SYNTHESIS, CHARACTERIZATION AND ANALGESIC ACTIVITY OF MITRAGYNINE ANALOGUES

by

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LIST OF SYMBOLS AND ABBREVIATIONS

σ	: Sigma
%	: Percentage
°C	: Degree celcius
1D	: One-dimensional
2D	: Two-dimensional
3D	: Three dimensional
7-OHMG	: 7-hydroxymitragynine
ANOVA	: One way analysis of variance
ATR	: Attenuated total reflection
cm^{-1}	: Recripocal centimeter (unit of wavenumber)
COSY	: Correlation spectroscopy
DCC	: Dicyclohexylcarbodiimide
DCU	: Dicyclohexylurea
DEPT	: Distortionless enhancement by polarization transfer.
DMAP	: 4-Dimethylaminopyridine
EI	: Electron impact
FTIR	: Fourier transform infrared spectroscopy
g	: Gram
g/mL	: Gram per mililiter
g/mol	: Gram per mol
GCMS	: Gas chromatography mass spectrometry
i.p	: intraperitoneal
K 7-OHMG	: Potassium 7-hydroxymitragynine

LCMS	: Liquid chromatography mass spectrometry
M	: Molar
m/z	: Mass-to-charge ratio
MCPBA	: *Meta*-chloro perbenzoic acid
MeOH	: Methanol
mg	: miligram
MG	: mitragynine
mg/kg	: miligram per kilogram
mg/ml	: Miligram per mililiter
MHz	: Megahertz
min	: Minute
ml/min	: Mililiter per minute
mm	: Milimiter
mmol	: Milimol
Na 7-OHMG	: Sodium 7-hydroxymitragynine
ng/ml	: Nanogram per mililiter
nicotinic ester 7-OHMG	: nicotinic ester 7-hydroxymitragynine
NMR	: Nuclear magnetic resonance
NOESY	: Nuclear Overhauser effect spectroscopy
PAG	: The periaqueductal gray
pH	: Measure of the acidity or basicity of an aqueous solution
PIFA	: (Bis(trifluoroacetoxy)iodo)benzene
ppm	: Parts per million
PTLC	: Preparative thin layer chromatography

reduced 7-OHMG	: reduced 7-hydroxymitragynine
RSD	: Relative standard deviation
s.c	: Subcutananeous
S.E.M	: Standard error of the mean
SAR	: Structure activity relationship
SD	: Sprague-Dawley
sd	: Standard deviation
TBHP	: *Tert*-butyl hydroperoxide
THF	: Tetrahydrofuran
Ti-Beta	: Titanium beta
TLC	: Thin-layer chromatography
TMS	: Tetramethylsilane
USA	: United States of America
v/v	: Volume per volume
β	: Beta
μ	: Micro
μg/ml	: Microgram per mililiter
μl	: Microliter
μm	: Micrometer
μmolar	: Micromolar

LIST OF PUBLICATIONS

1. Ramlee, N. I., Mordi, M. N., & Indurkar, J. (2011). *Synthesis of potassium salt of 7-hydroxymitragynine.* Paper presented at the 2nd International Seminar on Chemistry 2011, Universitas Padjadjaran, Bandung, Indonesia.

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SINTESIS, PENCIRIAN DAN AKTIVITI ANALGESIK UNTUK ANALOG-ANALOG MITRAGININA

ABSTRAK

Pokok Ketum (*Mitragyna speciosa*) mempunyai kandungan alkaloid yang tinggi di mana mitraginina (MG) adalah alkaloid yang paling menarik perhatian kerana sifat analgesiknya. Oleh itu, di dalam pembelajaran ini, satu siri analog MG seperti 7-hidroksimitraginina (7-OHMG), garam sodium 7-hidroksimitraginina (Na 7-OHMG), garam kalium 7-hidroksimitraginina (K 7-OHMG), penurunan 7-hidroksimitraginina (penurunan 7-OHMG) dan ester nikotinik 7-hidroksimitraginina (ester nikotinik 7-OHMG) telah berjaya disintesiskan dengan hasil yang tinggi, dicirikan dan kajian terhadap aktiviti analgesiknya dijalankan. Pelbagai agen pengoksidaan telah digunakan untuk menghasilkan 7-OHMG daripada MG. Tindak balas pengoksidaan menggunakan tert-butil hidroperoksida (TBHP), hidrogen peroksida (H_2O_2) atau asid meta-kloroperoksibenzoik (MCPBA) telah dijalankan dengan kehadiran palladium (Pd) sebagai pemangkin. H_2O_2 menunjukkan agen pengoksidaan yang paling baik, menghasilkan 7-OHMG sekitar 99% diikuti oleh TBHP, MCPBA and PIFA. Garam 7-OHMG telah disintesis dengan mencampurkan 7-OHMG dan natrium hidroksida (NaOH) atau kalium hidroksida (KOH) dan hasil terbaik diperolehi apabila nisbah molar 7-OHMG: NaOH/KOH, 1:1 digunakan dan menghasilkan sebanyak 59% untuk garam Na 7-OHMG dan 78% untuk garam K 7-OHMG. Tindak balas penurunan 7-OHMG menggunakan $NaBH_4$ telah berjaya menghasilkan penurunan 7-OHMG sebanyak 99%. Tindak balas pengesteran asid nikotinik dan 7-OHMG dengan kehadiran DCC sebagai agen gandingan dan DMAP sebagai pemangkin menghasilkan sekitar 94% ester nikotinik 7-OHMG. Kelarutan sebatian-sebatian diuji di dalam air dan memberikan nilai seperti berikut, penurunan

7-OHMG (5727.5μM)> 7-OHMG (780.0μM)> K 7-OHMG (415,6 μM)> Na 7-OHMG (404,8 μM)> MG (43.8 μM)> ester nikotinik 7-OHMG (3.8 μM) dan ini menunjukkan kumpulan OH and H sangat penting untuk meningkatkan kelarutan dalam air. Aktiviti analgesik telah dijalankan dengan menggunakan ujian plat panas ke atas tikus Sprague-Dawley pada dos 10.5 mg/kg. MG, 7-OHMG, penurunan 7-OHMG dan ester nikotinik 7-OHMG menunjukkan kesan analgesik wujud apabila dibandingkan dengan kumpulan kawalan. Antara analog-analog yang dihasilkan, 7-OHMG adalah sebatian yang paling tinggi aktiviti analgesik berbanding morfin. Kestabilan 7-OHMG dalam asetonitril telah dikaji pada suhu bilik (25), 4, dan -20°C disebabkan ketidakstabilan 7-OHMG ketika proses pengoksidaan. 7-OHMG menunjukkan kestabilan yang tinggi selama 3 bulan analisis apabila diuji pada kepekatan 800 dan 4000 ng/ml.

