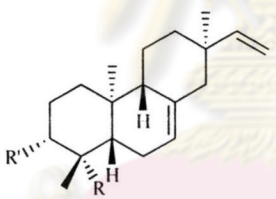
Chapter 1

Introduction

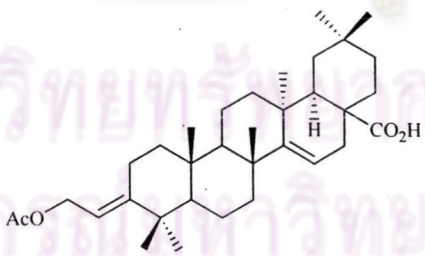
1.1 Chemical Components of *Croton oblongifolius* Roxb.

Croton oblongifolius Roxb. (Euphorbiaceae) is a perennial herb widely distributed throughout Thailand. It has been used as a traditional medicine for many applications such as to alleviate dysmenorrhea, as a purgative, and to treat dyspepsia and dysentery. Additionally, this plant has been used in conjunction with *C. sublyratus* to treat gastric ulcers and gastric cancers.

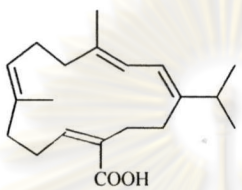
During the late 1960s and early 1970s, Seshadri *et al* reported the isolation and structure elucidation of 6 pimarane diterpenes (**1-6**) and an oleane-type triterpene (**7**) from stem bark of *C. oblongifolius* [1-5].



1, R = CH_2OH ; R' = OH
2, R = CH_3 ; R' = OH
3, R = CO_2H ; R' = H
4, R = CH_3 ; R' = OH
5, R = CHO ; R' = H
6, R = CH_2OH ; R' = H



However, our recent investigation on the chemical constituents of this plant, which is available in various parts of Thailand, resulted in the isolation of several new diterpenoids. This is presumably due to the geographic variation. Three novel cembranoid diterpenes, crotocembraneic acid (**8**), neocrotocembraneic acid (**9**) and neocembranal (**10**), were obtained from a specimen which was collected in the central part of Thailand, Petchaboon province [6-7].



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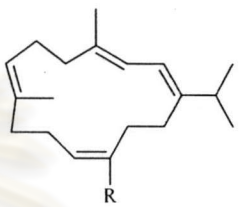
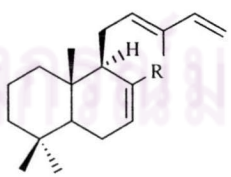
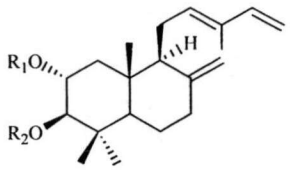


9, R = COOH
10, R = CHO

Four new labdane-type diterpenes, labda-7,12(*E*),14-triene (**11**), labda-7,12(*E*),14-triene-17-al (**12**), labda-7,12(*E*),14-triene-17-ol (**13**), and labda-7,12(*E*),14-triene-17-oic acid (**14**), were isolated from another specimen No.BKF 084729, collected from Prachuab Kirikhan province in the southern part of Thailand [8]. Moreover, different three new labdane diterpenoids, 2-acetoxy-3-hydroxy-labda-8(17),12(*E*),14-triene (**15**), 3-acetoxy-2-hydroxy-labda-8(17),12(*E*),14-triene (**16**), and 2,3-dihydroxy-labda-8(17),12(*E*)-14-triene (**17**), were obtained from a specimen collected from the northern part of Thailand, Loei province [9].



11, R = CH_3
12, R = CHO
13, R = CH_2OH
14, R = CO_2H



15, R_1 = Ac ; R_2 = H
16, R_1 = H ; R_2 = Ac
17, R_1 = H ; R_2 = H

1.2 Cancer-related Properties of Labdanes

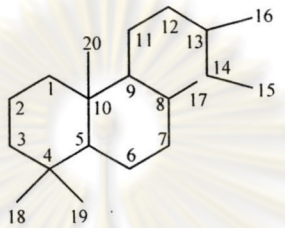
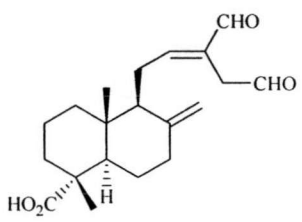


Figure 1.1 labdane skeleton

Labdane is a diterpene which its skeleton consists of a decalin system and a C-6 side chain at C(9) position (Figure 1.1). A very large number of diterpenoids possessing a labdane skeleton occur in nature [10]. The interest in studying labdanes is heightened due to the wide range of their biological activities [11] including antibacterial, antifungal, antiprotozoal, enzyme induction and anti-inflammatory modulation of immune cell functions. In addition, it is interesting that many labdane-type diterpenes also exhibit significant properties against cancer cells. A number of this diterpene families exhibit remarkable antiproliferative and cytotoxic activities.

