

## CHAPTER I

### INTRODUCTION

Medicinal plants are one of the natural products evolved from man's desperate attempt to conquer physical suffering, coupled with an overwhelming desire for eternal life. There is a worldwide trend towards the use of drugs of natural origin since they are believed to possess less harmful side effects than synthetic drugs. There has also been an effort to develop medicinal plants in order to make them safe and effective such as the development of an antipeptic ulcer drug from Plao Noi (*Croton sublyratus* Kurz.) and used as an anti-ulcer agent.[1]

From the Thai pharmacopoeia, many plants in the "Plao" (*Croton* genus) are useful as folk medicine. For example, the barks and roots of Plao Ngoen (*C. cascarilloides* Raeusch.) can be used as an antifebrile, the woods of Plao Lueat (*C. robutus* Kurz.) can be used as an antianemic agent, the barks and leaves of Plao Noi can be used as antiulceric agent.[2]

*Croton oblongifolius* is an interesting Thai medicinal plant because it is believed that all parts of the plant can be used as drugs, such as the leaves can be used as a tonic, the flowers are used as a teniacide, the fruits are used to treat dysmenorrhea, the seeds are used as a purgative, the barks are used to treat dyspepsia, and the roots are used to treat dysentery.[3]

### 1.1 Botanical aspects of *Croton oblongifolius* Roxb.

*Croton oblongifolius* Roxb. is a medium sized deciduous tree in the Euphorbiaceae family. There are about 700 species in this family. In Thailand, it is commonly called Plao Yai (central) or Plao Luang (Northern). It is distributed throughout forests or shrubs below 700 meters above sea level. Its calyx and ovary are clothed with minute orbicular silvery scales. Leaves are 5.6-12.0 by 13.0-24.0 cm in size. The shape of leaf blade is oblong-lanceolate. Its flowers are pale yellowish green and solitary in the axials of minute bracts on long erect racemes. The male flowers are located in the upper part of the raceme and the females in the lower part. The male flowers are slender and have the length of pedicels of 4.0 mm. The calyx is more than 6.0 mm. long and segments are woolly. The twelve stamens are inflexed in bud and the length of filaments is 3.0 mm. In female flowers, the pedicels are short and stout. Its sepals are more acute than in the male with densely ciliated margins. The diameter of the fruit is less than 1.3 cm., slightly 3-lobed and clothed with small orbicular and quite smooth on the back.[4,5] The picture of stem bark, leaf, flower and fruit of *Croton oblongifolius* Roxb. are shown in Fig.1.