SYNTHESIS, CHARACTERIZATION AND ANALGESIC ACTIVITY OF MITRAGYNINE ANALOGUES

ABSTRACT

Mitragyna speciosa contains many alkaloids and mitragynine (MG) is its most abundant alkaloid and has received much attention due to its analgesic property. In this work, a series of MG analogues, which includes 7-hydroxymitragynine (7-OHMG), Na salts of 7-hydroxymitragynine (Na 7-OHMG), K salts of 7-hydroxymitragynine (K 7-OHMG), reduced 7-hydroxymitragynine (reduced 7-OHMG) and nicotinic ester 7-hydroxymitragynine (nicotinic ester 7-OHMG) were synthesized, characterized and evaluated for their analgesic activity. Various oxidants were used to produce 7-OHMG from MG. Oxidation reaction using *tert*-butyl hydroperoxide (TBHP), hydrogen peroxide (H_2O_2) or *meta*-chloroperoxybenzoic acid (MCPBA) was carried out in the presence of palladium (Pd) as a catalyst while (bis(trifluoroacetoxy)iodo)benzene (PIFA) was used without any catalyst. H_2O_2 was found to be the best oxidant, producing around 99% yield of 7-OHMG, followed by TBHP, MCPBA and PIFA. Na 7-OHMG and K 7-OHMG were synthesized by neutralizing 7-OHMG with sodium hydroxide (NaOH) or potassium hydroxide (KOH) and the best yield was observed when the molar ratio of 7-OHMG: NaOH/KOH at 1:1 was used with the yield of 59 and 78% for Na 7-OHMG and K 7-OHMG, respectively. The reduction of 7-OHMG using sodium borohydride ($NaBH_4$) successfully produced reduced 7-OHMG with 99% yield. Whilst, the esterification of nicotinic acid to 7-OHMG in the presence of *N,N'*-Dicyclohexylcarbodiimide (DCC) and 4-Dimethylaminopyridine (DMAP) as a coupling agent and catalyst, respectively has been carried out and produced around 94% yield of nicotinic ester 7-OHMG. The aqueous solubility of the compounds

synthesized in this study is reduced 7-OHMG (5727.5μM)> 7-OHMG (780.0μM)> K 7-OHMG (415.6 μM)> Na 7-OHMG (404.8 μM)> MG (43.8 μM)> nicotinic ester 7-OHMG (3.8 μM) suggesting OH and H are important functional group to increase aqueous solubility. Antinociceptive activity of MG, 7-OHMG, reduced 7-OHMG and nicotinic ester 7-OHMG was performed using hot plate test on Sprague-Dawley rats given oral administration at the dose of 10.5 mg/kg. All compounds tested exhibited antinociceptive effect when compared to the control group. Among the analogues, 7-OHMG was relatively more potent compound when compared to morphine. The stability of 7-OHMG in acetonitrile was studied at room temperature (25), 4, and -20 °C. Minimum degradation of 7-OHMG was observed after 3 months of storage at all temperatures, suggesting 7-OHMG is stable in acetonitrile at both concentrations of 800 and 4000 ng/ml.

CHAPTER ONE

INTRODUCTION

1.1 Research background

Mitragyna speciosa is prevalent to tropical Southeast Asia known as 'biak-biak' or 'ketum' in Malaysia and 'kratom' by Thailand natives (Takayama, 2004). *Mitragyna speciosa* leaves are traditionally used by natives for its psychoactive effect. It has been used as a substitute for opium and to overcome morphine addiction due to its narcotic effect (Matsumoto et al., 2004; Takayama, 2004). Moreover, due to range of medicinal properties offered by this plant, people especially in villages use the leaf to treat diarrhea, cough, hypertension and muscle pain (Chee et al., 2008; Reanmongkol et al., 2007). The leaves of *Mitragyna speciosa* contain mitragynine (MG) as the main constituent along with 44 indole alkaloids such as speciogynine, speciociliatine and paynantheidine etc. (Ponglux et al., 1994; Takayama, 2004). Among them, 7-hydroxymitragynine (7-OHMG) is a minor constituent found in the leaves of *mitragyna speciosa* which has been reported to exhibit the most potent antinociceptive effect (Kikura-Hanajiri et al., 2009; Matsumoto et al., 2006; Ponglux et al., 1994). 7-OHMG has a higher affinity for μ-opioid receptor relative to the other opioid receptors (Matsumoto et al., 2006). 7-OHMG also produced higher antinociceptive effect when compared to morphine (Matsumoto et al., 2004). Morphine is a strong analgesic used clinically that play an important role in pain relieves. However, morphine produced various side effects such as vomiting, nausea, respiratory depression, loss of appetite, headaches, confusion and others. As an alternative, 7-OHMG can be studied for its analgesic activity, however, it being a minor constituent within the leaves. Therefore, in this study an alternative method is carried out to produce 7-OHMG using semi-synthetic

approach. In previously reported method, 7-OHMG was synthesized by the oxidation of MG using (bis(trifluoroacetoxy)iodo)benzene (PIFA) reagent with 50% yield and no catalyst was used in the reaction (Ishikawa et al., 2002). However, the purification steps involving column and preparative thin layer chromatographies produced only 10 % yield. In this study, a novel route for a laboratory scale synthesis of 7-OHMG through oxidation of MG using various oxidants and palladium (Pd) as a catalyst was explored in order to produce 7-OHMG more efficiently. Oxidizing agents such as *tert*-butylhydroperoxide (TBHP), hydrogen peroxide (H_2O_2) and *meta*-chloroperbenzoic acid (MCPBA) was used. Several semisynthetic derivatives of MG have been reported in the literature and exhibited various analgesic activities suggesting structure-analgesic activity relationship of the MG derivatives. Therefore, in the present study, various MG analogues were synthesized and tested for their analgesic activity. Physical properties of the MG analogues were studied such as their solubility in aqueous and stability in acetonitrile.

1.2 Research objectives

1. To optimize the oxidation reaction of mitragynine (MG) to 7-hydroxymitragynine (7-OHMG) using various oxidants such as (bis(trifluoroacetoxy)iodo)benzene (PIFA), *tert*-butyl hydroperoxide (TBHP), hydrogen peroxide (H_2O_2) and *meta*-chloroperoxybenzoic acid (MCPBA).

2. To synthesize sodium and potassium salts of 7-hydroxymitragynine (Na/K 7-OHMG), reduced 7-hydroxymitragynine (reduced 7-OHMG) and nicotinic ester 7-hydroxymitragynine (nicotinic ester 7-OHMG).

3. To determine analgesic activity of mitragynine, 7-hydroxymitragynine, reduced 7-hydroxymitragynine and nicotinic ester 7-hydroxymitragynine using hot plate test.

4. To evaluate solubility of mitragynine, 7-hydroxymitragynine, sodium 7-hydroxymitragynine, potassium 7-hydroxymitragynine, reduced 7-hydroxymitragynine and nicotinic ester 7-hydroxymitragynine in aqueous.

5. To evaluate stability of 7-hydroxymitragynine in acetonitrile at temperatures of -20, 4 and 25 °C (room temperature).

1.3 Research frame work

Figure 1.1 shows the frame work of the research activities that were carried out in this thesis. Mitragynine (extracted from *Mitragyna speciosa*) was used as a starting material.