Itokawa *et al* [12] found that 8(17),12(*E*)-labdadiene-15,16-dial (**18**) and four new coronarins A-D (**19-22**) isolated from the rhizomes of *Hedychium coronarium* (Zingiberacae), which exhibit cytotoxic activity, particularly coronarin A and B. 8(17),12(*E*)-Labdadiene-15,16-dial (**18**) also showed a moderate cytotoxicity when tested against KB cells (epidermoid carcinoma) with IC_{50} value of 40 μg/ml [13]. Recently, Kingston *et al* [14] reported another labdane diterpene, 11-hydroxy-8(17),

12(*E*)-labdadien-15,16-dial-11,15-hemiacetal (**23**), from *Renelmia alpinia* Zingiberacae, which possessed good cytotoxicity activity against M109 (Madison Lung Carcinoma cell line).



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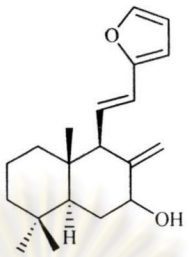

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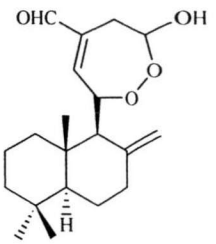

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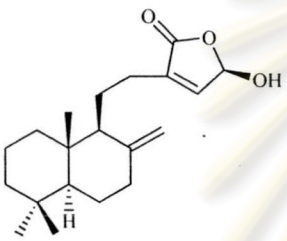

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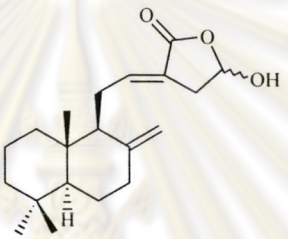

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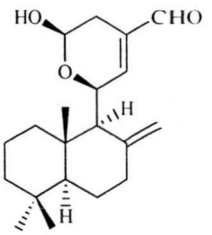
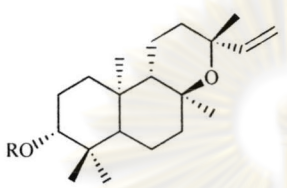


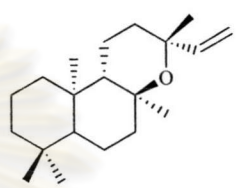
23

It is known that labdane diterpenes are the predominant metabolites in various parts of *Cistus* sp., as well as in the resin of the same species. Seven labdane diterpenes (**24-30**) were isolated from *Cistus creticus* and it was revealed that most of them showed strong cytotoxic activity in a preliminary screening of the chromatographic fractions [15]. After *in vitro* testing, it was found that the diterpenes **27**, **29** and **30** were active against RAJI cells, a pre-B-cell line. These cell lines were obtained from a patient with Burkitt lymphoma; MOLT3, a human cell T cell line originating from a patient with acute lymphoblastic leukemia (ALL); and H-9 cells originating from a patient (ALL) [16]. These diterpenes were also found to be active against the murine leukemia P-388 and KB cell lines. Moreover, extensive studies were carried out on a number of labdane-type diterpenes. semisynthetic and naturally occurring in *Cistus* sp. for their activity against a panel of 14 human leukemic cell lines [17-19]. Diterpene **27**

showed a significant cytotoxic activity, exhibiting IC_{50} values below 20 μg/ml in 13 cell lines. Furthermore, compound **27** was also tested against NCI-H460 (Lung), MCF-7 (Breast) and SF-268 (CNS) cell lines at a single dose of $1.0x10^{-4}$ M at the National Cancer Institute (NCI), USA. It was found to be considerably active as it caused the death of 80%, 89%, and 88% of the cell population, respectively [20].



