CHAPTER II

LITERATURE REVIEWS

2.1 General characterization of plants in the Genus Croton [11].

The genus Croton comprises 700 species of trees or shrubs. Their flowers are solitary or clustered in the rachis of a terminal raceme and the bracts are small. Male flowers contain 5-calyx, 5-petals. There are many stamens inserted on a hsiry receptacle. In female flowers, sepals are usually more ovate than the male, petals are smaller than the sepals or missing and disk annular of 4-6 glands are opposite the sepals. There are three ovaries with a solitary ovule in each cell. Seeds are smooth, albumen copious and broad cotyledons. Leaves are usually alternate with 2-glandular stipule at the base.

2.2 General characterization of *Croton oblongifolius* Roxb. [12]

Croton oblongifolius Roxb. is a medium sized tree. 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. Flowers are pale yellowish green and solitary in the axial of minute bracts on long erect racemes. The male flowers are locates in the upper part of the raceme and the females in the lower part. Male flowers are slender and have of pedicels the length of 4.0 mm. The calyx is more than 6.0 mm long and the segments are ovate, obtuse and more than 2.5 mm long. Petals are 3.0 mm long, elliptic-lanceolate and woolly. The twelve stamens are inflected in the bud and the lengths of the filaments are 3.0 mm. Inflames 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 scales.

<u>Figure 1</u> ***Croton oblongifolius*** **Roxb.**

2.3 The chemical constituents of *Croton oblongifolius* Roxb.

From the literature surveys *C. oblongifolius* Roxb. has been widely studies and many diterpenoid compounds have been isolated and characterized as in the following examples.

The chemical constituents from the barks of *C.oblongifolius* Roxb. were first investigated by Rao, P.S, Sachdev, G.P., Seshadri, T.R and Singh, H.B. [13] in 1968. They found a new diterpene alcohol, oblongifoliol.

In 1969, Aiyar, V.N, Rao, P.S, Sachdev, G.P. and Seshadri, R.R. [14] found deoxyoblongifoliol from the stem barks of *C. oblongifolius* Roxb.

Aiyar, V.N and Seshadfri, T.R. [15] in 1970 investigated of the structure of oblongifolic acid, the major diterpene acid component of the structure of the barks of *C. oblongifolius*. They proposed its structure to be isopimara-7(8),15-diene-19-oic acid.

In 1971 Aiyar, V.N. and Seshadri, T.R. identified the structure of oblongifoliol and deoxyoblongifoliol led to the assignment of their structures of *ent*-isopimara-7,15-diene-13β, 19-diol and *ent*-isopimara-7,15-diene-3β-ol,respectivel[16]

In the same year, Aiyar, V.N. and Seshadri, T.R. discovered three new minor components from the barks which were identified as *ent*-isopimara-7,15-diene, 13-hydroxy-*ent*-isopimara-7,15-diene and *ent*-isoppimara-7,15-diene-19-aldehyde [17]. Moreover, acetyl aleuritolic acid and 36-acetoxy-olean-14(15)-ene-28-oic acid were found in the barks [18].

In 1972, Aiyar, V.N. and Seshadri, T.R. reported the discovery of two furanoid diterpenes from the barks, namely *ent*-15,16-epoxy-3,11,13(16), 14-clerodatetraen-19-oic acid or 11-dehydro(-)-hardwickiic acid and (-)-hardwickiic acid [19].

In addition, in the same year the other parts of *C.oblongifolius* Roxb., including the root-bark, wood and leaves were studied. The stem bark was found to give a poorer yield of diterpene compounds, while the leaves gave only waxy materials [20].

The wood and leaves of *C.oblongifolius* Roxb. were investigated by Roengsumran, S., Petsom, A., Pudhom, K., Surachedtapan, S., Archayindee, S., and Vilaivan, T., in 1998 led to the isolation of two new cembrane diterpenes namely crotocembraneic acid and neocrotocembraneic acid from barks and leaves [21].