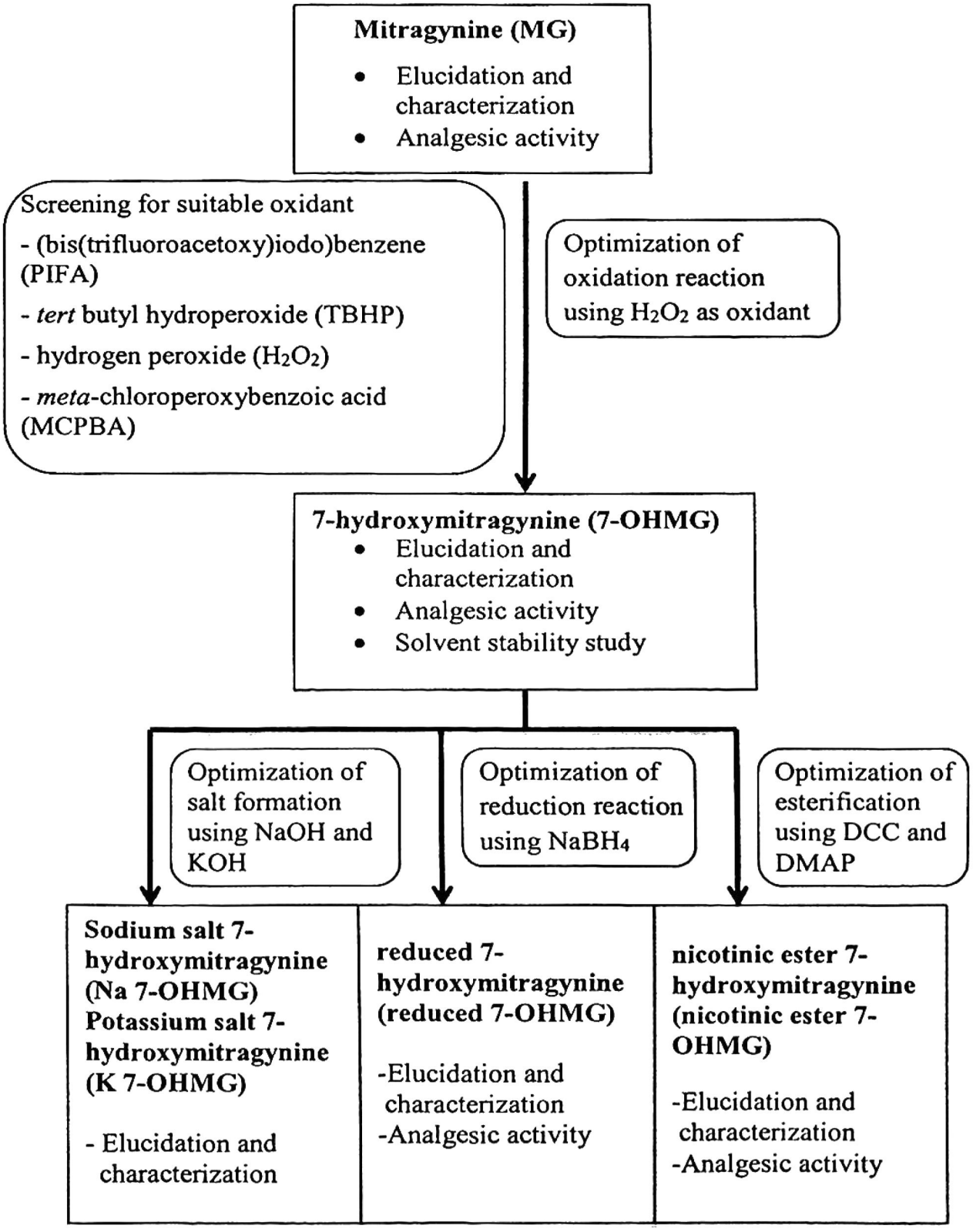


Figure 1.1: Research frame work.

CHAPTER TWO

LITERATURE REVIEW

2.1 Semi-synthetic drug

All over the world, natural products play an important role as a source in medicinal industry. It is thought that about half of pharmaceuticals are based on it (Clark, 1996; Katiyar et al., 2012; Newman et al., 2000). Most of the natural sources were modified in order to produce highly potent drug (Katiyar et al., 2012). Modification of natural sources that is usually used is through semi-synthetic approach. Semi-synthetic drugs that have been produced are combination of natural and synthetic compounds which complement each other since natural product usually contain complex structural feature that is not easily found in synthetic process (Topliss et al., 2002). One of the most popular semi-synthetic drug is heroin (Hay, 1993). Heroin is a semi-synthetic drug derived from morphine developed by C. R. Alder Wright in 1874 (Furunes et al., 2003). It is also known as diacetylmorphine, morphine acetate or diamorphine. Over 200 years ago, morphine was isolated from opium poppy plant (*Papaver somniferum*) (Katiyar et al., 2012). Morphine molecule went for chemical modification by the addition of two acetyl groups to produce heroin as shown in Figure 2.1. Heroin has a strong analgesic activity given via various route such as subcutaneous, intramuscular, intrathecal or intravenous (Sawynok, 1986). The semi-synthetic drug was reported as 2-4 times more potent than morphine (Sawynok, 1986). Furthermore, heroin has a better aqueous solubility when compared with morphine (Furunes et al., 2003). There are other semi-synthetic drugs derived from natural sources such as antibiotics (penicillin, tetracycline,

erythromycin), anticancer drugs (paclitaxel, irinotecan) and antiparasitics (avermectin) (Harvey, 2008).

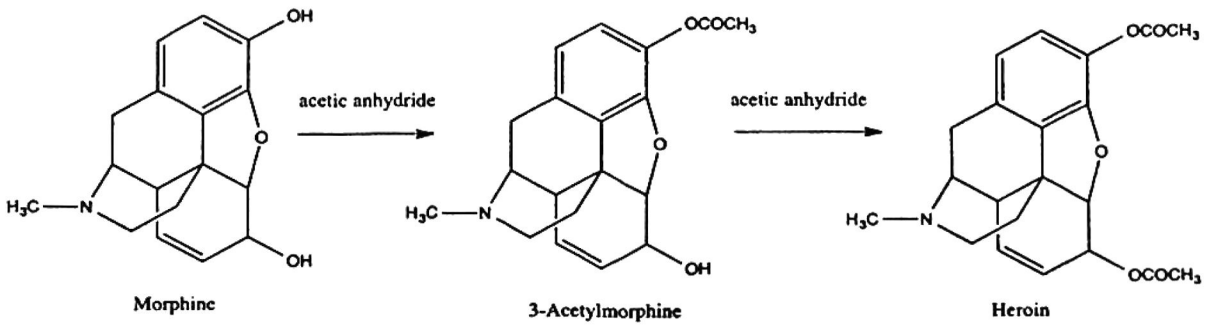


Figure 2.1: Synthesis of heroin from morphine.

2.2 *Mitragyna speciosa*

2.2.1 The plant

Over the last 50 years, many researchers were conducted on the *Mitragyna speciosa* due to its medicinal properties (Jansen & Prast, 1988b; Macko et al., 1972; Singh, 1932). *Mitragyna speciosa* belonged to the family of Rubiaceae (Joshi et al., 1963). It is indigenous to Malaysia and Thailand peninsula. The genus Mitragyna was named by botanist Pieter Korthals in 1839 due to the shape of its stigmas that is similar to the bishop's mitre (Shellard, 1974). Mitragyna is a small genus, which only contains 10 species worldwide (Adkins et al., 2011). Six out of the ten species of Mitragyna are found in Southeast Asia (Beckett et al., 1965). *Mitragyna speciosa* is able to grow up to 12-30 feet in height and 15 feet in width. The trees are illustrated in Figure 2.2. The leaves are commonly in dark green color as shown in Figure 2.3. *Mitragyna speciosa* plant is locally known as 'kratom' in Thailand and 'biak-biak' or 'ketum' in Malaysia (Takayama, 2004). Mitragynine (MG) is the major constituent in *Mitragyna speciosa* extract (Takayama et al., 2002).

Figure 2.2: The plant of *Mitragyna speciosa*

Figure 2.3: The leaves of *Mitragyna speciosa*

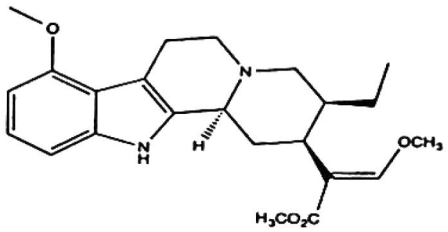
2.2.2 History and use of *Mitragyna speciosa*

In Thailand and Malaysia, extract of the leaves of *Mitragyna speciosa* has been used as medicinal herbs for over 100 years (Jansen & Prast, 1988a, 1988b). Commonly, the leaf was ingested via various techniques such as chewing, infusing as tea concoction or smoking. To date, natives still consume these leaves to combat fatigue and increase their working efficiency under harsh condition (Suwanlert, 1975). It is claimed that energy and strength are developed within 5-20 minutes after consumption of the leaves (Chee et al., 2008). Moreover, due to the range of

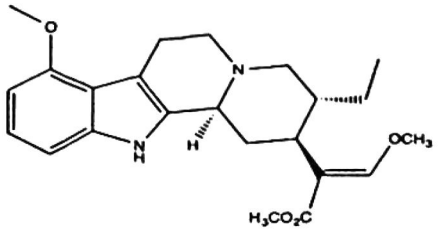
medicinal properties offered by this plant, people especially villagers are using the leaves to treat diarrhea, cough, hypertension and muscle pain (Chee et al., 2008; Reanmongkol et al., 2007). The leaves also had been used as a substitute for opium and to overcome morphine addiction due to its narcotic effect (Takayama, 2004). However, the study done by Suwanlert (1975) showed the chronic consumption of the leaf of *Mitragyna speciosa* lead to addiction. The addiction symptoms that normally occured are weight loss, anorexia, darkening of the skin, insomnia, aching of muscles and bones and inability to work (Chan et al., 2005; Suwanlert, 1975). Although the medicinal properties of the plant were widely studied by many researchers in the past, it has been banned by the Thailand's government in 1939 due to its narcotic effect (Adkins et al., 2011).

