PART I

BIOLOGICAL ACTIVITIES AND CHEMICAL COMPOSITIONS OF THREE THAI MEDICINAL PLANTS

CHAPTER 1

Introduction

Herpes simplex virus (HSV) infections are common viral diseases in the world. HSV is in the Herpesviridae family. It can spread via infection secretions from one person to another without an animal vector. There are two important serotypes of HSV e.g. serotype 1 (HSV-1) and serotype 2 (HSV-2). HSV-1 is transmitted via oral secretions and often detected as symptoms on facial sites, while HSV-2 is usually transmitted via sexual contact and results in genital infections (1,2). Smith and Robinson summarized the data from peer reviewed articles of type-specific HSV seroepidemiologic surveys and reported that the prevalence of HSV-1 infection was high in most geographical areas worldwide and was more prevalent than HSV-2 infection in all non-high-risk populations surveyed except groups of HIV-positive persons and Commercial Sex Workers HSV-2 prevalence was found to be the highest in Africa and in the part of the Americas, was lower in western and southern Europe than in northern Europe and North America and was the lowest in Asia (3).

The HSV incubation period for the primary infection is about 6 days and range from 1-26 days before showing symptoms (1). Recurrent infections are found in some patients because HSV still hides in the ganglia without replication and when HSV was reactivated from its latent state, it will cause a recurrent infection which may show serious symptoms. Medical treatment generally uses acyclovir, valacyclovir or famciclovir as the first-choice drugs (4). However, HSV can develop resistance to acyclovir through viral gene mutation which decreases the efficacy of these drugs (5). Due to HSV resistant acyclovir and higher cost for long-term treatment, medicinal plants are another choice for the patients; some examples are *Plantago major* L., *Prunella vulgaris* and *Clinacanthus nutans* (6,7). Researchers still continue to find new medicinal plants which are effective against herpes simplex virus.

Cancer is the major cause of death of the world's populations. In 2012, 8.2 million people died from cancer, 14.1 million people were new cases and 32.6 million people were living with cancer (within 5 years of diagnosis) (8). The most commonly diagnosed cancers are lung, liver, stomach, colorectal, breast and esophageal cancers (9). In Thailand, cancer is the leading cause of death. The incidence rates of cancer in Thailand from 1990 to 2002 tended to increase in both males and females. The most common cancers in Thai males were liver and lung cancers, while Thai females mainly had cervical and breast cancers (10). Cancer is a disease in which normal cells are rapidly transformed into abnormal cells. These cells can invade other nearby cells and spread from the origin to other organs. The risk factors for cancers are genetic, smoking, drinking alcohol, unhealthy food intake, low fruit and vegetable consumption, no physical activity and chronic hepatitis diseases. Current treatments of cancers, are surgery, radiotherapy, chemotherapy and anticancer drugs, depending on the type of cancer, period of diagnosis and the patients' health (9). High costs and side effects of standard treatments are still found. Thus, cancer drug investigators are interested to search for new and more effective anticancer drugs. Researchers have found that some medicinal plants had anticancer activities; for example, *Vitis vinifera*, *Allium sativum* and *Hypericum perforatum* (11). They have been continuing to explore more effective anticancer activity from natural product sources.

Tuberculosis (TB) is a serious infectious disease which is caused by the bacteria, *Mycobacterium tuberculosis*. TB can be transmitted from one person to another through the air. TB bacteria will be spread into the air after people, who are infected with TB, cough, sneeze, split or talk. People nearby may inhale these bacteria and become TB infected (12). In 1993, TB was announced as a global emergency by the World Health Organization (WHO). One-third of the world's population has been infected by TB bacteria. WHO set Thailand in a group of twenty-two countries which have the largest number of TB cases since 1998. In 2012, WHO reported that 8.6 million people got sick with TB and 1.3 million people passed away. The WHO also expected that Thailand will have approximately 86,000 new TB cases per year or an incidence rate of 124 cases per 100,000 population (13). TB can be treated with standard anti-TB drugs. Multidrug-resistant tuberculosis, which does not respond to the standard anti-TB drugs,

has been commonly found. This result is a cause of the TB treatment failure. Another alternative treatment is the use of second-line drugs but they are restricted and the recommended drugs are not usually available (12,13). Consequently, the worldwide mortality rate of TB will gradually increase. Therefore, novel anti-tubercular drugs are the target for new drug discovery challenges. In Indonesia, people used the medicinal plants, such as *Andrographis paniculata*, *Brucea javanica* and *Caesalpinia sappan*, as traditional use for the treatment of tuberculosis symptoms (14). This data showed that other medicinal plants might possess more potent activities against *Mycobacterium tuberculosis.*