In 1999, Roengsumran, S., Singtothong, p., Pudhom, K., Ngamrojnavanich, N., Petsom, A., and Chichantipyuth, C., discovered new cembrane diterpene compound, neocrotocembranal from *C. oblongifolius* Roxb.[22]

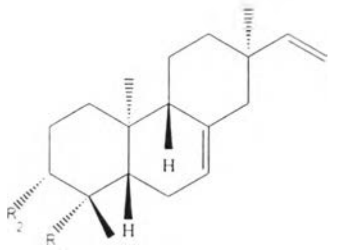
In the same year, Roengsumran, S., Petsom, A., Sommit, D., and Vilaivan, T., reported the discovery of four new labdane compounds which were identified as labda-7,12(E),14-diene, labda-7,12(E),14-triene-17-al, labda-7,12(E),14-triene-17-ol and labda-7,12(E),14-triene-17-oic acid.[7]

Table 1 The chemical constituents of *Croton oblongifolius* Roxb.

Organic compounds	Location	Reference
Oblongifoliol	India	13
19-Deoxyoblongifoliol	India	14
Oblongifolic acid	India	15
Ent-isopimara-7,15-diene	India	16
3-Deoxyoblongifoliol	India	16
Ent-isopimara-7,15-diene-19-aldehyde	India	16
Acetyl aleuritolic acid	India	17
19-Hydroxy-ent-isopimara-7,15-diene	India	16
Labda-7,12(E),14-diene	Prachaubkhirikhan	7,11
Labda-7,12(E),14-triene-17-al	Prachaubkhirikhan	7,11
Labda-7,12(E),14-triene-17-ol	Prachaubkhirikhan	7,11
Labda-7,12(E),14-triene-17-oic acid	Prachaubkhirikhan	7,11
Crotocembraneic acid	Petchaboon	5,21,23
Neocrotocembraneic acid	Nakonratchasima	5,21,24
Crotohalimaneic acid	Kanchanaburi	5,22
Benzoyl crotohalimaneic acid	Nakonrratchasima	5,22
Crovatin	Kanchanaburi	5
Isokolavenol	Kanchanaburi	5
Nidorellol	Loei, Sakolnakorn	5
Poilaneic acid	Chaingmai	5
Hardwickiic acid	India, Chonburi,	5,8,17
11-Dehydrohardwickiic acid	Udonthani	17
Labda-7,13(Z)-diene-17,12-olide	India	8
Labda-7,13(Z)-diene-17,12-olide-5-ol	Udonthani	8
(-)-20-Benzyloxyhardwickiic acid	Udonthani	8

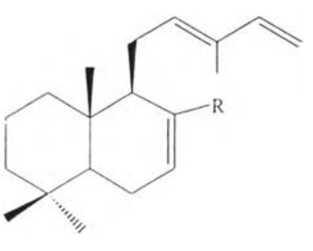
Organic compounds	Location	Reference
(-)-Pimara-9(11),15-diene-19-oic acid	Uttaradit	9
(-)-Pimara-9(11),15-diene-19-ol	Uttaradit	9
(2E,7E,11E)-1-Isopropyl-1,4-dihydroxy-4,8-dimethylcyclotetradeca-2,7,11-triene-12-carboxylic acid	Uttaradit	9
Methyl-15,16-epoxy-12-oxo-3,13(16),14-clerodatriene-20,19-olide-17-oate	Uttaradit	9
3-Acetoxy-labda-8(17),12(E)-triene-2-ol,	Loei	10
2-Acetoxy-labda-8(17),12(E)-triene-3-ol	Loei	10
Labda-8(17),12(E)-triene-2,3-diol	Loei	10

Isopimarane Group



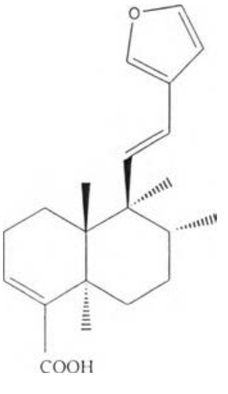
R_1	R_2	
CH_2OH	OH	:Oblongifoiol
CH_3	OH	:Deoxyoblongifoliol
COOH	H	:Oblongifolic acid
OH	H	:*ent*-isopimara-7,15-diene-3,19-diol
CH_3	H	:*ent*-isopimara-7,15-diene