2.2.3 Alkaloids of *Mitragyna speciosa*

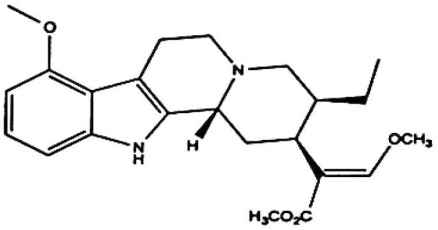
Currently, over 40 compounds have been isolated from *Mitragyna speciosa* (Adkins et al., 2011). Alkaloids content in the leaf of *Mitragyna speciosa* vary depending on geographical and batches of the species (Houghton et al., 1991). In Malaysia, alkaloids that have been isolated from the leaf of *Mitragyna speciosa* are MG, speciogynine, speciociliatine, paynantheine, 7-hydroxymitragynine (7-OHMG), 3,4-dehydro derivative of mitragynine, mitragynaline, corynantheidaline, mitragynalinic acid and corynantheidalinic acid (Takayama, 2004) and their structures are shown in Figure 2.4. Although MG is the major constituent of *Mitragyna speciosa*, the leaves originated from Malaysia plant exhibit less amount of MG which is only 12% from the total alkaloids when compared to plant originated from Thai (66%) (Takayama, 2004).



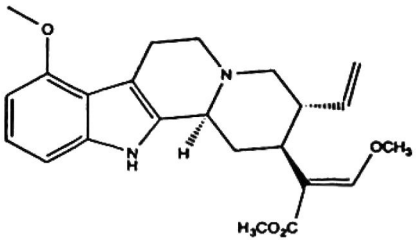
mitragynine



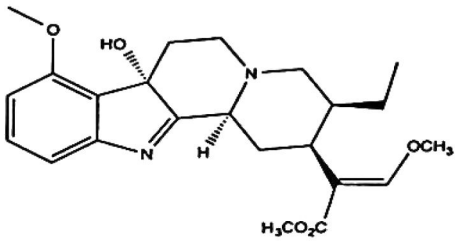
speciogynine



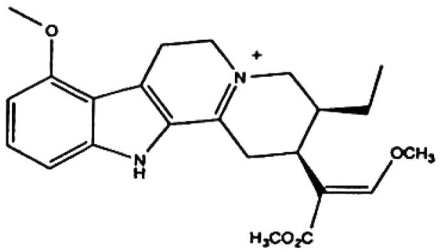
speciociliatine



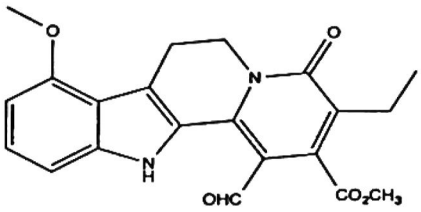
paynantheidine



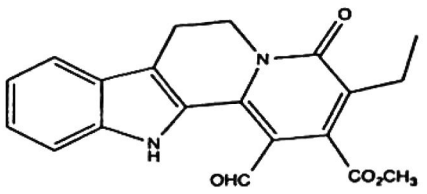
7-hydroxymitragynine



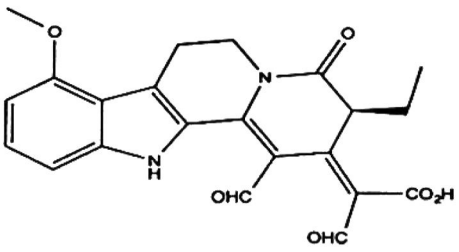
3,4-dehydro derivative of mitagynine



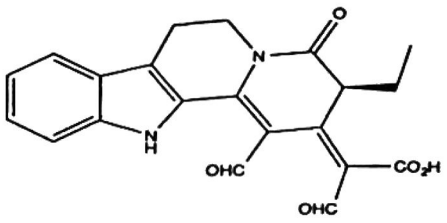
mitragynaline



corynantheidaline



mitragynalinic acid



corynantheidalinic acid

Figure 2.4: Alkaloids of *Mitragyna speciosa*

2.2.4 Analgesic activity of constituent of *Mitragyna speciosa*

Several studies had been conducted on the pharmacology of *Mitragyna speciosa* (Jansen & Prast, 1988b). Most of the studies were focused on MG since it is the most abundant constituent within the leaf. MG had been demonstrated to possess antinociceptive effect when administered via oral, subcutaneous (s.c.) and intraperitoneal (i.p.) routes (Macko et al., 1972). Mice treated with 5-30 mg/kg intraperitoneally showed maximum activity after 15-40 min in tail flick and hot plate tests (Matsumoto et al., 1996). The result suggests that MG can induce antinociceptive activity most probably by acting on the brain. Most recently, its minor alkaloid which is 7-OHMG has attracted attention from many researchers (Matsumoto et al., 2004; Takayama et al., 2006). Even though 7-OHMG is a minor constituent in the extracts however this compound can be easily synthesized from MG by oxidizing MG with (bis(trifluoroacetoxy)iodo)benzene (PIFA) (Ishikawa et al., 2002). 7-OHMG tends to show selectivity for μ-opioid receptors. The activity of 7-OHMG was 13-fold and 46-fold was higher than morphine and MG, respectively. 7-OHMG when administered 5-10 mg/kg in mice via oral route showed higher antinociceptive activity in tail-flick and hot plate test compared to morphine via the same route (Takayama, 2004).

2.3 Chemistry of mitragynine and its analogues

2.3.1 Total synthesis of mitragynine

Currently there are two reports that have been published to synthesize MG using total synthesis approach. The first published route was described by Takayama and co-workers (1995) whereby the synthesis step started from optically pure alcohol involving 10 steps before MG was successfully obtained. The second route was reported by Ma and co-workers in 2007 whereby the route involved 11 steps starting

with 9-methoxy substituted tetracyclic intermediate. The total synthesis of MG is an alternative approach to produce pure MG.

2.3.2 Structure activity relationship (SAR) of mitragynine

SAR can be defined as the functional group or region that influences the activity of the compounds (Patrick, 2005). SAR study is important to discover which part of the compounds is involved in biological activity. Several semi synthetic derivatives of MG have been reported and their SAR has been discussed (McCurdy & Scully, 2005; Takayama, 2004).

Takayama and co-workers (Takayama, 2004) investigated the SAR of MG and found that it is an agonist for opioid receptor. However, corynantheidine which is the 9-demethoxy derivative of MG, is an antagonist. This finding suggests that the methoxy group at C9 of MG is necessary for producing analgesic activity. Next, the demethylated derivative of MG, 9-hydroxycorynanthedine shifts activity from fully agonist in MG to partially agonist in 9-hydroxycorynantheidine. Further study has been carried out by converting phenolic function of 9-hydroxycorynantheidine into ethyl, i-propyl and methoxymethyl ether derivatives. Longer alkyl ether at C9 eliminates opioid activity. Similarly, the N-oxide derivative also eliminates opioid activity. Another derivative without opioid activity was observed when O at C17 was replaced with NH. An alcohol derivative at C23 region of MG reduced opioid activity suggesting an ester group is important for opioid activity.