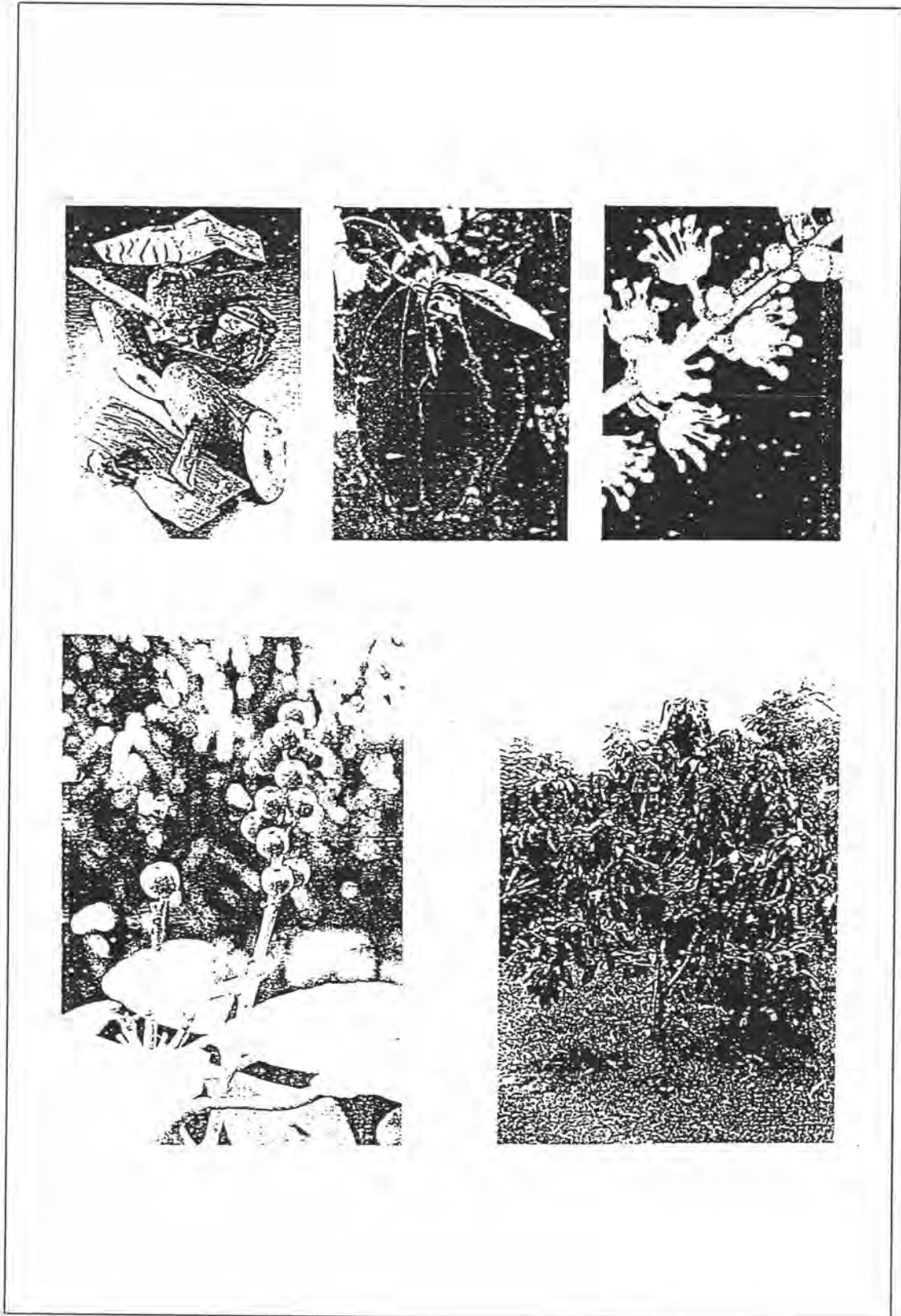


Fig. 1 *Croton oblongifolius* Roxb.

## 1.2 The chemical constituents of *Croton oblongifolius* Roxb.

From the literature surveys, *Croton oblongifolius* Roxb. have been widely studied and many diterpenoid compounds have been isolated and characterized in table below.

In 1968, Rao, P. S., Sachdev, G. P., Seshadri, T. R., and Singh, H. B.[6] studied chemical constituents from the bark of *Croton oblongifolius* Roxb. They found a new diterpene alcohol, oblongifoliol together with  $\beta$ -sitosterol.

In 1969, Aiyar, V. N., Rao, P. S., Sachdev, G. P., and Seshadri, T. R.[7] found deoxyoblongifoliol from the stem bark of *Croton oblongifolius* Roxb.