Labdane Group

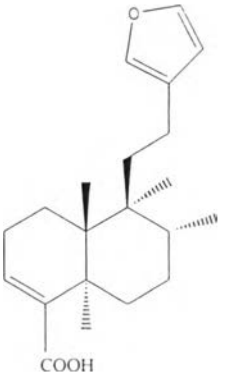


R	
CH_3	Labda-7,12(E),14-triene
CHO	Labda-7,12(E),14-triene-17-al
CH_2OH	Labda-7,12(E),14-triene-17-ol
COOH	Labda-7,12(E),14-triene-17-oic-acid

Clerodane Group



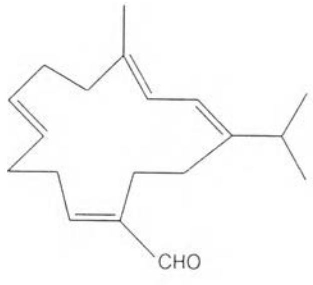
11-dehydro--(-)-hardwickiic acid



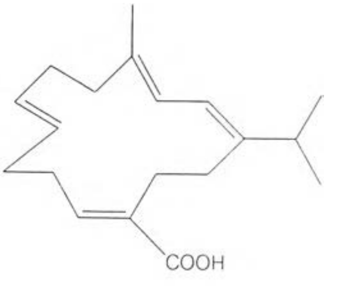
(-)-hardwickiic acid

Figure 2 : The structure of Diterpene Compounds from *C. oblongifolius*

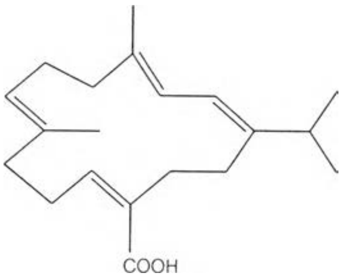
Cembrane Group



neocrotocembranal

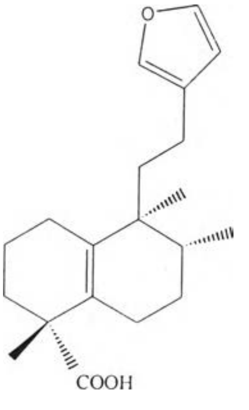


neocrotocembraneic acid

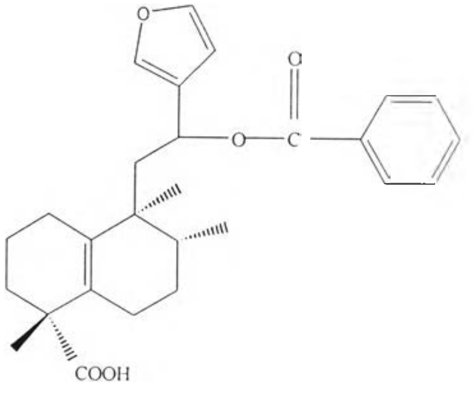


crotocembraneic acid

Halimane Group



crotohalimaneic acid



benzoyl crotohalimaneic acid

2.4 Biological activity of Diterpene compounds.[25]

Various biological activities have been reported for diterpenoids, principally from the members of the Ericaceae, Euphorbiaceae, Lamiaceae and Compositae. Each of the major diterpenoid classes from this last family will be discussed below in terms of their biological properties. (Table 2)

Table 2 Plant diterpene biological activity.

Activity compound	Source	Family
Antifeedant		
Ajugarin I-III	*Ajuga remota*	Limiaceae
Grindelanes	*Chrysothamnus Nauseosus*	Compositae
	Grindelia humilis	Compositae
	Haplopappus camporum	Compositae
Ciliaric acid	*Helianthus annuus*	Compositae
Kalmitoxins	*Kalmia latifolia*	Compositae
Grayanotoxins	*Kalmia latifolia*	Ericaceae
Kaurenoic acids	*Helianthus annuus*	Ericaceae
Trachylobanic acids	*Helianthus annuus*	Compositae
Kaurenoid	*Wedelia asperrima*	Compositae
Glycosides	*Xanthium strumarium*	Compositae
	Atractylis gummifera	Compositae
Teucjaponin A	*Teucrium japonicum*	Compositae
Antifungal		Lamiaceae
Casbene	*Ricinus communis*	
Pseudolaric acid	*Pseudolarix kaempferi*	Euphorbiaceae
Sclareol	*Nicotiana glutinosa*	Pinaceae
		Solanaceae