Another SAR study of MG derivative has been carried out by Zarembo and co-workers (1974). Microbial transformation of MG to mitragynine pseudoindoxyl by the fungus *Helminthosporum* sp. produced an analogue ten times higher opioid activity when tested using D'Amour–Smith test (tail flick test). Based on this study, oxidation of indole derivative was believed to increase the opioid activity of the

compound. Another oxidation of MG with lead tetraacetate, Pb(OAc) produced 7-acetoxyindolenine that is subsequently hydrolyzed to form 7-OHMG with 95% yield (Takayama, 2004). Another study showed that 7-OHMG can also be formed when MG was reacted with (bis(trifluoroacetoxy)iodo)benzene (PIFA) (Ishikawa et al. (2002). 7-OHMG showed a potent opioid agonist by investigating its opioid effects in an isolated ileum contraction test, antinociceptive tests and a receptor binding assay (Matsumoto et al., 2004). A relationship between structure of MG and its opioid activity is shown in Figure 2.5 as described by Adkins and co-workers (Adkins et al., 2011).

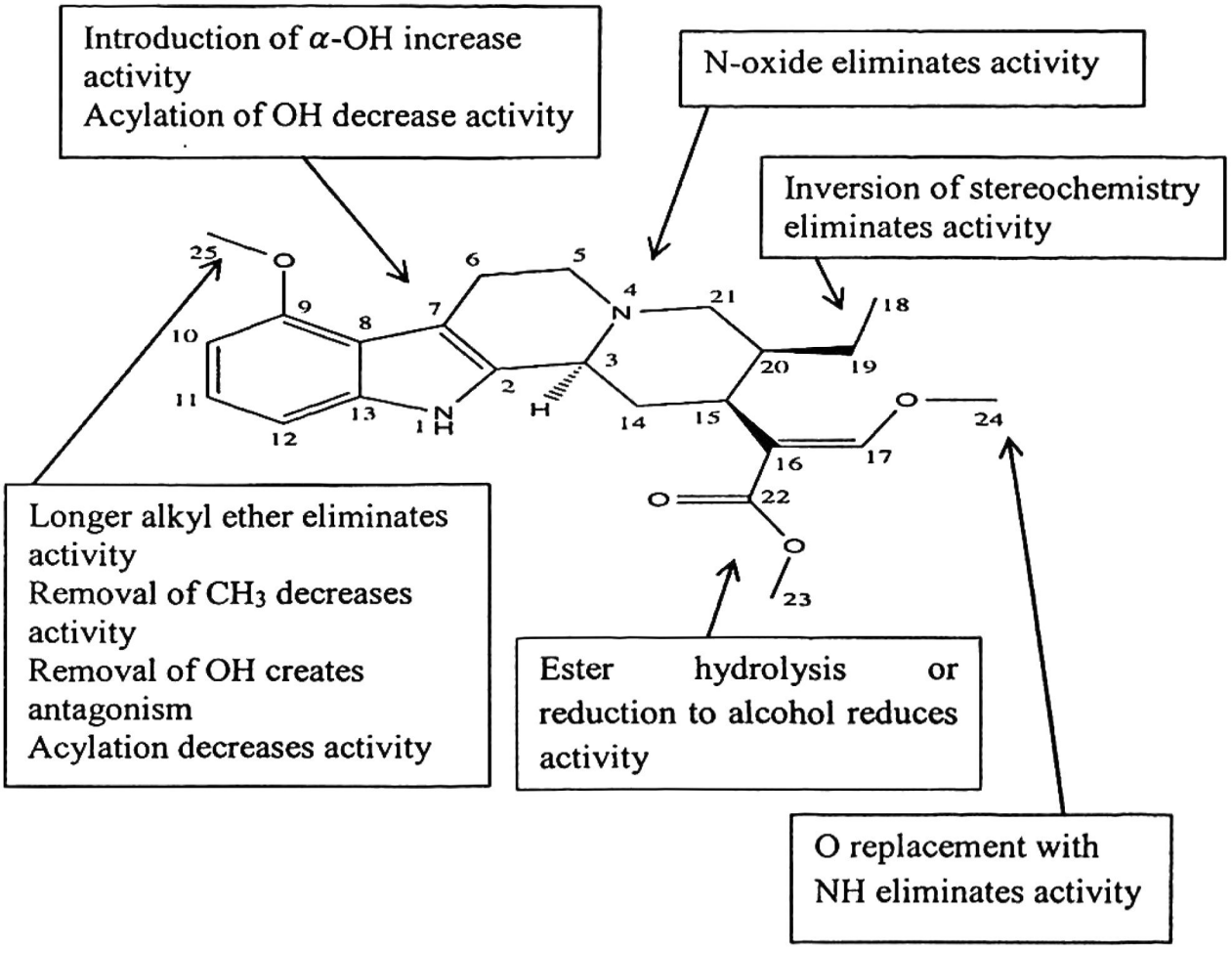


Figure 2.5: A structure-activity relationship of mitragynine and opioid activity.

2.4 Chemical reactions involve in the study

2.4.1 Oxidation of indole

Oxidation process can be defined as a loss of electrons by the addition of oxygen to a compound and popular in organic study (Wade, 2004). Various oxidizing agents have been reported in the literature such as (bis(trifluoroacetoxy)iodo)benzene (PIFA), *tert*-butylhydroperoxide (TBHP), hydrogen peroxide (H_2O_2) and *meta*-chloroperoxybenzoic acid (MCPBA) that can be used for the oxidation of compounds.

PIFA is a hypervalent iodine and has been used as oxidants since the past decades. A rapid development in the research related to PIFA has been reported due to its mild and selective oxidation reaction, environmental friendly and readily available (Zhdankin, 2009). A wide development has been done in synthesizing hypervalent iodine. The first investigation on hypervalent iodine was developed by Willgerodt (1886) involving (dichloroiodo)benzene as shown in Figure 2.6. Awang and Vincent (1980) had successfully developed an oxidation reaction of indole with iodosobenzene diacetate (IBD) and produced substituted indolenines as shown in Figure 2.7. The oxidation reaction enables to introduce primary alkoxy group at the β-position. PIFA in Figure 2.8 is also one type of hypervalent iodine. In 1984, Boutin and Loudon stated that PIFA is an excellent oxidizing agent for the oxidative conversion of aliphatic amides into amines. Work on PIFA has been developed in reaction of acyclic and cyclic alkenes into 1,2- and/or 1,3-bis(trifluoroacetoxy) derivatives (Çelik et al., 2006). Moreover, the oxidation of MG was also discovered using PIFA as an oxidant. The oxidation successfully introduced hydroxyl group at C7 position of MG which produced 7-OHMG with 50% percentage yield (Ishikawa et al., 2002).

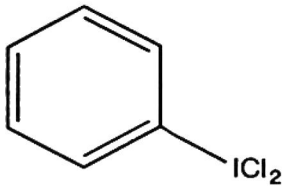


Figure 2.6: Structure of (dichloroiodo)benzene.

