88:12	18–20	20	36
3[a]	Hexane	85:15	30–32	20	48
4[a]	Hexane	87.:13	18–20	20	42
5[a]	DIE	83:17	30–32	20	42
6[a]	Acetone	85:15	30–32	20	57
7[a]	Ionic liquid[g]	89.:11	18–20	24	51
8[a]	Ionic liquid[h]	88:12	18–20	24	41.5
9[b]	Hexane	69:31	30–32	3.5	73
10[c]	Hexane	67:33	30–32	3.5	68
11[d]	Hexane	70:30	30–32	3.5	7
12[e]	ACN	93:7	30–32	72	20
13[e]	DME	90:10	30–32	72	31

Acylating agents [a]Vinyl acetate. [b]Isobutyric anhydride. [c]Butyric anhydride. [d]Propionic anhydride. [e]Isopropenyl acetate. [f]ACN, acetonitrile; DIE, diisopropyl ether; DME, dimethoxyethane. [g]1-Butyl-3-methylimidazolium tetrafluoroborate. [h]1-Butyl-3-methylimidazolium hexafluorophosphate.

Improved selectivity was observed for the propionate derivative followed by acetate derivative in enzymatic hydrolysis of acyl esters.[13] It has also been observed that pH played a key role during the course of the reaction, as little variation during hydrolysis effected the purity of the resolved products. Therefore, all the reactions were performed strictly in a narrow range of pH 7–7.1, that was maintained by adding 0.5 M NaOH solution. Table 1 summarizes the results obtained from the hydrolysis of acylates. The reactions were performed up to grams scale level. The enzyme to substrate ratio played a very important role in resolution, as at lower concentration of lipase e.g. lipase:substrate (1:49), higher resolution of the geometrical isomers was observed. Increasing the concentration of lipase was detrimental to the selectivity. Much lower concentration i.e. $<2\%$ decreased the rate of hydrolysis. The reason for the fall in selectivity at higher concentrations of biocatalyst may be attributed to the presence of other lipases in an organism in minor amounts which also played their roles during hydrolysis.

Besides, transesterification reactions of the isomeric mixture were also studied using various immobilized enzymes, however, *Pseudomonas fluorescens* lipase (PFL) displayed better resolution than other lipases, therefore detailed studies were undertaken with PFL.[14] Table 2 depicts the results of transesterfication reactions with PFL in the presence of various acyl transfer agents. Notably, vinyl acetate proved to be the best acyl transfer reagent that gave a product ratio (E:Z) of 89:11 in 24 h in an ionic liquid, as almost similar results were obtained in acetonitrile. Increasing the size of the acyl transfer agent did not improve the resolution efficacy, though rate of esterification increased considerably in some cases e.g. butyric/isobutyric anhydride. The progress of all the enzymatic reactions was monitored by GC as well as TLC.

In conclusion, the resolution of a mixture of geometrical isomers geraniol and nerol was successfully achieved by biocatalytic approach. In this study, we observed that during hydrolysis, selectivity depended largely on the biocatalyst–substrate ratio and pH, and the best results were obtained using geranyl propionate as a substrate, however, in transesterification reactions it also depended on the nature of the acylating agent while the effect of the organic solvent was not so significant.

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13 Lipase-catalyzed hydrolysis: In a typical experiment, a mixture of the substrate (1 g), organic solvent (5 mL), the crude powder of enzyme PS lipase (20 mg) in 25 mL of phosphate buffer (pH 7.0, 0.1 M) was stirred at 20–22 °C. pH of the reaction was maintained with 0.5 M NaOH solution. The course of the reaction was monitered by GLC. After a certain degree of conversion, the reaction was terminated and the contents extracted with hexane and organic phase washed with water, dried over sodium sulfate and concentrated in vacuo to get the product.
14 Lipase-catalyzed transesterification: Ester synthesis was performed in screw-capped test tubes in which 1 mmol of terpene alcohol and 1.2 mmol vinyl ester or alkanoic anhydride was added to 6-mL solvent followed by 0.5 times (w/w of reactant) of lipase. Samples were kept in a shaker at 320 rpm at room temp. After the incubation period, samples were removed and diluted with hexane and filtered to remove the enzyme that could be recycled again.

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