Other bacteria and fungi are the cause of many infectious diseases such as diarrhea, candidiasis and syphilis. The simple treatment will use antibacterial and antifungal medicines. However, natural products are still normally used as an alternative choice or as a supplement. For example, in Thailand, raw banana (*Musa sapientum Linn.*) has been used as an anti-diarrheal drug (15) and Galanga (*Alpinia nigra* (Gaertn.) B.L.Burtt) rhizome has been used to treat tinea (16).

Natural products have been widely used as medicines, food and dietary supplements since ancient times and till now. They are rich in bioactive constituents which are useful for human health. Some of them showed significant activities alone or increased their activities when combined with others (17,18). In Thailand, the biodiversity of medicinal plants are plenty and the traditional uses of these plants have been recorded (19). Due to their inexpensive price and commercial availability, many Thai people prefer to use Thai medicinal plants for the treatment common diseases or combine them with standard drugs. Therefore, medicinal plants are important resources for search for novel compounds for new drug development.

The aims of this project were:

1. To study the biological activities of the crude and pure extracts of three selected Thai medicinal plants.
2. To isolate, purify and elucidate the structure of the bioactive compounds from the selected Thai medicinal plants.

PART II

THE SYNTHESIS OF (–)-STEVIAMINE AND ANALOGUES

CHAPTER 1

Introduction

Medicinal plants have been used from ancient times as traditional medicines. The knowledge of many medicinal plants has been recorded or inherited from ancestor to descendant. The biological activities of some medicinal plants could not be supported scientifically and for this reason, many scientists have studied these plants in order to isolate and purify the pure natural product components for biological investigations. The quantities of active compounds obtained from these plants are often dependent on many factors, including, the landscape, the weather, the season and the time of harvest. Often large quantities of medicinal plants are required to obtain useful amounts of the bioactive natural product components, which is time consuming and costly. In some cases it is more cost effective to prepare these compounds using organic synthesis which also allows the preparation of analogues and stereoisomers for further biological testing and structure-activity studies.

Stevia rebaudiana Bertoni, belongs to the Asteraceae family, it is a native herb to the northern area of South America and is found growing in Brazil and Paraguay. It is commercially produced in Paraguay, Brazil, Uruguay, Central America, Israel, Japan, Korea, Thailand and China (186-187). The indigenous peoples of Brazil and Paraguay have used the *S. rebaudiana* leaves in tea and food as a sweetener. It has been used medicinally as a cardiogenic, a contraceptive, as a treatment for diabetes, obesity, hypertension, heartburn and decreased uric acid levels. In the World War II, Stevia was planted in England using as a possible sugar substitute (186-187). The biological activities of Stevia e.g. antibacterial, antifungal, antiviral, antitumor and antidiabetic activities have been investigated (186). The leaf extracts of *S. rebaudiana* contains constituents, which are responsible for Stevia's sweetness. They are stevioside, rebaudiosides A and C, and dulcoside A. The other compositions have been identified as, rebaudiosides B, D and E, steviolbioside, jhanol, austroinulin, 6-*O*-acetylaustroinulin, β-amyrin acetate, lupeol, β-sitosterol, stigmasterol, tannins and

volatile oils (187). Stevioside is sweeter approximately 300 times than sugar and non-caloric sweetener. This compound has been reported to possess the properties of hypoglycemia, antihyperglycemic, lowering high blood pressure, anti-inflammatory and immune modulator and also found to be nontoxic in acute and subacute toxicity (188). From the biological activities of crude Stevia extracts and stevioside, they can be applied for the treatment of many diseases including diabetes and high blood pressure (186-188).