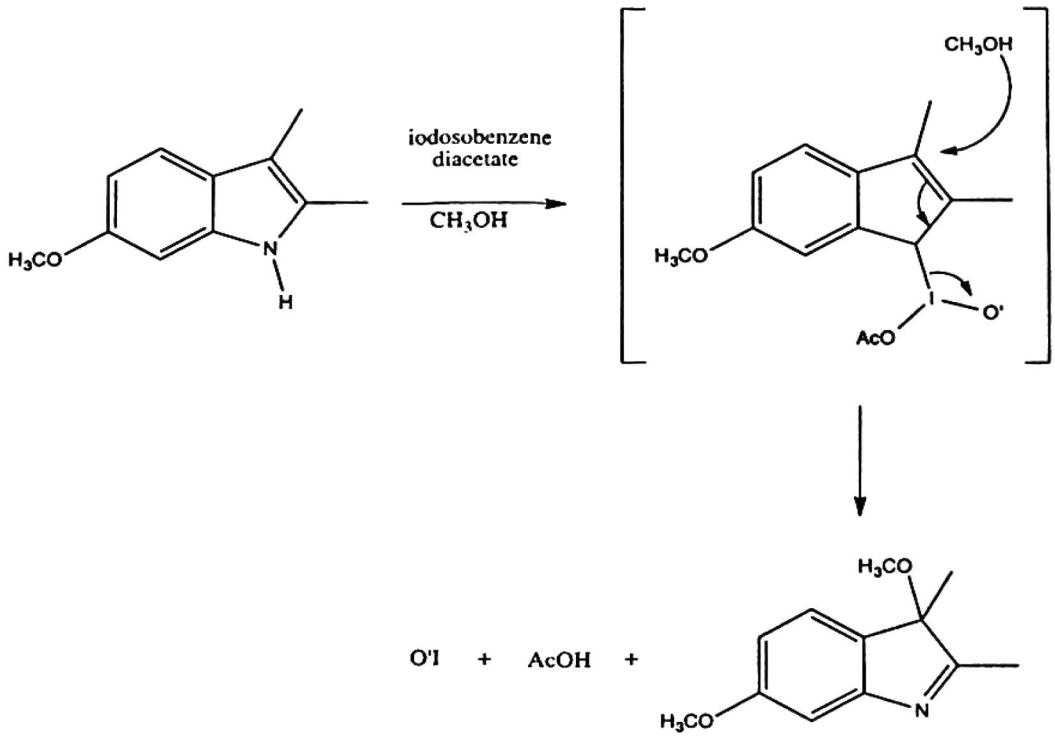


Figure 2.7: The scheme for oxidation of indole using iodosobenzene diacetate (IBD).

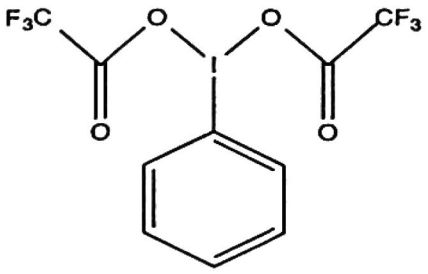


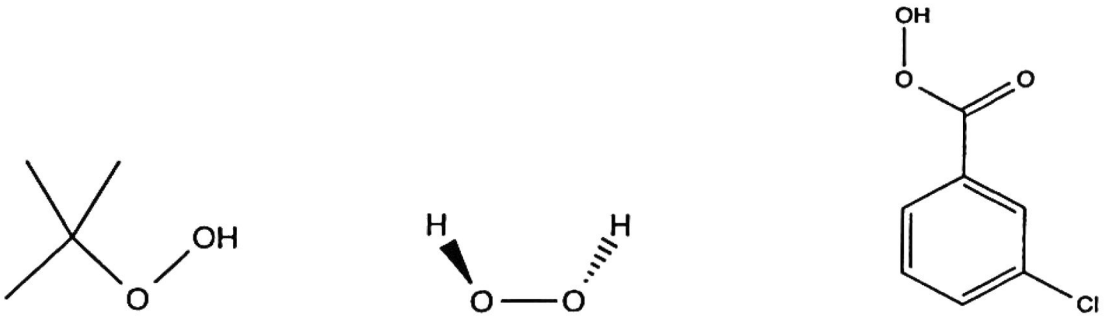
Figure 2.8: The structure of (bis(trifluoroacetoxy)iodo)benzene (PIFA).

TBHP and H_2O_2 are from organic peroxide group while MCPBA is from peroxycarboxylic acid group. TBHP and H_2O_2 are colorless liquid while MCPBA is in white powder form. All of them have slightly pungent odor. The density of TBHP, H_2O_2 and MCPBA are 0.94, 1.44 and 0.93 g/ml, respectively at 25°C. The chemical structures are depicted in Figure 2.9 and their molecular weights are 90.12, 34.01 and 172.57 g/mol, respectively. All of them are being used widely as an oxidant in variety of oxidation reaction under mild condition (Bawaked et al., 2011; Corma et al., 1995). Typically, reactions involving TBHP, H_2O_2 or MCPBA are carried out in catalytic environment. Catalyst is added in order to accelerate the reaction rate of chemical reaction. The oxidation product varied depending on type of oxidant and catalyst.

Grootboom and Nyokong (2002) used two types of catalyst in oxidation of cyclohexane, which are iron perchlorophthalocyanine ($Cl_{16}PcFe^{II}$) and tetrasulfophthalocyanine ($[Fe^{II}TSPc]^{4-}$). When ($[Fe^{II}TSPc]^{4-}$) was applied as a catalyst, it produces higher yields compared to ($Cl_{16}PcFe^{II}$) due to high solubility of ($[Fe^{II}TSPc]^{4-}$). In addition, a research in 1980 has reported that the oxidation of terminal olefins to methyl ketones using TBHP catalyzed by palladium (Pd) has successfully been developed (Roussel & Mimoun, 1980).

H_2O_2 is also one of the strong oxidants. It is preferred as an oxidant since it is proven as a clean and green oxidizing agent (Choudhary et al., 2001). H_2O_2 can lead to environment friendly, non-toxic and economical oxidation (Linley et al., 2012; Yin & Liu, 2008). H_2O_2, which is a clean oxidizing agent gives benefit in converting organic compounds into value-added products (Choudhary et al., 2001). Razmi et al. (2009) also stated H_2O_2 plays an important role in the pharmaceutical and chemical industries where it act as an oxidizing, bleaching and sterilizing agent. The reaction

product of oxidation is mostly depend on steric hindrance of oxidant. Study carried out by Corma and co-workers (1995) revealed that oxidation product of TBHP was lower than H_2O_2. TBHP forms complex Ti-OO-$C(CH_3)_3$ when catalyzed by titanium which indicates bulky intermediate compared to H_2O_2 which only form simpler complex, Ti-OO-H. MCPBA is also being reported as strong oxidizing agent as well. One of several advantages of MCPBA is ease of handling since it is present in powder form. It is commonly used in oxidation of alkene in order to form epoxide (Birman & Danishefsky, 2002; Boyer et al., 2004).



tert-butyl hydroperoxide hydrogen peroxide *meta*-chloroperoxybenzoic acid

Figure 2.9: The structures of *tert*-butyl hydroperoxide (TBHP), hydrogen peroxide (H_2O_2) and *meta*-chloroperoxybenzoic acid (MCPBA).

2.4.2 Reduction of double bond

Reduction can be defined as the addition of hydrogen to a compound and is important in synthetic chemistry. A compound can be reduced by using a variety of reducing agent. The most effective reducing agent is lithium aluminum hydride ($LiAlH_4$) and sodium borohydride ($NaBH_4$) (Brown et al., 1982; Brown et al., 1966; Gerrance & P., 2004). Both reducing agents are different in their reactivity. $NaBH_4$ was discovered by Schlesinger in 1940 (Schlesinger et al., 1953). Subsequently, $LiAlH_4$ was discovered in 1945 by Schlesinger, Finholt and Bond (Finholt et al., 1947). $NaBH_4$ is less reactive than $LiAlH_4$. It only reduces aldehyde and ketone, while $LiAlH_4$ has ability to reduce all including polar multiple bonds (Brown, 1951).

However, many researches focused more on $NaBH_4$ due to negative property of $LiAlH_4$. The reduction reaction using $LiAlH_4$ is hard since the reduction has to be performed in non-hydroxylic solvents such as toluene or ether. It is because $LiAlH_4$ reacts violently with water. Since $LiAlH_4$ is a strong reducing agent, the selectivity in reduction is limited (Chaikin & Brown, 1949). In conclusion, $NaBH_4$ is a mild and selective reducing agent. For example, indolo[2,3-a]quinolizidine alkaloid compounds which contain a basic nitrogen atom have been selectively reduced at the indole double bond with 90% percentage yield using $NaBH_4$ as shown in Figure 2.10 (Gribble, 1998).