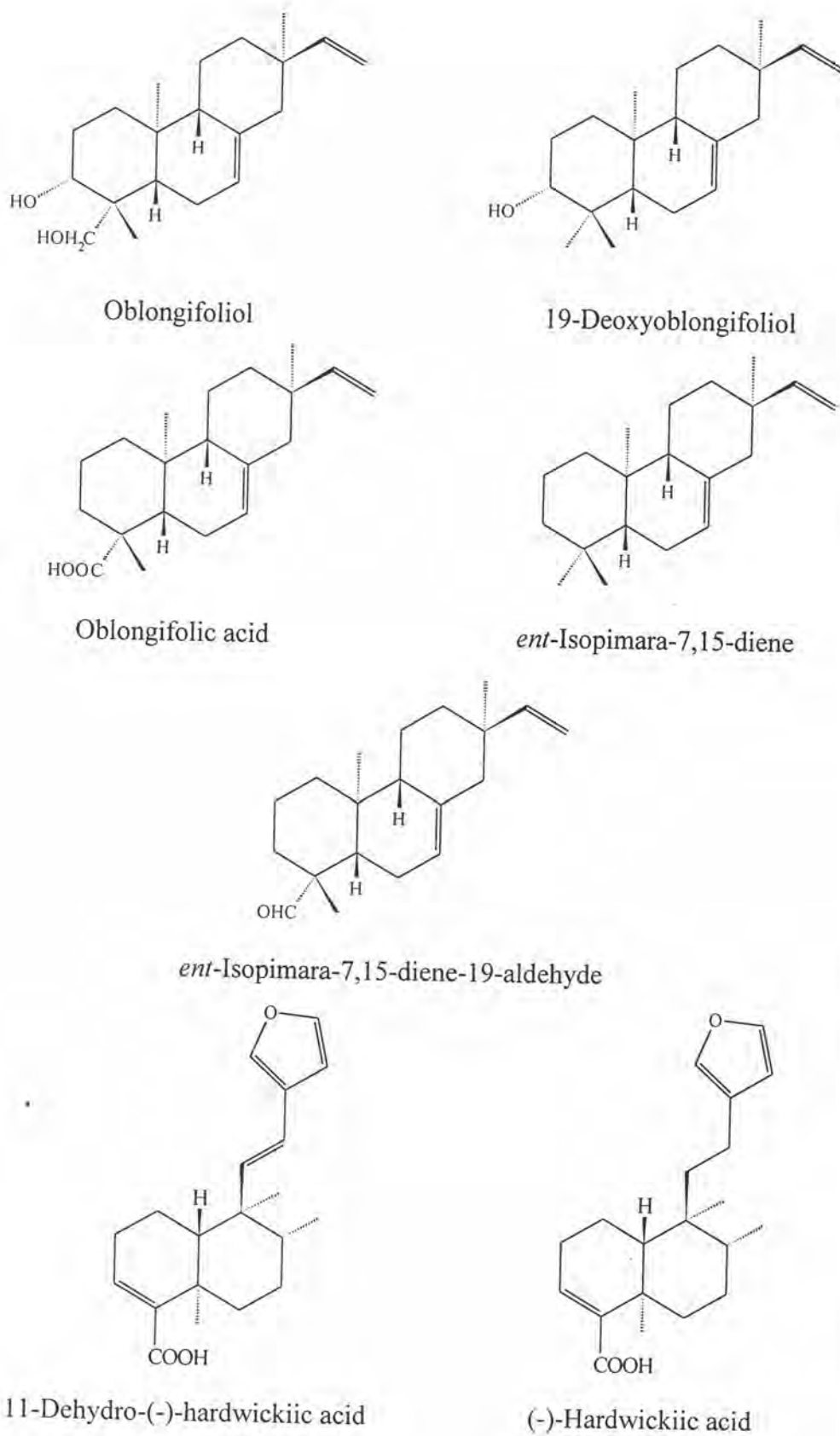
In 1970, Aiyar, V. N. and Seshadri, T. R.[8] studied the structure of oblongifolic acid, the major diterpene acid component of the bark, it was assigned as isopimara-7(8),15-diene-19-oic acid.