Activity compound	Source	Family
Fish poison		
Eremone	*Emerocarpus setigerus*	Solanaceae
Hautriwaic acid	*Emerocarpus setigerus*	Solanaceae
Insecticidal		
Ajugarin IV	*Ajuga remota*	Lamiacaea
Antibacterial		
Longikaurins	*Rabdosia longituba*	Lamiaceae
Kaurenoic acid	*Mikania monagasensis*	Compositae
Antibiotic		
Przewaquinone	*Salvia przewalskii*	Lamiaceae
Sonderianol	*Croton sonderrianus*	Euphorbiaceae
Antimicrobial		
7,8-dihydroxy-sandaracopimaradiene	*Iboza riparia*	Lamiaceae
Antiviral		
Bacchotricuneatin	*Baccharis tricuneata*	Compositae
Plant growth inhibitor		
Carboxyatractyloside	*Xanthium strumarium*	Compositae
Sweetening agent		
Stevioside	*Stevia nemoralis*	Compositae

2.5 Biological activity of diterpene compounds isolated from *C. oblongifolius* Roxb.

Diterpenoids isolated from *C. oblongifolius* had been investigated for many biological activities such as cytotoxicity, antimicrobial, antiplatelet aggregation, cAMP phosphodiesterase inhibition, antioxidant, antibacterial, and so forth. For instance, cembranoid compounds (crotocembraneic acid and neocrotocembraneic acid) exhibited cytotoxic activity against P 388 cell line and poilaneic acid exhibited cAMP phosphodiesterase inhibition activity [(5)]. Clerodane and hardwickiic acid exhibited antimicrobial activity [(8)]. Some labdane compounds [(6)] were active against tumor cell line and showed antiplatelet aggregation. Moreover, some pimarane compounds [(9)] showed cytotoxic activity against tumor cell line, as well.

2.6 Cytotoxic activity of some Diterpene compounds of *C. oblongifolius* Roxb.

Previous studies in cytotoxic activity of some diterpene compounds from the stem barks of C. oblongifolius against 6 tumor cell lines: L 929(fibroblast), Hep-G2 (hepatoma), SW 620 (colon), Chago(lung), KATO (gastric) and BT 474 (breast) have been summarized in Table 3.

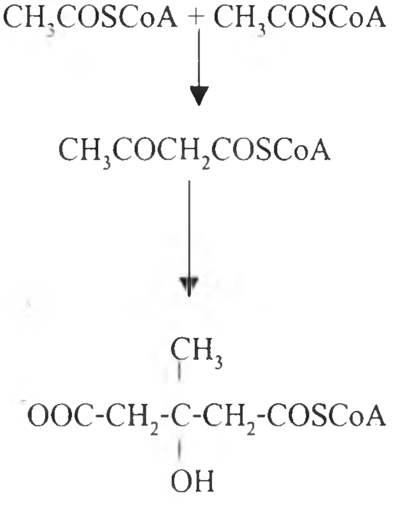
Table 3 Cytotoxic activity against cancer cell lines of some diterpene compounds from *C. oblongifolius*

Compound	% survival					
	L929	HepG2	Sw620	Chago	Kato	BT474
(-)-20-benzyloxyhardwickiic acid	100	74	58	100	65	82
Labda-7,12(E),14-triene-17-al	6	7	3	3	7	13
Labda-7,12(E),14-triene-17-oic acid	73	57	88	59	70	91
Labda-7,13(Z)-diene-17,12-olide-15-ol	100	61	73	72	47	75
Labda-7,12(E),14-triene-17-ol	64	7	3	82	6	11
Crotocembraneic acid	82	71	96	93	6	97
Neocrotocembraneic acid	46	37	96	97	90	5
Neocrotocembranal	82	71	8	12	10	45
Crotohalimaneic acid	64	7	3	82	6	11