In December 2008, the U.S. FDA gave a ‘‘no objection” approval for GRAS (Generally Recognized As Safe) status to Truvia (developed by Cargill and the Coca-Cola Company) and PureVia (developed by PepsiCo and the Whole Earth Sweetener Company, a subsidiary of Merisant), as a new zero-calorie sweetener. Both products consisted of rebaudiosides A which were derived from the Stevia plant (189). Due to the problem of a bitter aftertaste associated with Stevia, the flavor companies have been trying to find ways to mask it without devaluation any benefits of its natural condition. From a result of a research investigating the bitter composition, they have reported a new water-soluble iminosugar alkaloid in Stevia which was identified as “steviamine”. Alkaloids have not been formerly reported from Stevia species. During a continuing research for new iminosugars of *Veltheimia capensis* (Sand Lily, Hyacinthaceae family), which is a native to the Western Cape and South Africa, the same alkaloid was also found incidentally. There is no previously report of indolizidine alkaloids from the Hyacinthaceae family (189).

(−)-Steviamine [**1**] is the most recent member of the polyhydroxylated indolizidine natural products (Figure 1.1). Its absolute configuration was established by X-ray crystallographic analysis of its hydrobromide salt (189-190). (−)-Steviamine [**1**] is the first polyhydroxylated indolizidine to have a methyl group at C-5 and a hydroxymethyl group at C-3. This group of alkaloids which includes, swainsonine [**2**], castanospermine [**3**] and lentiginosine [**4**] (Figure 1.1) have potential utility as antidiabetic, antiviral, anticancer and immunoregulatory agents (191-192). Unlike swainsonine [**2**], steviamine [**1**] and its synthesized enantiomer ((+)-steviamine), have shown relatively weak to modest glycosidase inhibitory activity against a number of

different glycosidases (193). The most potent activity found in this study was against β-galactosidase (from rat intestinal lactase), where *ent*-steviamine had an IC_{50} value of 35 μM (193). While, *ent*-steviamine [**5**] (193) and some of its analogues, including 10-*nor*-steviamine [**6**] (194) (and some of its 1,2,3,8a-epimers) (194), 5-*epi*-*ent*-steviamine [**7**] (193) and 1,3-di-*epi*-10-(4-methoxyphenyl) steviamine [**8**] (195) have been synthesized recently, (−)-steviamine [**1**] itself has not been previously prepared.

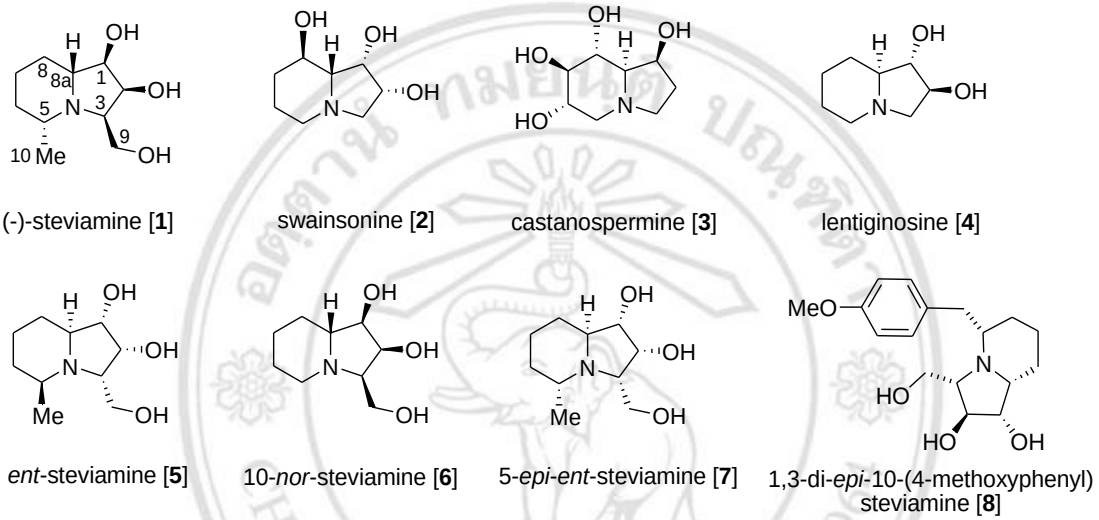

Figure 1.1 Representative polyhydroxylated indolizidine natural products [**1-4**] and some of synthetic analogues [**5-8**]

The aims of this project were:

1. To develop a new concise synthetic strategy for the preparation of (−)-steviamine and its analogues.
2. To test the synthesized compounds as glycosidase inhibitors.