24, R = H
25, R = Ac



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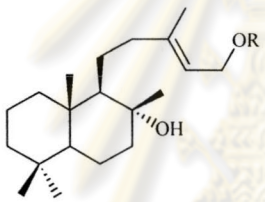
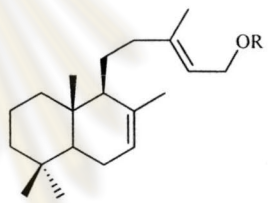
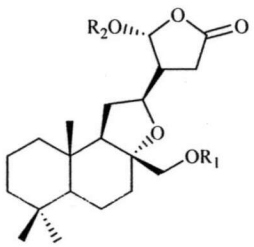


27, R = H
28, R = OAc



29, R = H
30, R = Ac

A cytotoxic labdane diterpene with a new type of carbon skeleton, acuminolide (**31**) was isolated from the stem bark of *Neouvaria acuminatissima* along with its congeners 17-O-acetylacuminolide (**32**) [21]. These two diterpenes were evaluated against a panel of human cancer cell lines and cultured P-388 cells, which both compounds showed broadly cytotoxic activities, exhibiting ED_{50} values ranging from 10^{-1} to 10^{0} μg/ml in several cell lines. Among the human cell lines, the most potent activity was observed with melanoma (Mel2) (ED_{50} = 0.7 μg/ml) and prostate (LNCaP) (ED_{50} = 0.8 μg/ml) cell for diterpenes **31** and **32**, respectively.



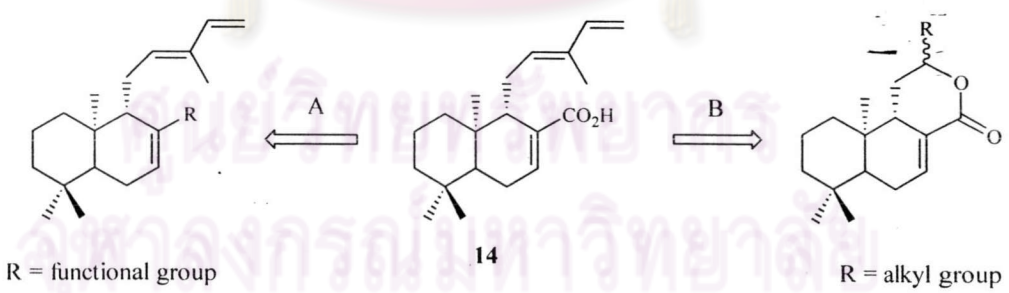
31, R_1 = H, R_2 = H
32, R_1 = Ac, R_2 = H

As aforementioned, our group has recently reported the isolation of three new labdane diterpenoids (**15-17**) from stem bark of *C. oblongifolius* collected at Loei province in the northern part of Thailand. These three diterpenes were tested for their cytotoxicities against 5 human tumor cell lines; human gastric carcinoma (KATO-3), colon adenocarcinoma (SW620), breast ductol carcinoma (BT474), liver hepatoblastoma (HEP-G2), and undifferentiated lung carcinoma (CHAGO). It was found that diterpene **17** showed non-specific moderate cytotoxicities against all of tested cell lines (2.2-4.6 μg/ml). Diterpene **15** showed weak activity against gastric (5.7 μg/ml) and colon adenocarcinoma (7.1 μg/ml) while diterpene **16** showed moderate activity against gastric (3.3 μg/ml) and weak activity against breast ductol carcinoma (5.9 μg/ml) [9].

1.3 The Proposed Chemical Transformation of Labdane Diterpene from *C. oblongifolius*

By considering the chemical structures of labdane diterpenoids and their cytotoxic activities against human cancer cell lines as mentioned above, it was suggested that the side chain bearing allylic alcohol or allylic acetate moiety and cyclic lactone ring of labdane skeleton plays important roles for their cytotoxicity. Therefore, we decided to modify the side chain of natural labdane diterpene, from *C. oblongifolius,* into substances which might possess inhibitory activity against cancer cell lines, possibly exhibiting anticancer activities. Furthermore, the cytotoxic activities of the modified substances would bring to the evaluation of the structure and activity relationship.

In this study, labda-7,12(*E*),14-triene-17-oic acid (**14**) was selected as a starting material due to large quantity of the compound. The modification of diterpene **14** was proposed to perform by two pathways. The first pathway is path A, the C(17) carboxylic acid moiety of **14** would be transformed into related functional groups and the second pathway is path B, compound **14** would be converted to substances containing 6-membered lactone ring on decalin system as shown in Scheme 1.1.