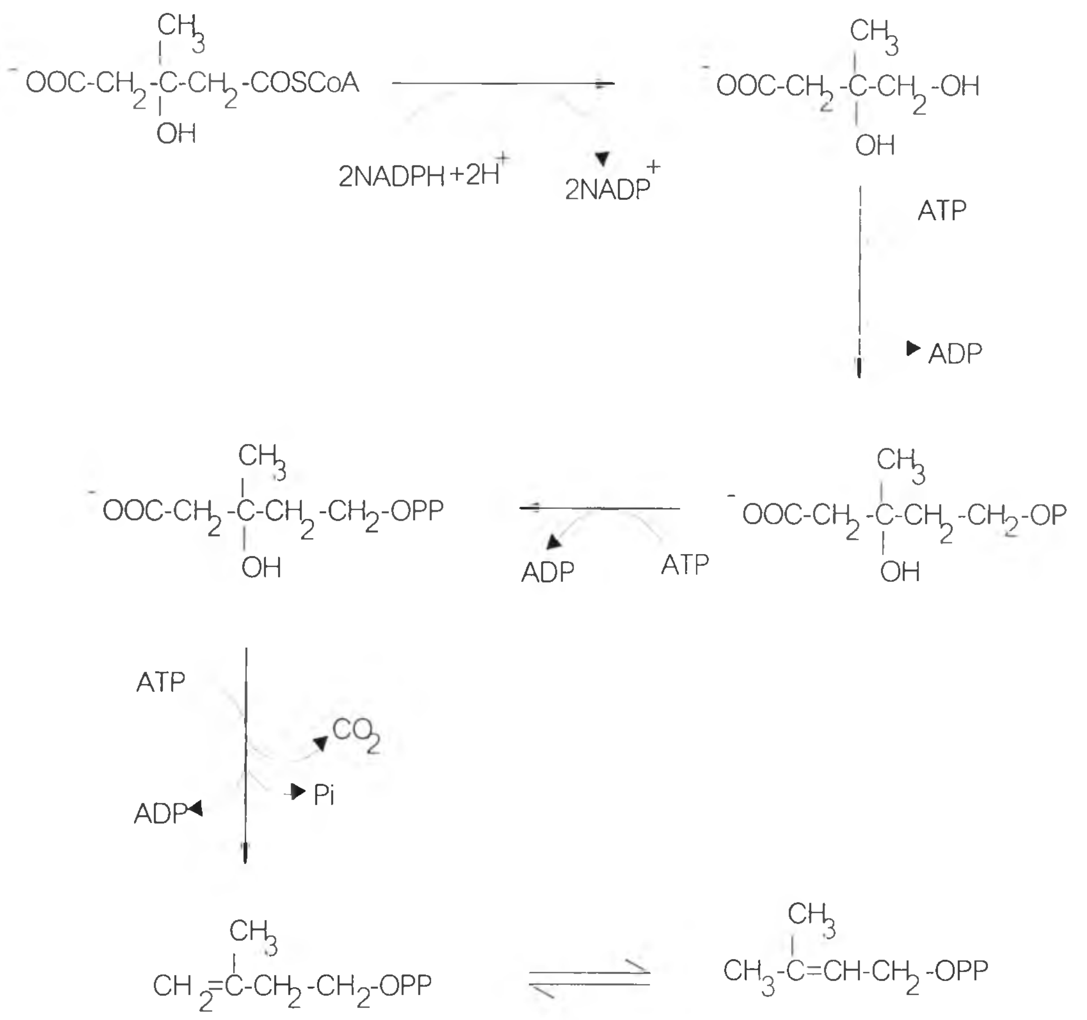
2.7 Biosynthesis of diterpenoid compounds

Biosynthesis of diterpenoid compounds is shown in Scheme 1-3 [26]