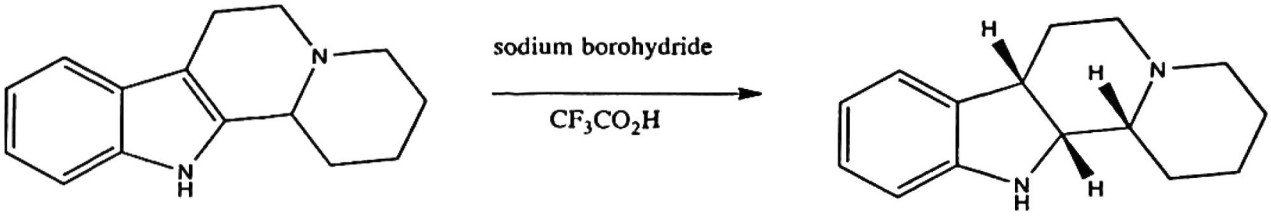


Figure 2.10: The reduction of indolo[2,3-a]quinolizidine alkaloid by using sodium borohydride ($NaBH_4$).

2.4.3 Esterification of alcohol and acid

Esterification is a chemical reaction that produce an ester from two reagent normally alcohol and acid. To date, esterification can be performed in two ways, which are Fischer and Steglich esterifications. Fischer esterification is carried out in reflux condition in the presence of catalyst such as sulphuric acid, tosic acid, or Lewis acids (Kabza et al., 2000). On the other hand, Steglich esterification was performed by introducing the alcohol and acid with dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) (Neises & Steglich, 1990). DCC is widely used as an activating agent and DMAP acting as a catalysts in the synthesis of ester (Perrone et al., 1999; Vanhaecht et al., 2000). Steglich esterification is more

favorable due to its mild condition, and high percentage conversion compared to Fischer esterification. For example, esterification of fatty acid with phenyllalkanols in presence of DCC and DMAP carried out in dichloromethane produced more than 90% percentage yield. The esterification was carried out by stirring the fatty acid, alcohol, DCC and DMAP at room temperature until the esterification reaction was complete (Rauf & Parveen, 2004).

2.5 Physicochemical properties of compound

2.5.1 Solubility of compound

Solubility is one of the most important physicochemical parameters of a new compound that requires to be understood in drug development (Glomme et al., 2005; Stegemann et al., 2007). Solubility can be defined as the concentration of a solute such as solid, liquid or gas in a saturated solution at certain temperature (Mukherjee et al., 2012). Aqueous solubility is one of the drug physicochemical properties needed during drug development process especially for drug formulation (Nanjwade et al., 2011). Numerous factors contributed to solubility such as composition of the aqueous media, pH, temperature, ionic strength and others. Solubility determination can be done by shake-flask method (Glomme et al., 2005). An excess of samples were dissolved in distilled water, then shaken continuously for 24 hours at room temperature in order to form saturated solution (Banerjee et al., 2005). Stegemann and co-workers (2007) reported that the duration of equilibrium solubility as between 4 to 24 hours. Molecular composition of the compound can also influence its solubility. Compound that is composed of functional groups such as fluorine (F), hydrogen (H) and oxygen (O) indicates the formation of hydrogen bond (Patrick, 2005). Hydrogen bond can be defined as a bond that interacts between H (as a hydrogen bond donor) and F, N, or O (as a hydrogen bond acceptor). The hydrogen

bond interaction generates more polar compound and improves its solubility (Abraham & Le, 1999).

Approximately, half of the active compounds used for medicinal purpose are consumed in the form of salts (Balbach & Korn, 2004; Gardner et al., 2004). Salt formation is one of the most preferred approaches to improve solubility of acidic and basic drugs (Stahl & Wermuth, 2008; Stegemann et al., 2007; Sweetana & Akers, 1996). Generally, salts have higher solubility than their corresponding free acid or base forms (Serajuddin, 2007). A salt formation is generated by neutralization reaction of an acid with a base (Patel et al., 2009). There are numerous reported literatures that demonstrated the solubility enhancement for acidic and basic drugs in salt form. For example, Ware and Lu (2004) present the solubility of various salts of trazodone drug such as hydrochloride, tosylate and pamoate salts.. Additionally, solubility of terfenadine salts derived from methanesulfonic acid, lactic acid, hydrochloric acid and phosphoric acid generated up to 10 fold of solubility from the original free parent base (Streng et al., 1984). Furthermore, 90% of the literatures for basic salts are on formation of salts with sodium, potassium, calcium, magnesium, ammonia or meglumine and 55% of it are formed with sodium cation (Stahl & Wermuth, 2008). Potassium is rarely used compared to sodium. However, there are some studies that indicated the importance of potassium in the formation of salt compared to sodium (Remington, 2005). For example, potassium salts of penicillin G is preferred over sodium due to hygroscopic properties of sodium (Swarbick, 2006). The potassium salt of chlorothiazide has showed up to 400 fold more soluble than sodium salt of chlorothiazide which indicates larger enhancement in solubility compared to sodium salt (Paluch et al., 2011).

2.5.2 Stability of compound

Stability is the ability of a compound to remain within specifications to ensure its character and purity. Several factors affect the stability such as temperature, light, or moisture. Liquid chromatography (LC) is an essential instrument determining stability (Li et al., 2011). A stability study was carried out after storage at various temperatures. In a study developed by Gannu and co-workers (2007), a stock solution containing 5 μg/ml of doxofylline was analysed. After storage over 15 days at 2-8 °C for and 8 hours at room temperature, more than 98% of the compound remained unchanged. The stability was measured by comparison of liquid chromatography mass spectrometry (LCMS) chromatogram peak areas of compound with that of freshly prepared solution of compound studied. This suggests that the standard solution was stable over those storage time and temperature.

2.6 Pharmacological activity

2.6.1 Pain

Pain is defined as unpleasant sensory and emotional experience associated with actual or potential tissue damage according to International Association for the Study of Pain's (1994). Pain is being categorized into acute and chronic pain.

Acute pain usually an effect from injury or specific disease. Acute pain can be caused by many reasons such as surgery, childbirth, burns, cuts and broken bone. Acute pain may last just a moment, but may be severe and lasts for weeks or months (Walsh et al., 1993). Unrelieved acute pain could lead to chronic pain (Carr & Goudas, 1999).

Chronic pain is defined as pain that extends beyond the expected period (Turk & Okifuji, 2001). Common chronic pain complaints are migraine, headache,

cancer pain, arthritis pain, herpes zoster, diabetic neuropathy, low back pain, psychogenic pain and neurogenic pain (Basbaum et al., 2009). Pain signal can remain in the nervous system for long time as it may last for weeks, months or years (Shipton & Tait, 2005).

2.6.2 Mechanism of pain pathway

The pathways that carry information about noxious stimuli to the brain are complex. Generally, pain pathway involves four processes which are transduction, transmission, modulation and perception (Herbert, 2000).

Transduction is the process where afferent nerve endings are involved in converting the noxious stimuli into nociceptive impulses. There are three types of afferent nerve endings which are A-beta, A-delta, and C fibers. The A-delta and C fibers are involved in nociception process. A-delta fibres (myelinated nerve) are in medium sized and carry rapid and sharp pain. Whereas, C fibres (unmyelinated nerve) are small in diameter and demonstrate slow conduction of impulse (Patel, 2009).

Transmission is the process where nerve impulses are sent to the dorsal horn of the spinal cord via peripheral nerve and the signal is transmitted to the brain. The neurotransmitters that are involved in transmitting nerve impulse from one neuron to another neuron such as glutamate, neurokinin A and substance P (Hemmings & Hopkins, 2006). A-delta and C fibers terminate in the dorsal horn of the spinal cord. The pain impulse that is transmitted from spinal cord to the thalamus and brain stem via nociceptive ascending pathways uses spinothalamic and spinoparabrachial pathways.

