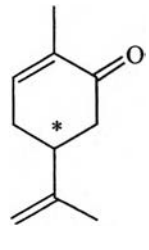
CHAPTER I

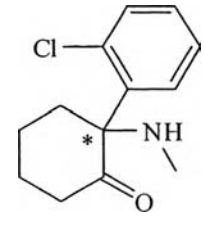
INTRODUCTION

The separation of chiral compounds has been of great interest because the majority of bioorganic molecules are chiral. Living organisms are composed of chiral biomolecules such as amino acids, sugars, proteins and nucleic acids. In nature, these biomolecules exist in only one of the two possible forms, e.g. amino acids are in the L-form and sugars are in the D-form [1]. These two forms are not superimposable like the image and mirror image of each other and are called enantiomers. Generally, enantiomers have the same of physical and chemical properties, which make it difficult to separate them. Whenever enantiomers are in chiral environment, they may show different bioactivity and/or toxicity [2]. The distinct of those behaviors depends on the stereochemistry of enantiomers, for instance, (4*S*)-(+)-carvone has a caraway odor, while (4*R*)-(–)-carvone has a characteristically sweet spearmint odor [3].

In pharmacology, chirality is an important factor in drug efficacy. About 56% of the drugs currently in use are chiral compounds, and about 88% of these chiral synthetic drugs are used therapeutically as racemates [3]. Many racemic drugs showed different in the stereospecificity of the metabolism and/or the pharmacodynamic effects between two enantiomers, such as (*S*)-enantiomer of ketamine drug is responsible for the anesthetic effects while the (*R*)-enantiomer causes the hallucinogenic effects [4].



carvone



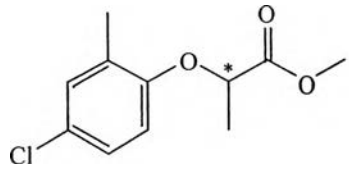
ketamine

Figure 1.1 Structures of carvone and ketamine

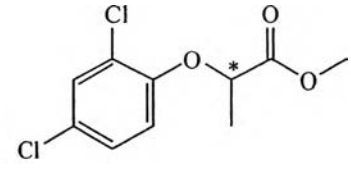
From the difference in pharmacological activity of enantiomers, the pure drug in the active form has been increasingly required to market. In 1992, the U.S. Food and Drug Administration issued a guideline for chiral drugs that only active forms could be brought to market, and that each enantiomer of the chiral drugs should be separated before being used [2, 5]. From the survey of worldwide pharmaceutical data through the last decade indicated that the use of racemic drugs has been decreased from 32% to 8% [5].

Likewise in agrochemical industry, many pesticides were produced and brought to the market as racemates. Generally, only one enantiomer is target-active or more target-active than the other. Using the racemic pesticides may contribute an extra pollution load to the environment. Furthermore, additional costs are involved in both production and removal processes of the inactive isomers [6].

Phenoxy herbicides have been commercially available and are the most widely used family of herbicides worldwide. Phenoxypropionates are the most common group of phenoxy herbicides and are mostly used as the ester form because of their higher herbicide activity to control the growth broad-leaved weeds than the acid form [7, 8]. Considering the biological activity of each enantiomer, (*R*)-enantiomers are normally more active than (*S*)-enantiomers and in some cases only (*R*)-enantiomers are active, such as mecoprop-methyl and dichlorprop-methyl [6, 7]. From the difference in biological activity of each enantiomer of mecoprop-methyl and dichlorprop-methyl, several European governments required that only the active (*R*)-enantiomers of these compounds can be used. Furthermore, many scientists revealed that using the active enantiomer at lower application is more effective and/or more selective toward a targeted pest than using racemates. Other advantages include greater environmental safety, reduced cost and extended patent life [9-11].



mecoprop-methyl



dichlorprop-methyl

Figure 1.2 Structures of some widely used herbicides: mecoprop-methyl and dichlorprop-methyl

Asymmetric synthesis is the well-known method to obtain purely single enantiomers using chiral auxiliary agents or chiral catalysts. Nonetheless, other reliable methods are still required to examine the purity of the synthesized products. For analytical scale, separations of chiral compounds by chromatography and electrophoresis are widely developed [12]. Both indirect and direct separation approaches can be used. Indirect approach involves the conversion of chiral compounds to diastereomers with chiral reagents before separating them. On the other hand, the direct approach utilizes a chiral selector as a stationary phase or an additive to form a temporary diastereomeric complex with chiral compounds [13-15].

Gas chromatography (GC) is the technique used mostly for the separation of volatile and thermally stable organic compounds. Capillary GC combines the advantages of high efficiency, sensitivity, reproducibility and short analysis time. The success of direct GC separation of enantiomers relies on utilizing chiral stationary phases (CSPs), which can rapidly and reversibly form transient diastereomers with the targeted chiral molecules. Direct methods are straightforward to conquer all the problems associated with chiral derivatization process in the indirect approach [13, 16]. Using CSPs for the separation of enantiomers are now considered as standard analytical practice. Cyclodextrins (CDs) and their derivatives have been widely and successfully used as chiral stationary phases because they can separate enantiomers of various chiral analyte structures by forming an inclusion complex, dipole-dipole interactions, or other specific mechanisms [17-20]. However, most chiral separations have been performed through trial and error, and extensive experience is generally required. Since the enantioseparation mechanisms of cyclodextrins are extremely complicated and are not well understood, the

investigation onto the relationship between the structure of each cyclodextrin derivative and analytes is still needed.

Enantiomeric separation using cyclodextrins depends on several parameters such as size of CD, type of substituents, position of the substituents on the CD, concentration of CD as well as the analyte structure. Among these parameters, the analyte structure is the important parameter that can generate a large change of enantiomeric separation system. Nevertheless, there are only a few previous studies into the relationship between enantioselectivities of CD derivatives and structure of chiral analytes [7, 21-25].

Phenoxy acid methyl esters were selected as the analytes of interest since they are widely used as herbicides in agriculture. Furthermore, they are highly toxic and their long term degradation cause environmental problem [6-11]. Phenoxy acid methyl esters with various substituent type and number on the aromatic ring are used as chiral analytes and were separated directly by capillary GC using cyclodextrin derivatives of different size as chiral selectors: hexakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-α-cyclodextrin (or ASiMe) and octakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-γ-cyclodextrin (or GSiMe). Both derivatized α- and γ-CDs were separately dissolved in polysiloxane before using as chiral stationary phases. These two phases have never been reported to use for chiral separation of phenoxy acid methyl esters.

This research thus aims to systematically examine the influence of analyte structure and the size of CD towards the enantioseparation. Through retention factor and enantioselectivity obtained from chromatograms, thermodynamic parameters will be calculated through van't Hoff equation to suggest the interaction between phenoxy acid methyl esters and CD derivatives. Hopefully, the interpretation of the data obtained from this work will provide some knowledge about the influence of analyte structure and the size of cyclodextrin on enantioseparation. Moreover, this work would enhance the possibility of selecting the most suitable chiral stationary phase and separation condition for the chiral separation of these phenoxy acid methyl

esters, including other phenoxy acid methyl esters having similar structure to the studied compounds.