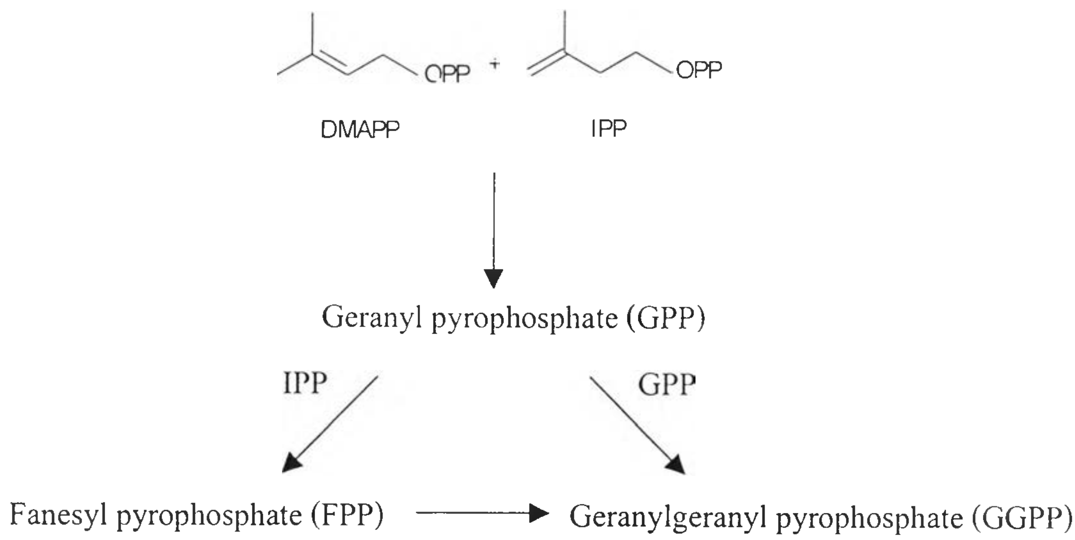
<u>Scheme 1</u> : Biosynthesis of (s)-3-hydroxy-3-methylglutaroyl coenzyme A



Scheme 2 : Origin of the isopentenyl-pyrophosphate (IPP) and dimethyl allylpyrophosphate (DMAPP) units



Scheme 3 : Assembly of Isoprenes



The geranylgeranyl pyrophosphate is, then through cyclize to give many diterpene compounds such as labdane compounds, clerodane compounds, trachylobane compounds, and so forth.