Modulation of pain is the process that decreases, changes or trasmits pain impulse in dorsal horn of the spinal cord (Glynn, 1999). Modulation pathway is

referred as descending modulating pain pathway. Descending pathways involved the propagation of impulse from cerebral structures to the dorsal horn. This pathways can lead to either decrease in transmission (inhibition) or potentiation in transmission (facilitation) of pain impulses to the brain (Millan, 2002). The periaqueductal gray (PAG) is the most important control center in the brain that is involved in descending inhibition. Stimulation of PAG produces analgesia.

The perception of pain is a conscious awareness of the experience when the pain threshold is reached. The pain threshold is the point at which sufficient pain transmits to the brain and the pain starts to be felt. The major pathway of pain is shown in Figure 2.11.

2.6.3 Hot plate test

Hot plate test was proposed by Eddy and Leimbach in 1953. The hot plate test is a test that evaluate pain response of animal, caused by heat. In this test, animal is placed on the hot plate and the time is measured when the animal starts jumping and hind paw-licking (Carter, 1991; Espejo & Mir, 1993). The hot plate test is normally used to study the possible involvement of supraspinal receptors (Yaksh,1999).

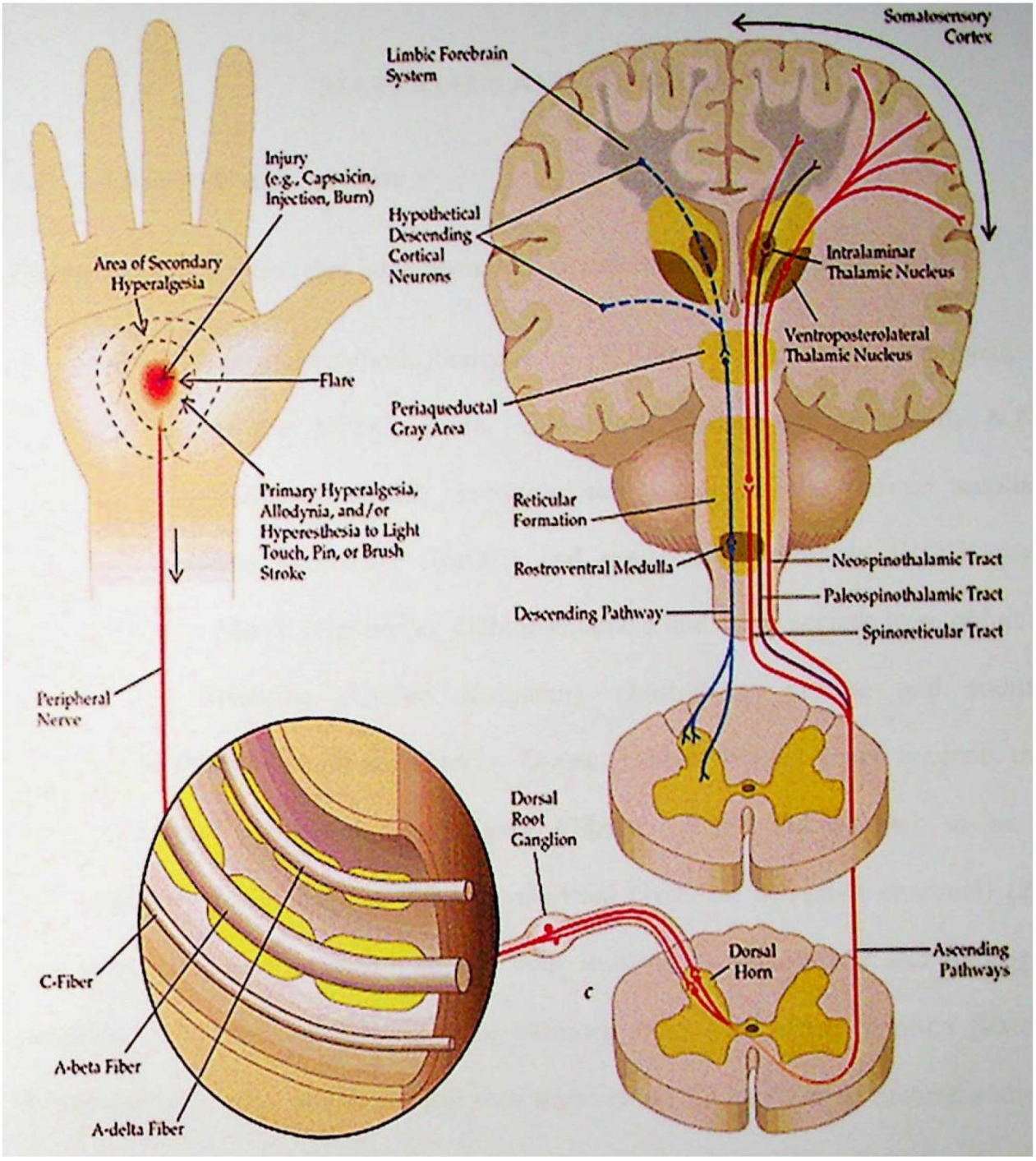


Figure 2.11: The pain pathway (adopted from The Functional Role of Pain, 2012)

CHAPTER THREE

MATERIALS AND METHODS

3.1 Chemicals and solvents

Chemicals and solvents that have been used in this research are as listed below.

(Bis(trifluoroacetoxy)iodo)benzene (PIFA), 4-dimethylaminopyridine (DMAP), acetonitrile HPLC grade, chloroform deuterated, methanol, N,N'-dicyclohexylcarbodiimide (DCC), nicotinic acid for synthesis, sodium sulphate anhydrous, sodium hydroxide (NaOH) and potassium hydroxide (KOH) were purchased from Merck (Germany), dichloromethane and ethyl acetate were obtained from Fisher Scientific (United Kingdom), chloroform, hexane and sodium borohydride ($NaBH_4$) were supplied by Q-Rec (United States). Some reagents that were used were purchased from Sigma Aldrich (United States) such as *meta*-chloroperoxybenzoic acid (MCPBA), palladium (10% on activated charcoal) (Pd) and *tert*-butyl hydroperoxide (TBHP, 70% in water). Acetonitrile and hydrogen peroxide, 30% in water (H_2O_2) were obtained from J.T. Baker (United States), formic acid (for mass spectroscopy) was supplied by Fluka (United States), sodium hydrogen carbonate ($NaHCO_3$) and Tween 20 were purchased from Systerm (Classic Chemical Sdn. Bhd., Malaysia).

3.2 Equipments and instruments

Equipments and instruments that have been used in this research are listed below.

Analytical balance (Mettler Toledo AL204 laboratory balance) was purchased from Mettler Toledo Inc. (United States), Fourier Transform Infrared spectroscopy (FTIR) (Nicolet 6700) was purchased from Thermo Scientific (United States), freeze dryer was supplied by Labconco (United States), Gas Chromatography Mass Spectrometer (Agilent 6890N) was purchased from Agilent Technologies (United States), Hot plate analgesia meter (Series 8, PE34) was supplied by IITC Life Science, Victory Blvd, Woodland Hill, CA 91367 (United States), hot plate stirrer was obtained from Fischer Scientific (United States), Liquid Chromatography Mass Spectrometer (Finnigan LCQ Duo) was purchased from Thermofinnigan (United States), melting point, SMP10 was supplied Bibby Scientific (United Kingdom), Nuclear Magnetic Resonance (Avance III 500 MHz) was supplied by Bruker (United States), oral feeding needle (Curve: 16 ga x 3 in.) was purchased from Pooper & Son Inc. (United States), rotary evaporator (RV 10 HB 10) was purchased from IKA (United States).