Scheme 1.1

The another pathway is the chemical conversion of labdane diterpene **14** into limonidilactone (**33**), a natural labdane diterpene isolated from the leaves of *Vitex*

limonifolia in 1995 by Aphaijitt *et al* [22]. The structure and relative configuration of this labdane diterpene was established based on spectroscopic and X-ray diffraction experiments. The absolute configuration of this compound has bee established as shown in Figure 1.2 by comparing its spectroscopic data and optical rotation with limonidilactone (**34**) synthesized by Marcos *et al* [23-24]. In this synthetic method, limonidilactone was synthesized from a natural labdane **35**, zamoranic acid, isolated from *Halimium viscosum* via the six-step sequence as shown in Scheme 1.2.

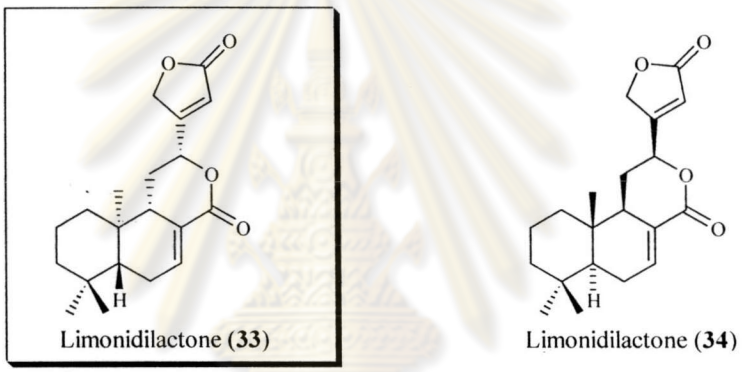
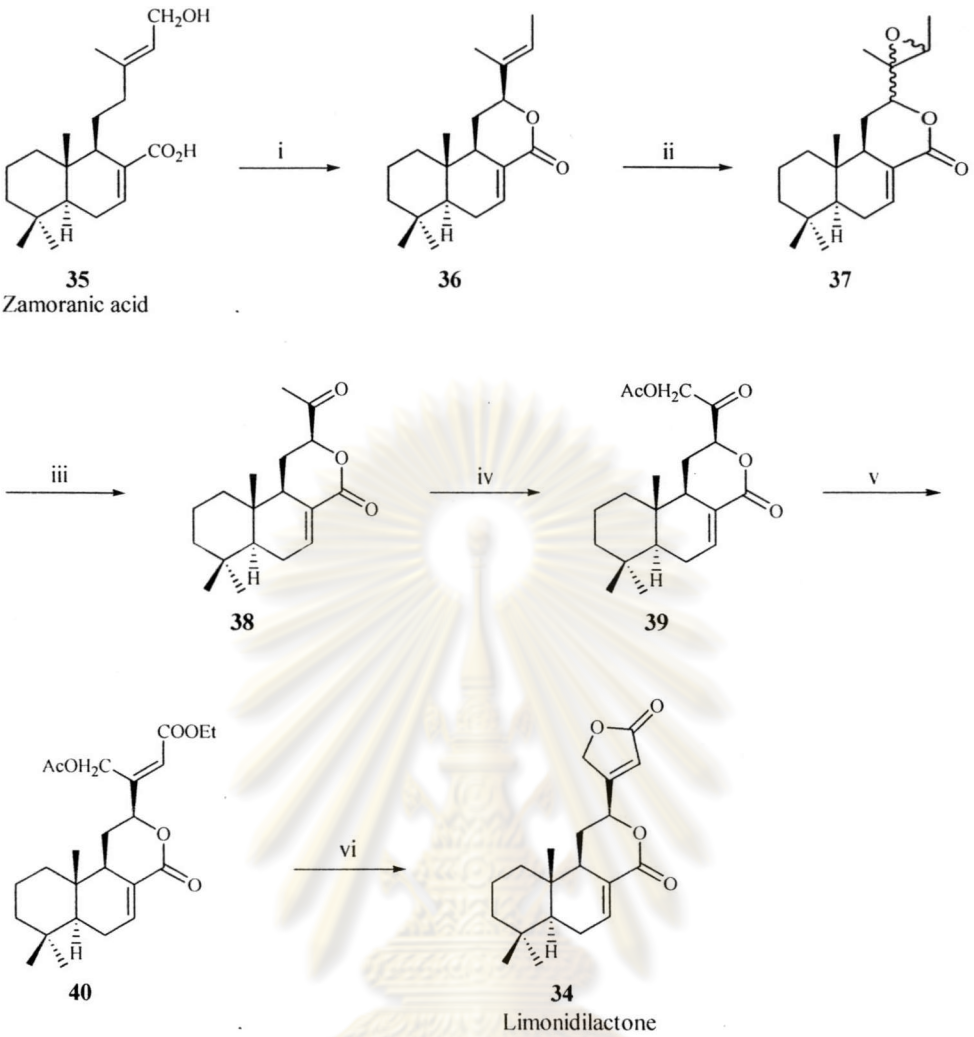