2.7.1 Biosynthesis of Labdane compounds [27]

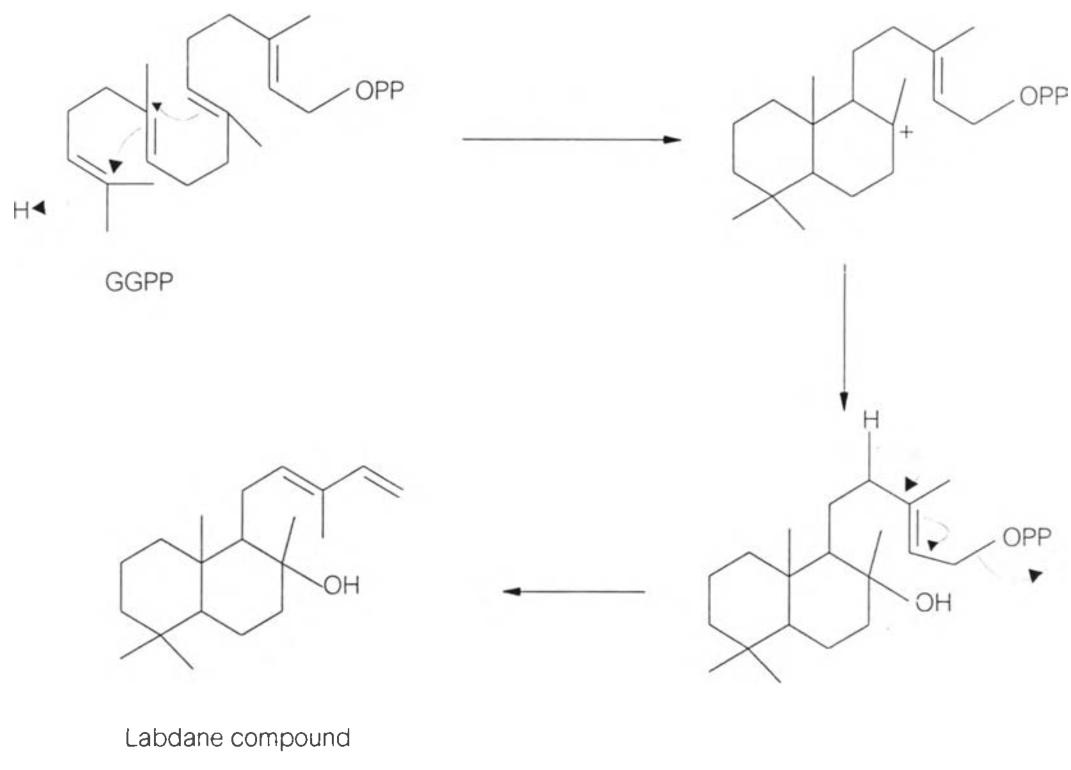


Figure 3: Biosynthesis of Labdane compounds

2.7.2 Biosynthesis of Trachylobane diterpene (28,29)

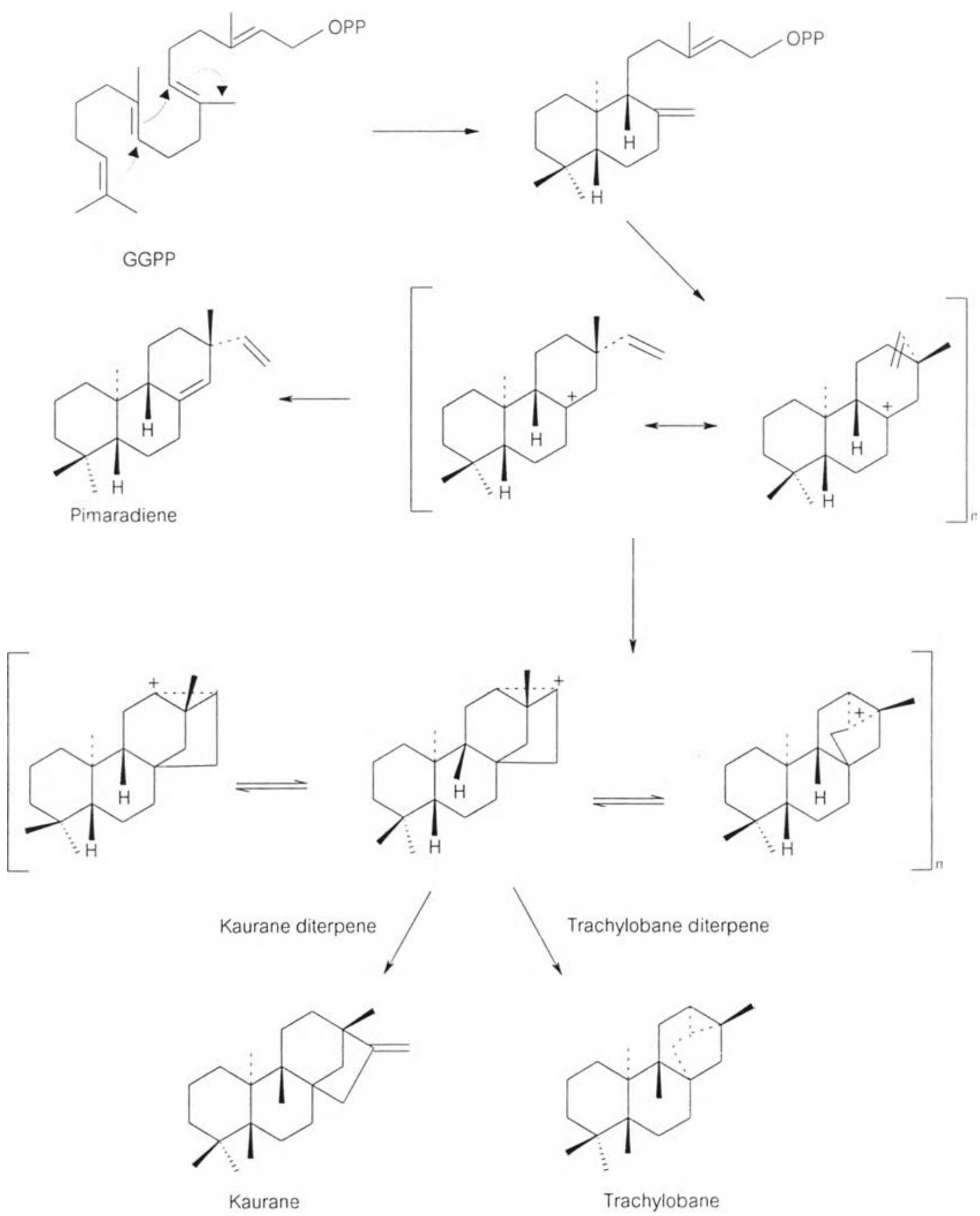


Figure 4: **Biosynthesis of Trachylobane compounds**