In 1971 Aiyar, V. N. and Seshadri, T. R. determined the structures of oblongifoliol and deoxyoblongifoliol again. Two components have been assigned their structure as *ent*-isopimara-7,15-diene-3 $\beta$ ,19-diol and *ent*-isopimara-7,15-diene-3 $\beta$ -ol, respectively.[9] In the same year, they found three new minor components from the bark. One was *ent*-isopimara-7,15-diene, the second was 19-hydroxy-*ent*-isopimara-7,15-diene and the third was *ent*-isopimara-7,15-diene-19-aldehyde.[10] Moreover, Acetyl aleuritolic acid, 3 $\beta$ -acetoxy-olean-14(15)-ene-28-oic acid, has been obtained from the bark.[11]

In 1972, Aiyar, V. N. and Seshadri, T. R. found two closely related furanoid diterpenes from the bark. One was *ent*-15,16-epoxy-3,11,13(16),14-clerodatetraen-19-oic acid or 11-dehydro(-)-hardwickiic acid and the second was (-)-hardwickiic acid.[12] They studied other parts of *Croton oblongifolius* Roxb. including the root-bark, wood, and leaves. Most compounds reported were isolated from the stem-bark were in poorer yields, while the leaves gave only waxy materials.[13]

**Table 1** The Chemical Constituents of *Croton oblongifolius* Roxb.

Plant parts	Substances	References
Bark	Oblongifoliol	6
	19-Deoxyoblongifoliol	7
	3-Deoxyoblongifoliol	9
	Oblongifolic acid	8
	<i>ent</i> -Isopimara-7,15-diene	9
	<i>ent</i> -Isopimara-7,15-diene-19-aldehyde	9
	19-Hydroxy- <i>ent</i> -isopimara-7,15-diene	9
	(-)-Hardwickiic acid	10
	11-Dehydro(-)-hardwickiic acid	12
Wood	Oblongifoliol	6
	19-Deoxyoblongifoliol	7
	Oblongifolic acid	8
	<i>ent</i> -Isopimara-7,15-diene	9
	3-Deoxyoblongifoliol	9
	<i>ent</i> -Isopimara-7,15-diene-19-aldehyde	9
	(-)-Hardwickiic acid	10
	Acetyl aleuritolic acid	11
	11-Dehydro(-)-hardwickiic acid	12
	Leaves	Waxy materials



**Fig. 2** The structures of diterpenoid compounds from *Croton oblongifolius* Roxb.

### 1.3 Significance of the Problem

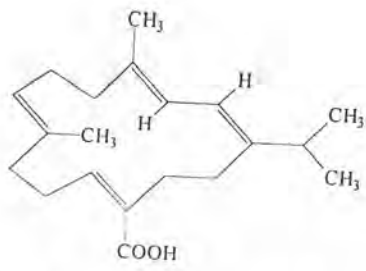
From the above information, it can be seen that the chemical constituents in *Croton oblongifolius* Roxb., which were found in specimens from India, are mainly categorized into the isopimarane and clerodane groups. When these chemical constituents were compared with the chemical constituents found in *Croton oblongifolius* Roxb. from Thailand, it is found that the main constituents are different. It was discovered that the main chemical constituents in *Croton oblongifolius* Roxb. found in Amphur Vicheinburi, Petchaboon province are cembranoid diterpene compounds.[14,15] On the other hand, specimen from Amphur Pranburi, Prachuabkirikhan province gave labdane type of diterpenoids. [16]

Since many cembranoid compounds have been shown to inhibit the growth of cancer cells, therefore, the new cembranoid compounds, isolated from *Croton oblongifolius* Roxb. found in Thailand have been tested for their cytotoxicity against P 388 cell line. These compounds show interesting cytotoxicity (this is further discussed in latter parts of the thesis). Furthermore, quite a large amount of the cembranoid compounds had been isolated from stem bark of *Croton oblongifolius* Roxb. Thus, it permits further study of biological activity of the cembranoid compounds as well as their derivatives. The newly discovered cembranoid compounds, posses carboxylic acid, a functional group that can easily be changed into another functional group. In this study, the carboxylic acid group was changed into methyl ester, alcohol and aldehyde and they were tested for their biological activities.