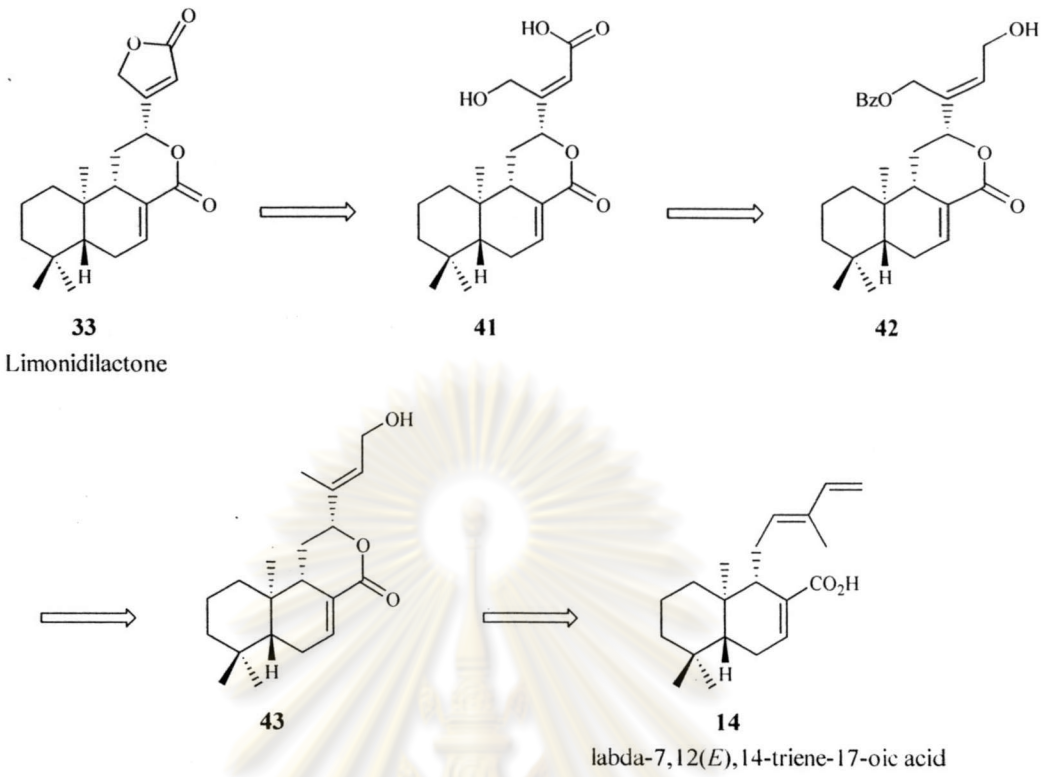
Figure 1.2



Reagents and conditions: (i) *p*-TsOH, benzene, 60°C; (ii) *m*-CPBA, DCM, rt; (iii) H_5IO_6, THF:H_2O (2:1), rt; (iv) $Pb(OAc)_4$, benzene, $BF_3.OEt_2$, MeOH; (v) $Ph_3PCHCOOEt$, benzene, reflux; (vi) *p*-TsOH, MeOH, rt

Scheme 1.2

On the basis of the retrosynthetic analysis of limonidilactone (**33**) from labda-7,12(E),14-triene-17-oic acid (**14**) as shown in Scheme 1.3, the closure of 6-membered lactone ring would occur first to provide compound **41** with stereochemical control at C(12) position. Following the functionalization of C(15) and C(16), γ-lactone ring would then be cyclized to give rise to target compound **33**.



