CHAPTER I

INTRODUCTION

In the present day, research in pharmaceutical and chemical agriculture concentrates on development of products with high optical purity* because of the discovery that optical isomers can give different effects (Crossley, 1992). Menthol, a racemic mixture**, is an economic product, since it was imported around 300,285 kg or 129,585,125 bht in 1995. However, (±)menthol has only half of the effective effects of that contained in (-)menthol which are primarily used for its physiological cooling effect and minty characteristics. (-)Menthol is also widely used in cigarettes, dentrifices, cosmetics, and pharmaceutical products. There are two methods of (±)menthol synthesis, first, (±)menthol is obtained synthetically by treating 3-menthene with a peracid to obtain a mixture of 3,4-epoxy-p-menthane and 3,4-dihydroxy-p-menthane, which is then converted to menthone by boiling with dilute sulfuric acid. Mixed menthol isomers are produced from menthone by hydrogenation. Second, (±)menthol could also be produced via hydrogenation of thymol (Mark et al., 1983).

*Optical purity is an estimate of degree of contamination of enantiomer in terms of another, expressed in percent (Kryt, 1980)

**A racemic mixture is a mixture of equal quantities of dextrorotary and levorotary isomers of the same compound, and therefore is optically inactive (Parker, 1989)

Up to now, (-)menthol is mostly obtained by crystallization from *Mentha arrensis* oil. However, the synthetic product is becoming increasingly popular. (-)Menthol can also be produced by stereospecific synthesis or by resolution of (±)menthol. Either chemical or biological catalysts can be used in these processes.

However, processes where enzymes are used as catalyst tend to give higher optical purity product than the chemical catalysed ones because of the more specific character of enzymes in comparison to synthetic catalysts. Since the starting materials ((±)menthol and an acyl donor) are only sparingly soluble in an aqueous solution which is a natural solvent of enzymes, it is of more advantage to incorporate suitable organic solvents into enzyme catalysed reaction media, and this leads to the development of modified reaction media called 'non-conventional media'. In this project, we are interested in investigating the resolution of racemic menthol in an aqueous/organic system. Enzymes are naturally soluble in an aqueous phase while the substrates are in an organic phase. The reaction occurs via substrate mass transfer from the organic to the bulk aqueous phase and to the enzyme active sites where the product produced is released and diffuse back to the organic phase. The advantages of this reaction media is, first, the prolongation of reaction time due to the continuous supply and extraction of substrates and product, respectively. This reduces substrate exhaustion and product inhibition. Second, downstream separation is facilitated due to the separate product containing organic phase and catalyst containing aqueous phase. The product of this process is (-)menthyl acetate which is useful in food industry as additives (Croteau, 1980). (-)Menthyl acetate can be further hydrolysed to (-)menthol which is a more economic product.

The reaction studied here in this project was the production of (-)menthyl acetate via the transesterification of (±)menthol and an ester. Alcoholysis (cleavage by an alcohol) of an ester is called transesterification (Morrison & Boyd, 1983).

$$R_1COOR_2 + R_3OH \underset{}{\overset{\text{acid,base}}{\rightleftharpoons}} R_3COOR_2 + R_1OH \qquad (1.1)$$

Menthol and acyl donor (such as : ethyl acetate) are reactants.

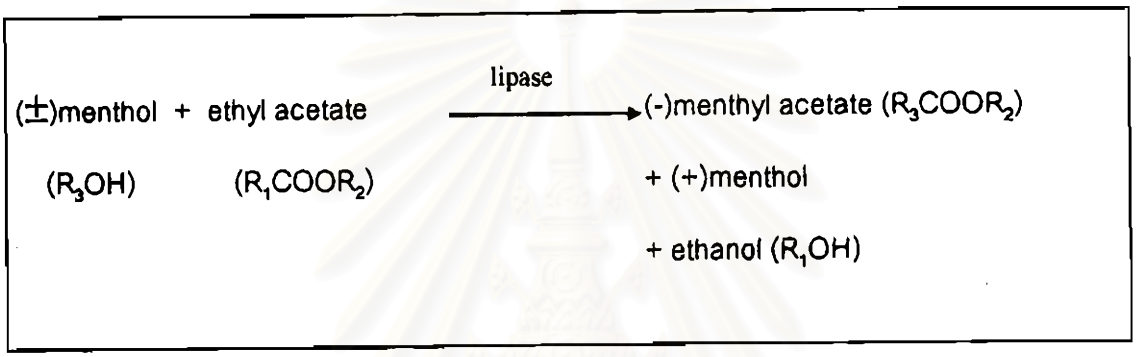

Figure 1.1 The reaction of racemic menthol using ethyl acetate as acyl donor.

1.1 Project objectives

1.1.1 To select suitable types of lipases, acyl donor and various organic solvents for resolution of racemic menthol ((±)menthol) in the aqueous/organic system.

1.1.2 To optimise operating conditions and study kinetics of lipase resolution of racemic menthol in the aqueous/organic system.

1.2 Scope of the project

Resolution of racemic menthol via lipase catalysis in the aqueous/organic system will be studied according to the following parameters:

1. 3 types of lipases : -*Candida cylindracea*
   -Porcine pancreas
   -Hog pancreas
2. 3 types of acyl donors : -ethyl acetate
   -butyl acetate
   -hexyl acetate
3. Five kinds of organic solvents:-hexane, heptane, iso-octane, benzene, and cyclohexane
4. Resolution of racemic menthol in organic solvent was studied according to the following conditions: -temperature
   -volume ratio of buffer solution and organic solvent
   -substrate concentration
   -stirring speed
5. Kinetic study of the reaction