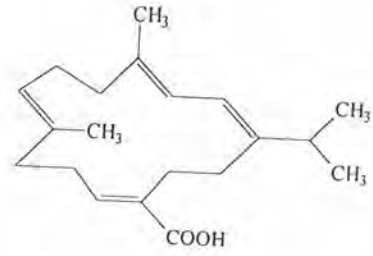
In order to find reliable sources of cembranoid compounds, *Croton oblongifolius* from various locations in Thailand were investigated. Using NMR screening of the crude hexane extract, it turns out that a large variety of diterpenoid compounds were found. For example, cembranoid compounds were found in the stem barks of *Croton oblongifolius* Roxb. from Amphur Sai Yok, Kanchanaburi province while specimen from Amphur Pranburi, Prachuabkirikhan province gave four new labdane diterpenoids. [16]

Therefore, the investigation of the chemical components of stem bark of *Croton oblongifolius* Roxb. from the different locations including Amphur Sai Yok, Kanchanaburi province (location 2, approximately 3 km away from the first location), Amphur Pakchong, Nakornratchasima province, Amphur Panusnikom, Chonburi province, and Amphur Muang, Prachuabkirikhan province, was undertaken. In addition, the biological effects of the chemical components from the stem bark of *Croton oblongifolius* Roxb., obtained from different locations were tested, and they showed many interesting results. Therefore, it is of much interest to study chemical components as well as their biological activities in *Croton oblongifolius* Roxb. available from various locations in Thailand.

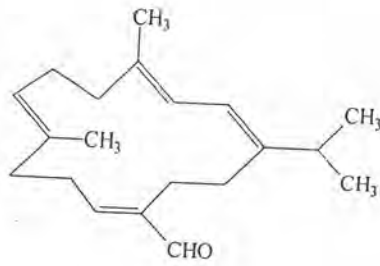




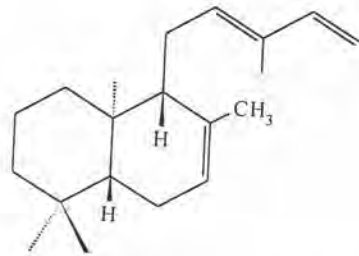
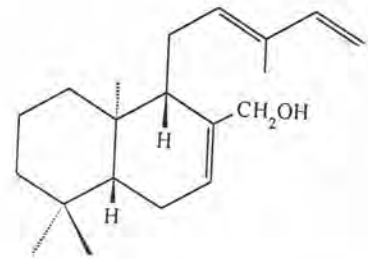
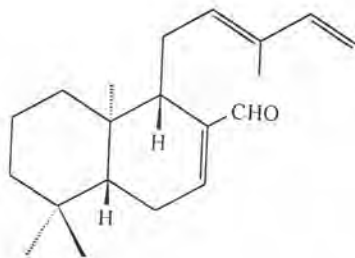
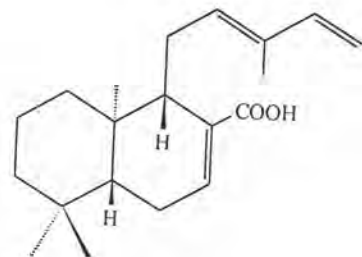
Crotocebraneic acid



Neocrotocebraneic acid



Neocrotocebranal

Labda-7,12(*E*),14-trieneLabda-7,12(*E*),14-triene-17-olLabda-7,12(*E*),14-triene-17-alLabda-7,12(*E*),14-triene-17-oic acid

**Fig. 3** The structures of diterpenoid compounds from *Croton oblongifolius* Roxb. in Thailand.

#### 1.4 The objectives of this research

The objectives of this research can be summarized as follow:

1. To extract, isolate, and purify the organic constituents from the stem bark of *Croton oblongifolius* Roxb.
2. To elucidate the structure of the isolated compounds obtained from the stem bark of *Croton oblongifolius* Roxb.
3. To modify the structures of cembranoid compounds as well as other diterpenoid compounds which were isolated from *Croton oblongifolius* Roxb.
4. To investigate the biological activity of the compounds thus obtained.