33
Limonidilactone

41

42

43

14
labda-7,12(*E*),14-triene-17-oic acid

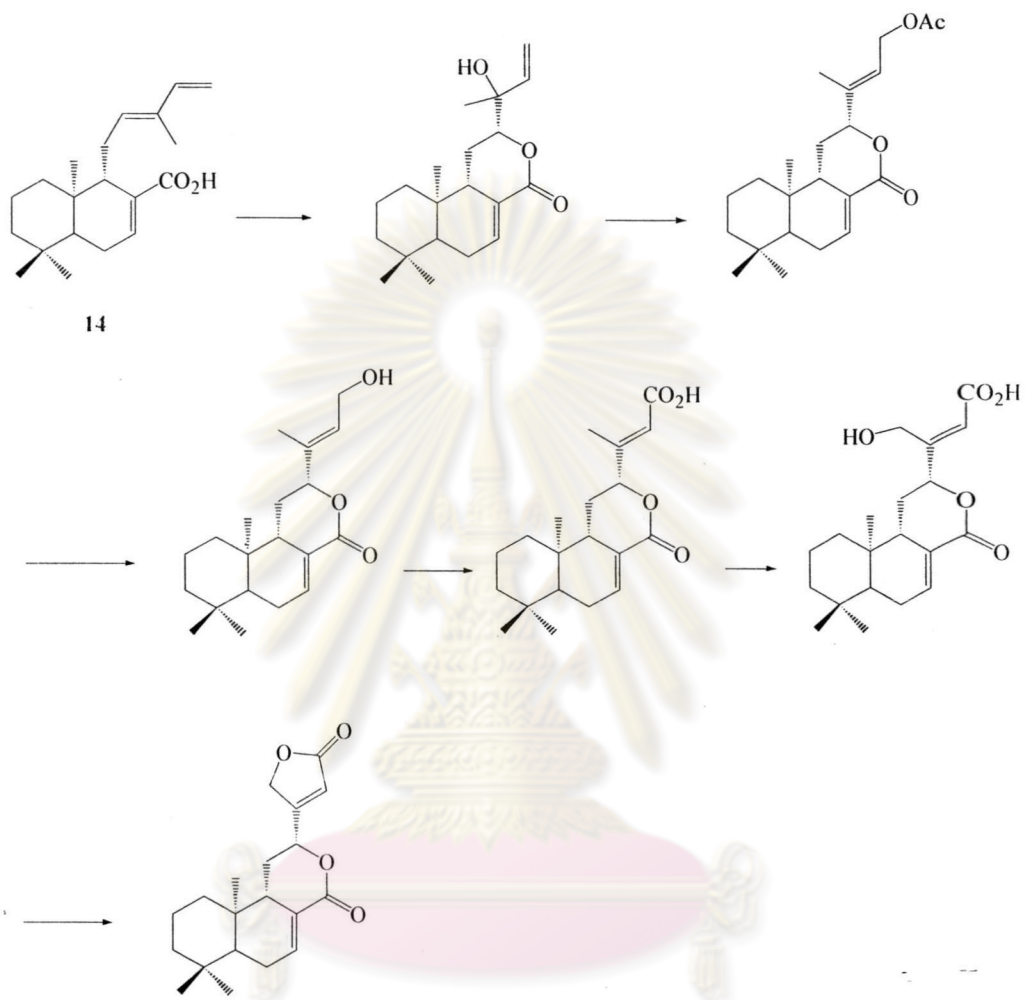
Scheme 1.3

Although the biological activities of (-)-limonidilactone (**33**) have never been reported, by investigation of structure possessing γ-butenolide and δ-lactone system on labdane skeleton, we might anticipate that this diterpene should exhibit cytotoxic activity against some human cancer cell lines.

The objectives of this research is as follows :

1. To synthesize limonidilactone (**33**) from labda-7,12(*E*),14-triene-17-oic acid (**14**).
2. To change the functional group of labda-7,12(*E*),14-triene-17-oic acid (**14**) to other functional groups.
3. To investigate cytotoxic activity of compounds which obtained from chemical reactions.

We proposed the pathway for synthesizing limonidilactone (**33**) from compound **14** by a series of chemical reactions as shown in scheme 1.4.



Scheme 1.4